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Novel Therapeutic Approaches of Sildenafil Against Rhabdomyolysis-Associated Acute kidney Injury

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ABSTRACT

Objectives: Rhabdomyolysis-associated acute kidney injury (AKI) is a manifestation frequently observed in many cases. Sildenafil (Sild) is a phosphodiesterase-5 inhibitor selected in this study for its experimental protective effects in muscle and kidney injury experimental models. The current study aimed to hypothesize that Sild had a renoprotective effect through the anti-oxidant, anti-inflammatory, and anti-apoptotic mechanisms in glycerol (Gly)-induced rhabdomyolysis rat model. **Methods:** Male Sprague Dawley rats were allocated into four groups: control group (saline, i.p); Sild control group (5 mg/kg, orally); glycerol (Gly) group (50%, 10 ml/kg, i.m.); prophylactic Sild plus glycerol group (5 mg/kg, orally, 1 hr before glycerol). All treatments were applied as a single dose. Blood samples and renal tissues were collected 24 hr following glycerol injection. **Results:** Gly produced renal morphological changes, muscle, and renal dysfunction, in addition to increased mortality rates, oxidative stress, renal inflammatory responses, and renal apoptosis. Sild reduced muscle/kidney function disturbances (serum total CK, CK-MB, creatinine, BUN, in addition to urinary creatinine levels), reduced oxidative stress in renal tissue, increased antioxidant defense (TAC, SOD, NRF-2/HO-1), decreased mortality rates, and accelerated renal histological recovery. Additionally, inflammatory mediator levels of TNF-α, NF-κB, and COX-2 were suppressed. Moreover, the study revealed new insights into protection from rhabdomyolysis-associated AKI, through reduction of renal apoptosis by decreasing levels of BAX and increasing levels of BCL-2. **Conclusions:** Sild protected against rhabdomyolysis-linked renal morphological damage, renal and muscle function disturbances, by decreasing renal oxidative stress, inflammation, and apoptosis. Therefore, Sild proved its muscle/reno-protective impacts, and possibly can be used as a new therapeutic approach for acute muscle and kidney injuries, which represents a new benefit for a common and widely used medication.

Keywords: *Sildenafil; Rhabdomyolysis; BCL-2; BAX; NF-κB; NRF-2; HO-1*

INTRODUCTION

Rhabdomyolysis is a life-threatening disorder, which is expressed by skeletal muscle damage and the liberation of intracellular components into circulation. This potentially occurs, as a result of infections, medications, crush injury, hormonal disturbances, and muscle overactivity[1]. Acute kidney injury (AKI) is a usual indication of rhabdomyolysis, as a consequence of liberation of myoglobin, which accumulates in the kidney and scavenges nitric oxide, causing initiation of oxidative stress, promotion of inflammation, apoptosis, vasoconstriction, and endothelial deterioration[2].

Rhabdomyolysis-damaged muscle cells liberate molecules that penetrate tissues of the kidney, leading to the initiation of the nuclear factor kappa beta (NF-κB) cascade, which promotes the production of inflammatory cells and cytokines, including tumor necrosis factor (TNF-α)[3]

One of the most essential antioxidant defense mechanisms is nuclear factor E2-related factor 2 (Nrf-2) signaling that controls many anti-inflammatory and antioxidant genes expression, including heme oxygenase-1 (HO-1). The importance of Nrf-2 against multiple kidney diseases was confirmed[4]. Protection against rhabdomyolysis-induced AKI is triggered by HO-1, which is the antioxidant enzyme that contributed to heme degradation and is initiated in the existence of reactive oxygen species (ROS) and inflammatory mediators, as tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6)[5]. HO-1 generates ferritin, which decreases free iron and provides cell protection from ROS and apoptosis[6]. Additionally, rhabdomyolysis-associated oxidative stress promotes lipid peroxidation, which amplifies ROS generation, caspases-3 initiation, and tubular-cell apoptosis[7]

Apoptosis is a consequence of caspase activation. The anti-apoptotic family, including B-cell lymphoma 2 (BCL-2) is involved in AKI. BCL-2 is an anti-apoptotic protein that mainly regulates homeostasis and caspase activation[8]. Bax, the apoptotic protein is vital for the release of cytochrome c, in the context of apoptosis, as it associates with apoptosis activating factor 1 (APAF-1), which triggers activation of caspase and apoptosis[9]. Additionally, HO-1, the Nrf-2 downstream gene, could elevate BCL-2 expression, and reduce caspase-3 expression. Furthermore, Nrf-2 could downregulate Bax expression, and reduce caspase-3 expression[10]

Sildenafil (Sild), a distinguished phosphodiesterase-5 (PDE-5) inhibitor, is commonly used for treating erectile dysfunctions and pulmonary hypertension. It also prompted protective properties throughout muscle injury on numerous organs[11-13], supporting its likely usefulness as a drug for the treatment of muscle injury and can be used in comparison with others in reducing muscle oxidative stress and/or inflammation. In addition, its effect on some experimentally-induced kidney injury models favored the selection of Sild to be used in this study[14-16]

In this context, the current work investigated the potential involvement of Sild in renal and muscle protection against AKI secondary to rhabdomyolysis induced by glycerol (Gly) injection in rats, in addition to the potential involvement of the apoptotic (BAX)/anti-apoptotic (BCL-2), Nrf-2/HO-1 and TNF-α/NF-κB pathways in the protective mechanisms exerted by Sild.

MATERIAL AND METHODS

Drugs and chemicals

Glycerol (Gly) was obtained from (EL-Goumhouria Co., Egypt), and was dissolved in normal saline. Sildenafil (Sild) was purchased from (Pfizer, Egypt), and was dissolved in distilled water. All chemicals were at fine grade.

Animals

Male Sprague Dawley rats with weight 250 ± 20 gm were kept 7 days in cages for adaptation at suitable conditions and were fed with regular food and water. The experiment and animal handling were approved by Institutional Animal Care and Use Committee (IACUC) at Faculty of Pharmacy, Delta University for Science and Technology, Gamasa, Egypt (approval number: FPDU 150321/5)

Experimental design and induction of AKI secondary to rhabdomyolysis

Random separation of rats into 4 groups (n = 9) was applied: **1) Control group:** injected once with normal saline i.p.; **2) Sild control group:** received a single oral administration with sildenafil (5mg/kg); **3) Gly group:** received a single i.m. injection with 50% glycerol (10 ml/kg), equally divided into the two hind limbs muscles of each rat; **4) Sild group:** received a single oral administration with sildenafil, one hour before glycerol with the same mentioned doses.

The dose of Sild is chosen based on a previous study[17]. Animals were placed in metabolic cages to collect the urine. Water deprivation with access to food was applied for 24 hrs during the experimental period, during which urine was collected. Then, rats were sacrificed at the end of the study. Blood was collected and centrifuged, for ten minutes at 3,000 rpm. The serum was obtained and used for serological tests. Additionally, kidneys were collected, and weights are measured for calculating the renal somatic index (RSI):

$$RSI = \frac{\text{kidney weight (gm)}}{\text{final body weight (gm)}} \times 100$$

The left kidneys were homogenized in phosphate buffer (PBS), then centrifuged for fifteen

minutes at 4000 ×g. The supernatants were used for enzyme linked immune assay (ELISA) tests. While right kidneys were kept in formalin (10%) until use in histopathological and immunohistochemical assessments.

Assessment of kidney function and rhabdomyolysis

In serum and urine, creatinine (Cr) levels were measured using a colorimetric kit obtained from (Diamond, Egypt). In urine, total protein and albumin levels were measured using colorimetric kits obtained from (SPINREACT Co., Egypt) and (Human, Egypt), respectively.

In serum blood urea nitrogen (BUN), and the rhabdomyolysis markers: total creatine kinase (CK) and creatine kinase-MB (CK-MB) levels were measured, using colorimetric kits purchased from (Biomed diagnostic, Egypt), following the manufacturer's protocol.

Assessment of oxidative stress markers

Malondialdehyde (MDA), nitric oxide (NO), total antioxidant capacity (TAC), superoxide dismutase (SOD), and reduced glutathione (GSH) levels were detected in renal homogenates by colorimetric kits obtained from (Biodiagnostic, Egypt), following the manufacturer's protocol.

Assessment of the antioxidant-defense signaling (NRF-2 / HO-1)

In kidney homogenates, nuclear factor E2-related factor 2 (NRF-2) and heme oxygenase-1 (HO-1) levels, were assessed with ELISA kits purchased from (Bioassay Technology Laboratory, Shanghai Crystal Day Biotech Co., Ltd., Shanghai, China). The assay was done following the manufacture procedure using specific biotinylated antibody, followed by adding the homogenized renal tissues, then incubating for two hours at 37◦C. Washing with PBS, followed by streptavidin horseradish peroxidase (HRP) addition, and incubation for half an hour at 37◦C were the last steps. Lastly, at 450 nm, absorbance was recorded.

Immunohistochemical assessment

TNF-α, NF-κB p65, cyclooxygenase 2 (COX-2), Bax and Bcl-2 antibodies were obtained from (ABclonal Technology, MA, USA, catalog number: A0277, Bioss antibodies, MA, USA, catalog number: bs-20159R, ABclonal Technology, MA, USA, catalog number: A1253, Servicebio technology Co., China, catalog number: GB11007-1 and Genemed Biotechnologies, Inc., CA, USA, catalog number: 61005), respectively. Renal tissues were assessed using the staining protocol: Avidin-Biotin Complex (ABC), according to the directions of the manufacturer.

Briefly, deparaffinized tissue sections were treated with 3% hydrogen peroxide, then incubated with primary antibody. Slides were washed, incubated with biotinylated anti-rabbit secondary antibody, then complex avidin–horseradish peroxidase was added with diaminobenzidine (DAB; Sigma Chemical Co., St. Louis, MO), as the chromogen, to detect immunoreactivity[18]. Mayer's hematoxylin was used to counterstain the sections, then photographed with a microscope at 400× magnification and semi-quantified to detect the scores of positive cell counts.

Histopathological examination of renal tissues

Formalin (10%) was used for fixation of the right kidneys, which were then immersed in paraffin wax, cut into 5 µm sections, then stained with hematoxylin and eosin (H&E), followed by examination with a microscope at 400× magnification, and semi-quantified to detect the renal score injury for tubular necrosis, tubular dilation, cast formation, congestion and damaged glomeruli, using a 5-point scale: negative (0), minimal (1), moderate (2), severe (3), and very severe (4). Summation of scores for each rat in the group was applied, to obtain median scores of renal injury for all groups, and were semi-quantified[19].

Statistical analysis

Data are expressed as means ± standard error of the means (S.E.M). Statistical analyses were done with GraphPad Prism 5 (GraphPad Software, USA). The parametric test, one-way analysis of variance (ANOVA), followed by Tukey Kramer's post hoc was selected to compare the means at $p < 0.05$. The nonparametric test, Kruskal-Wallis, followed by Dunn's multiple comparison post-hoc was selected for scores analysis for mortality, histopathology, and immunohistochemistry at $p < 0.05$.

RESULTS

Effect of sildenafil on mortality rates and renal somatic index (RSI)

Mortality was increased significantly in Gly group to 44.44 %, in comparison with control group. Sild group produced a significant decreased in mortality to 11.11% compared with Gly group. Renal somatic index was markedly increased in Gly group, in comparison with control group. Sild group retained normal values for RSI, in comparison with Gly group (**Table 1**).

Effect of sildenafil on renal functions

Renal functions were significantly increased in Gly group, in comparison with control group. Serum levels of BUN and creatinine, in addition to urine level of creatinine, were markedly decreased in Sild group,

Table 1. Effect of Sildenafil on mortality rates and RSI

	Control	Sild control	Gly	Sild
% Mortality	**0%**	**0%**	**44.44% #**	**11.11% ***
RSI	**0.7 ± 0.04**	**0.71 ± 0.03**	**1.29 ± 0.16 #**	**0.78 ± 0.02 ***

Mortality rates are expressed as (%). Kruskal-Wallis test was used for analysis, followed by Dunn's multiple comparison. RSI are presented as means ± S.E.M. (n=9). One-way ANOVA was used for analysis, followed by Tukey Kramer's post hoc. #,, at p < 0.05, in comparison with control, and Gly groups respectively, Gly: Glycerol; Sild: Sildenafil; RSI: Renal somatic index; AKI: Acute kidney injury*

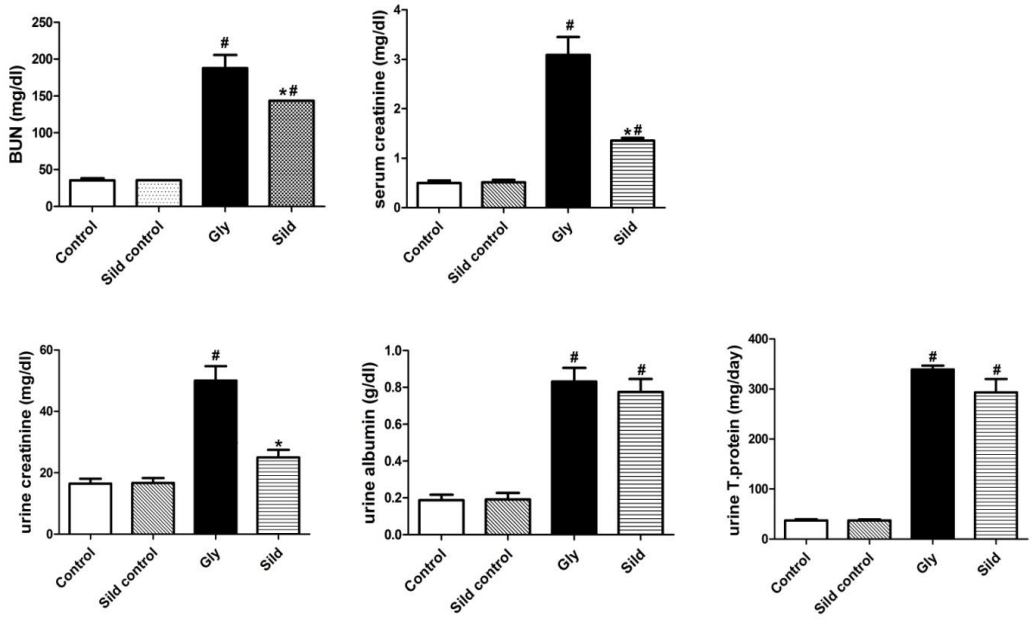

Figure 1. Effect of Sildenafil on kidney functions. Serum levels of BUN, creatinine, in addition to urine concentrations of creatinine, albumin and T. protein were measured in Gly-induced rhabdomyolysis rat model. Data are expressed as means ± S.E.M. (n=8). Statistical analysis was performed using one-way ANOVA, followed by Tukey Kramer's post hoc-test, #, *, at p< 0.05, in comparison with control and Gly groups, respectively, Gly: Glycerol; Sild: Sildenafil; BUN: Blood urea nitrogen; T. protein: total protein

while no effect was observed in urine levels of T. protein and albumin of Sild group, in comparison with Gly group **(Figure 1).**

Effect of sildenafil on rhabdomyolysis markers

Rhabdomyolysis markers are significantly increased in Gly group in comparison with control group and were significantly decreased in Sild group, in comparison with Gly group **(Figure 2).**

Effect of sildenafil on oxidative stress

Renal MDA and NO levels were increased significantly in Gly group, in comparison with control group. Meanwhile, renal TAC, SOD and GSH levels were decreased in Gly group, in comparison with control group **(Figure 3).**

Renal MDA level was decreased significantly in Sild group, in comparison with control group. Meanwhile, sildenafil had no significant effect on nitrosative stress, indicated by the slight decrease in NO level, in comparison with Gly group. Significant increase in levels of TAC and SOD with some amelioration in the level of GSH, were indicated in Sild group in comparison with Gly group **(Figure 3).**

Effect of sildenafil on the signaling of NRF-2/HO-1

Renal levels of NRF-2 and HO-1 were increased significantly in Sild group, in comparison with Gly and control groups, indicating its potent antioxidant activity **(Figure 4).**

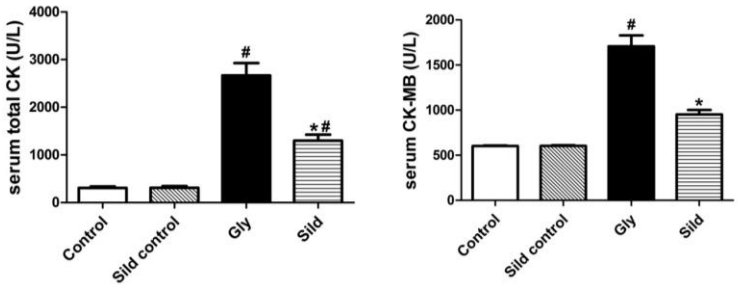

Figure 2. Effect of Sildenafil on rhabdomyolysis markers. Serum levels of CK and CK-MB are measured in Gly-induced rhabdomyolysis rat model. Data are expressed as means ± S.E.M. (n=8). Statistical analysis was performed using one-way ANOVA, followed by Tukey Kramer's post hoc-test, #, *, at p <0.05, in comparison with control and Gly groups, respectively, Gly: Glycerol; Sild: Sildenafil; CK: Creatine kinase; CK-MB: Creatine kinase isoenzyme MB

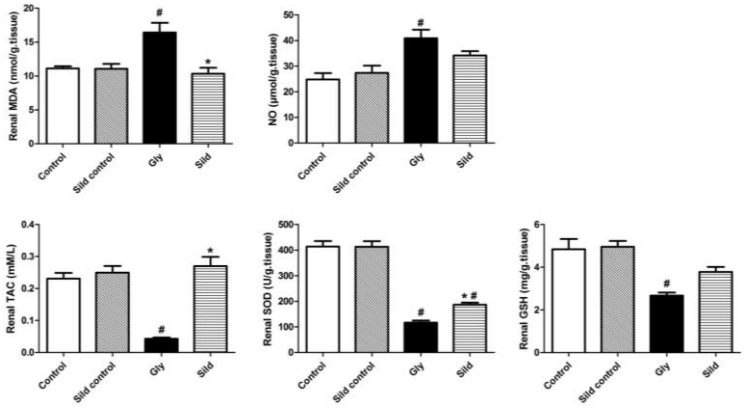

Figure 3. Effect of Sildenafil on oxidative stress markers. The lipid peroxidation marker (MDA) and nitrosative stress marker (NO), in addition to the antioxidant markers (TAC, SOD, and GSH), were measured in Gly-induced rhabdomyolysis rat model. Data are expressed as means ± S.E.M. (n=6). Statistical analysis was performed using one-way ANOVA, followed by Tukey Kramer's post hoc-test, #, *, at p<0.05, in comparison with control and Gly groups, respectively, Gly: Glycerol; Sild: Sildenafil; MDA: Malondialdehyde; NO: Nitric oxide; TAC: Total antioxidant capacity; SOD: Superoxide dismutase; GSH: Reduced glutathione

Effect of sildenafil on NF-κB signaling

Renal levels of TNF-α, NF-κB p65 and COX-2 were increased in Gly group, as shown by positive nuclear staining (black and yellow arrows) and glomeruli (black arrowheads). Sild group decreased levels of TNF-α, NF-κB p65 and COX-2, as shown by a low positive nuclear staining (black and yellow arrows) and glomeruli (black arrowheads) **(Figure 5(a)).** Semi-quantitative analysis indicated high expression scores for Gly group and low expression scores for Sild group, which confirmed these findings **(Figure 5(b)).**

Effect of sildenafil on apoptotic markers

Renal level of Bax, were increased in Gly group, as shown by positive nuclear staining (yellow arrows). Sild group decreased level of Bax as shown by a low positive nuclear staining (yellow arrows) **(Figure 6(a)).** Meanwhile, renal level of BCL-2 was decreased in Gly group, as shown by low positive nuclear staining (yellow arrows). Sild group increased level of BCL-2, as shown by a low positive nuclear staining (yellow arrows) **(Figure 6(a))**. Semi-quantitative analysis indicated that Gly group showed a significant increase in Bax/BCL-2 ratio in comparison with control values. The ratio was decreased significantly in Sild group, in comparison with Gly group **(Figure 6(b)).**

Effect of sildenafil on renal histological changes

Renal sections stained with H&E showed normal features in control group. Gly group showed tubular dilation with cast formation (yellow arrow) and acute tubular necrosis (black arrow). Sild group showed mild tubular dilation and cast formation (yellow arrow) with focal tubular necrosis (black arrow) **(Figure 7(a)).** Semi-quantitative analysis for renal injury scores indicated that Sild group showed significant restoration in renal histology, in comparison with Gly and control groups **(Figure 7(b)).**

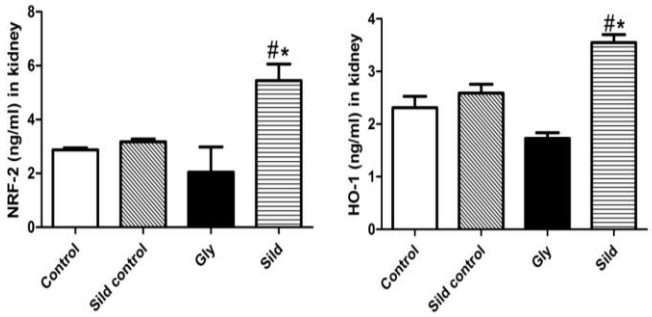

Figure 4. Effect of Sildenafil on signaling of NRF-2/HO-1. Protein expressions of the antioxidant related markers: NRF-2 and HO-1 are measured in Gly-induced rhabdomyolysis rat model. Data are presented as means ± S.E.M. (n=5). One-way ANOVA was used, followed by Tukey Kramer's post hoc-test, #, *, at $p<0.05$ in comparison with control and Gly groups, respectively, Gly: Glycerol; Sild: Sildenafil; NRF-2: Nuclear factor E2-related factor 2; HO-1: Heme oxygenase-1

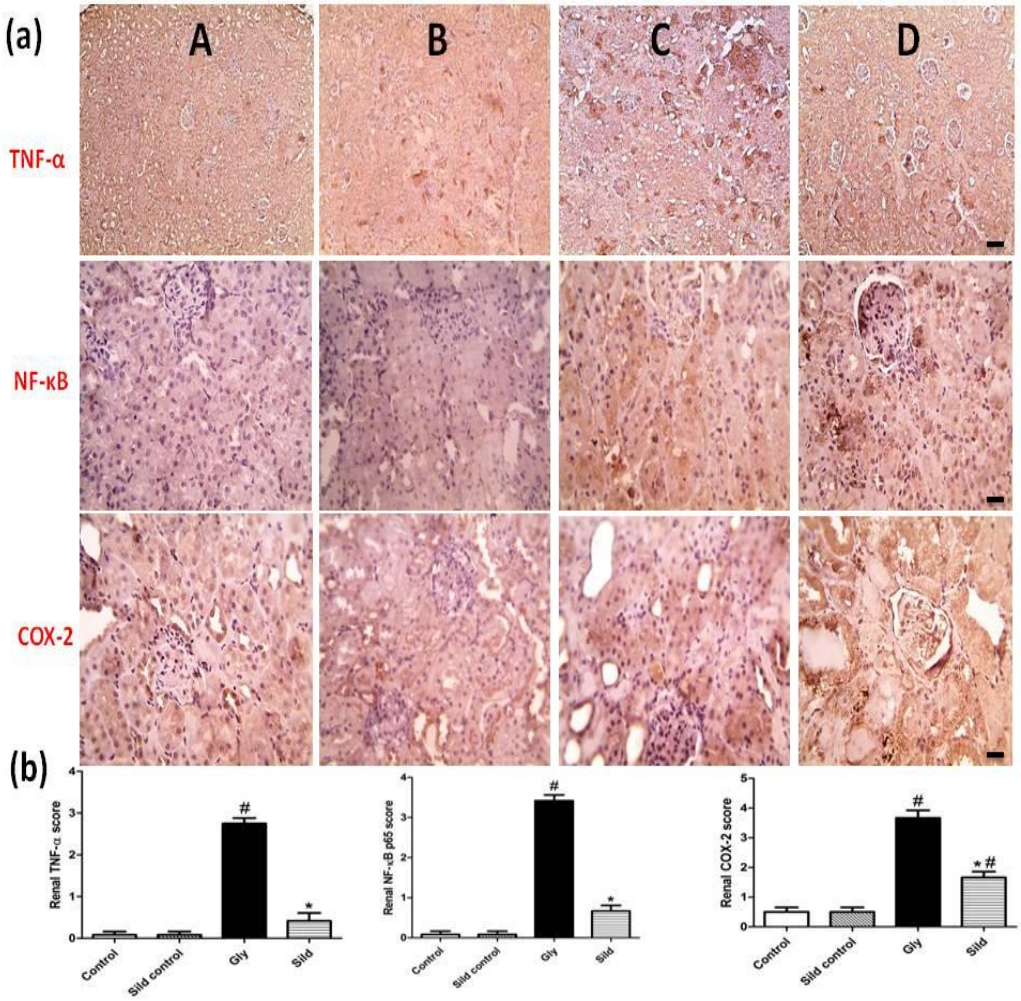

Figure 5. Effect of Sildenafil on TNF-α, NF-κB and COX-2 expressions. (a) Photos of renal TNF-α, NF-κB and COX-2 nuclear expression for: **A**; normal control, **B**; Sild control, **C**; Gly, and **D**; Sild groups. IHC counterstained with Mayer's hematoxylin, X: 400, bar: 50. **(b)** Semi-quantitative analysis of positive cell counts to detect TNF-α, NF-κB and COX-2 scores. Data expressed as medians (n=6), #, *, at $p< 0.05$, in comparison with control and Gly groups, respectively. Kruskal-Wallis test was used, followed by Dunn's multiple comparison test. Gly: Glycerol; Sild: Sildenafil; TNF-α: Tumor necrosis factor-alpha; NF-κB: Nuclear transcription factor.

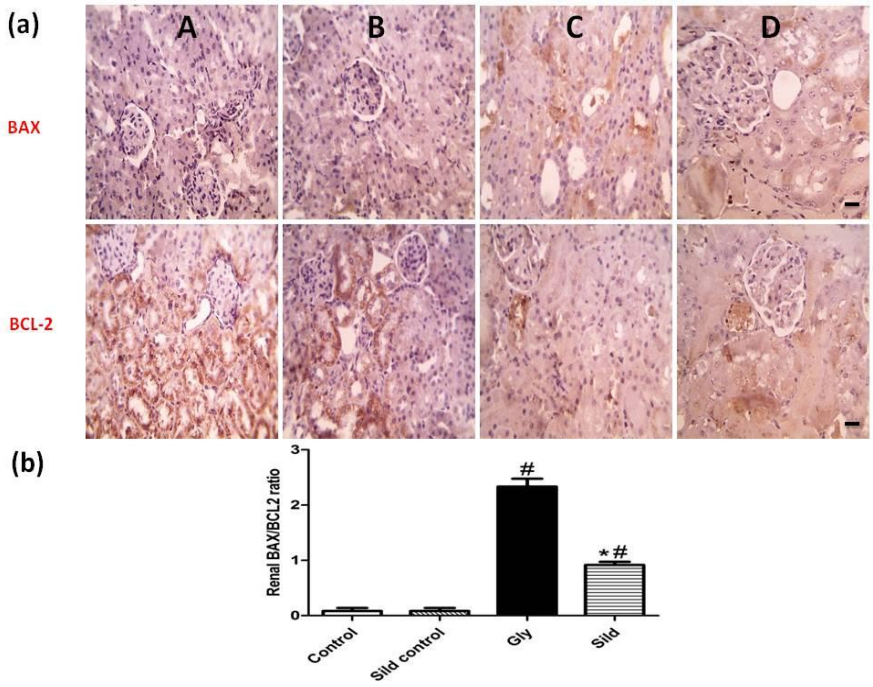

Figure 6. Effect of Sildenafil on apoptotic markers (BAX/BCL-2). (a) Photos of renal BAX and BCL-2 nuclear expression for: **A**; normal control, **B**; Sild control, **C**; Gly, and **D**; Sild groups. IHC counterstained with Mayer's hematoxylin, X: 400, bar: 50. **(b)** Graph illustrated effect of Sild on BAX/BCL-2 ratio. Data are expressed as mean ± S.E.M. (n=6). Statistical analysis was performed using one-way ANOVA, followed by Tukey Kramer's post hoc-test, #, *, at $p<0.05$, in comparison with control and Gly groups, respectively, Gly: Glycerol; Sild: Sildenafil; BAX: BCL-2 associated x protein; BCL-2: B-cell lymphoma 2

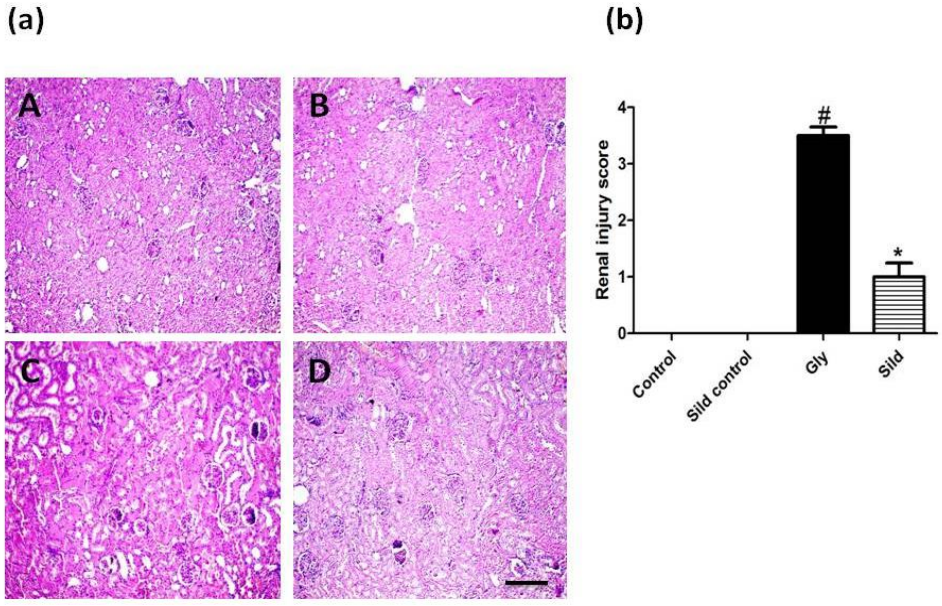

Figure 7. Effect of Sildenafil on renal histological changes. (a) H&E stained photos of renal sections for: **A**; normal control, **B**; Sild control, **C**; Gly, and **D**; Sild groups. X: 100, bar: 100. **(b)** Graph illustrated effect of Sild on renal injury score. Data are presented as medians (n=6), #, *, at $p< 0.05$, in comparison with control and Gly groups, respectively. Kruskal-Wallis test was used, followed by Dunn's multiple comparison test, Gly: Glycerol; Sild: Sildenafil.

DISCUSSION

The current work reported that Gly-induced AKI is secondary to rhabdomyolysis in rats. Glycerol can cause skeletal muscle injury and liberation of myoglobin into the blood leading to these toxicities[20]. Muscle injury was confirmed by the deterioration in muscle function: high levels of serum CK and CK-MB. Renal injury was confirmed by the disturbance in renal function: high levels of serum BUN, and creatinine, plus high levels of urine T. protein, albumin, and creatinine, with an increase in RSI. These changes were accompanied by renal histopathological changes, a decrease in levels of TAC, SOD, GSH, Nrf-2, HO-1, and an increase in levels of MDA NO NF-κB, TNF-α, and COX-2, in addition to activation of the pro-apoptotic BAX and down regulation of the anti-apoptotic BCL-2. Moreover, glycerol increased the rate of mortality.

The AKI that occurred with glycerol injection was previously confirmed to be the consequence of these inflammatory and oxidative changes[21-24]. Previous reports also confirmed the elevated NF-κB, TNF-α, and COX-2 expression levels in AKI and the experimental rhabdomyolysis model[25-28].

Our study revealed that single oral administration of Sild (5 mg/kg), one hour prior to Gly injection maintained the overall body health, as it achieved improvement in Gly-induced acute muscle injury. This study indicated that Sild could be a muscle protective drug, through a reversal of the elevated serum levels of total CK and CK-MB in the rhabdomyolysis model. Meanwhile, Sild protected against AKI, as it restored the normal renal histology, RSI, antioxidant balance, reversed AKI-related histopathological changes, oxidative stress, inflammatory actions, apoptosis, and improved survival.

These actions could be due to the impact of the Bcl-2 family, confirmed by the immunohistochemical observations: decline in the expression of pro-apoptotic BAX and augmentation in the expression of anti-apoptotic BCL-2. Incidentally, the decline in the pro-apoptotic BAX could be associated with an increase in the activation of Nrf-2, and an increase in the anti-apoptotic BCL-2 could be associated with an increase in the activation of HO-1.

The ability of Sild to improve Gly-induced elevation in free radicals is reliable to the outcomes of previous studies, which confirmed its antioxidant effects and presented that Sild decreased MDA in several experimentally-induced kidney injuries in rats[29-31]. Previous reports denoted that Sild was able to increase GSH[32], superoxide dismutase[33], and TAC[34] activities. Meanwhile, Sild had no remarked effect on NO level, as it is well known to increase its constitutive levels in several tissues, without producing nitrosative damage. Consequently, Sild can be considered an effective drug in reducing renal oxidative stress.

Nrf-2 is an important factor in fighting the renal injury initiated by oxidative stress, and inflammation[4]. Meanwhile, HO-1 depletion is related to AKI secondary to rhabdomyolysis, with elevated creatinine levels and incidence of mortality. In addition, normal levels of HO-1 could inhibit inflammatory cytokine expression, including TNF-α[21].

In the present experimental study, Sild could be an effective anti-inflammatory drug, as it produced a remarkable decrease in the production of TNF-α and COX-2. Additionally, treatment with Sild achieved improvement in rhabdomyolysis-induced disruption in the balance between NRF-2 and NF-κB, by decreasing the expression of NF-κB and increasing the expression of NRF-2 and HO-1 in rhabdomyolysis-induced AKI rat model. These consequences suggest that Sild efficiently diminishes inflammation via inhibition of the NF-κB signal transduction pathway, which was stated in previous reports as well. Other studies supported our results and confirmed that Sild activates the NRF-2/HO-1 pathway. Therefore, Sild may be considered as an NF-κB inhibitor, and an NRF-2 activator, possibly by its anti-inflammatory effect, as well as its antioxidant effect. In addition, the resulted modulation of these pathways proved the antioxidant and anti-inflammatory effects of Sild, which was confirmed in previous reports[35,36] kappa B; COX-2: Cyclooxygenase-*2*

Inhibition of apoptosis protected against renal injury experimentally in a rhabdomyolysis model[3,37]. The apoptotic BAX and the anti-apoptotic BCL-2 genes are expressed in regenerated tubular cells[38]. Previous models of cisplatin and ischemia-induced AKI documented the protective effects exerted by the decline of the BAX apoptotic gene, due to renal apoptosis inhibition[8,39,40]. In this study, Sild ameliorated glycerol-induced AKI via regulating BAX/BCL-2 proteins, thus suppressing apoptotic cell death, as noticed by immunolabeling.

Sildenafil exerted protective effects by multiple mechanisms: antioxidant, anti-inflammatory, and anti-apoptotic actions by regulating the BCL-2 protein family in the rhabdomyolysis model

CONCLUSION

In conclusion, AKI by glycerol is initiated by renal oxidative stress, inflammation, and apoptosis, while prevented by Sild pretreatment. Therefore, Sild could be used as an eventual medicine for recovery from clinical AKI secondary to rhabdomyolysis.

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Conflict of interest

The author declares that there isn't any conflict of interest regarding the publication of this paper.

Abbreviations

AKI, acute kidney injury; BAX, BCL-2 associated x protein; BCL-2, B-cell lymphoma 2; BUN, blood urea nitrogen; CKI, chronic kidney injury; CK, creatine kinase; CK-MB, creatine kinase-MB; Cr, creatinine; ELISA, sandwich enzyme-linked immunosorbent assay; Gly, glycerol; GSH, reduced glutathione; HO-1, heme oxygenase-1; HRP, streptavidin horseradish peroxidase; IL-, interleukin; MDA, malondialdehyde; NF-κB, nuclear transcription factor kappa B; NO, nitric oxide; NRF-2, nuclear factor E2-related factor 2; PBS, phosphate buffer saline; PDE-5, phosphodiesterase-5; RIAKI, rhabdomyolysis-induced acute kidney injury; RSI, renal somatic index; SOD, superoxide dismutase; Sild, Sildenafil; TAC, total antioxidant capacity; TNF-α, tumor necrosis factor-alpha**.**

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