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A study on the effect of cimetidine and L-carnitine on myoglobinuric acute kidney injury in male rats

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ABSTRACT

Myoglobinuric acute renal failure is the most important life threatening complication of rhabdomyolysis. Iron, free radicals, nitric oxide and cytochrome p450 are involved in the pathogenesis of mARF.

The aim of this study is to compare the effect of cimetidine, L-carnitine and both agents together on mARF in rats.

Forty rats were divided into 5 groups; group I: control rats, group II: myoglobinuric ARF rats, group III: mARF rats received L-carnitine (200 mg/kg, i.p.), group IV: mARF rats received cimetidine (150 mg/kg i.p.) and group V: mARF rats received both agents together. 48 h after glycerol injection, systolic blood pressure was measured. Urine and blood samples were collected to evaluate urine volume, GFR, BUN, creatinine, K, Na, serum creatine kinase, NO and glutathione levels. Kidney specimens were taken to investigate renal cytochrome p450 and for histological examinations.

Cimetidine treatment significantly decreased creatinine, BUN, K, Na, SBP and creatine kinase and increased GFR and urine volume compared to group II. L-carnitine exerted similar changes except for the effect on K and GFR. NO was significantly decreased, while renal glutathione and cytochrome p450 were significantly increased in groups treated with L-carnitine or cimetidine as compared to group II. Combined treatment further improved renal functions, creatine kinase, oxidative stress parameters and SBP as compared to each therapy alone. The histological changes confirmed the biochemical findings.

Cimetidine and L-carnitine have protective effects – almost equally – against mARF. Using both agents together, minimises the renal injury.

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Introduction

Rhabdomyolysis is a common cause of ARF, especially at times of conflict or after major disasters when crush injuries are frequent. The outcome of rhabdomyolysis is usually good provided that it doesn't result in myoglobinuric acute renal failure (mARF) [1].

The pathophysiology of mARF has been studied extensively in animal model of glycerol-induced ARF. The main pathophysiological mechanisms are renal vasoconstriction, intraluminal cast formation, and direct heme-iron-induced cytotoxicity [2]. Oxidative stress has a key role in this pathogenesis [3].

Iron (Fe) has been implicated to play an important role in myoglobinuric acute kidney injury [4]. In vivo studies suggest that heme Fe causes proximal tubular lipid peroxidation and cytotoxicity, without invoking release of free iron, and this process is due to redox cycling of the heme group from ferrous to ferric and to ferryl oxidation states [5]. L-Carnitine is an anti-oxidant and prevents the accumulation of end-products of lipid peroxidation [6].

Studies indicate that cytochrome p450 may also be an important source of the catalytic iron. Inhibition of this enzyme by the use of cimetidine may decrease rhabdomyolysis-induced myoglobinuric nephrotoxicity [7]. Cimetidine binds to cytochrome p450 and forms a stable complex [8], through the binding of the imidazole ring structure of cimetidine to the haem moiety of cytochrome p450 [9].

This collective body of evidence suggests an important role for reactive oxygen metabolites in toxic acute renal failure and may provide therapeutic opportunities for the prevention or treatment of mARF in human.

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The present work was designated to study the effect of cimetidine (cytochrome p450 inhibitor), L-carnitine (antioxidant) and both agents together on myoglobinuric ARF in rats aiming to establish a mechanism that may aid as a prophylactic treatment.

Materials and methods

This study was carried out in strict accordance with the approved guidelines. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Faculty of Medicine Cairo University.

The present study was carried out in Physiology Department, Faculty of Medicine, Cairo University on forty adult male Wistar Albino rats weighting 150–200 g. They were housed singly in wire mesh cages at room temperature (25 °C with 55% relative humidity), normal light and dark cycle and were allowed to acclimatise to their environment and to the blood pressure measurement procedures every day for 5 days before the beginning of the experiment. Veterinary care was provided by the Laboratory Animal House Unit of Faculty of Medicine, Cairo University. All animals had a free access to laboratory chow[1] and tap water.

The animals were divided into five groups, eight rats each. **Group I, (Control group)** served as normal control reference values for the measurements evaluated. They were injected with normal saline (10 ml/kg, i.m.). **Group II, (ARF group)** were injected with 50% glycerol[2] (10 ml/kg, i.m.) with no further treatment. **Group III, (ARF + L-carnitine group)** were injected with glycerol (10 ml/kg, i.m.) and L-carnitine[3] (200 mg/kg, i.p.) concomitant with, and 24 h after glycerol injection [6]. **Group IV, (ARF + cimetidine group)** were injected with glycerol (10 ml/kg, i.m.) and cimetidine[4] (150 mg/kg, i.p.) concomitant with glycerol injection [7]. **Group V,** (ARF + L-carnitine + cimetidine group) were injected with glycerol (10 ml/kg, i.m.) and L-carnitine (200 mg/kg, i.p.) concomitant with and 24 h after glycerol injection, with cimetidine (150 mg/kg, i.p.) concomitant with glycerol injection. All groups were water deprived 24 h before saline (control group) or glycerol (groups 2–5) injection [6].

Urine was collected separately for each rat, using special cages similar to those described by Demirkan and Melli [10], for 24 h, starting 24 h after glycerol injection. Forty-eight hours after glycerol injection, rat tail systolic blood pressure was measured by the Power Lab at Physiology Department, Faculty of Medicine, Cairo University. This system is an electronic version of the traditional sphygmomanometer cuff method [11].

The rats were anaesthetised and blood samples were withdrawn through retro-orbital route using capillary tubes [12] and serum was separated and stored at −70 °C until used. The serum was used for determination of serum creatinine, blood urea nitrogen, Na, K, creatine kinase, glutathione (GSH), and nitric oxide (NO) levels. At the end of experiment, rats were culled using chloroform inhalation and tissue samples from the right kidneys were dissected and kept frozen at −80 °C in liquid nitrogen until they were used to measure cytochrome p450 content in the kidney tissue. Tissue samples from the left kidneys were dissected and fixed in 10% formalin—buffered solution for histological examinations. Blood and tissue samples were collected 48 h after glycerol injection.

[1] Laboratory chow ingredients (g/kg): Carbohydrates: Corn starch 480, Sucrose 100, Fats: Soybean oil 50, animal fat 120, Protein: Casein 190.

[2] Glycerol was prepared in the Biochemistry Department Faculty of Medicine Cairo University.

[3] L-Carnitine was obtained from Mepaco Co., Egypt in the form of ampoules, each ampoule contains 1 g/5 mL.

[4] Cimetidine was obtained from Sigma Co., Alorich in the form of powder.

Biochemical studies

The biochemical studies were held at the Biochemistry Department, Faculty of Medicine, Cairo University.

Creatinine was estimated by QuantiChromTM creatinine Assay Kit [13]. Determination of GFR was done by calculating the creatinine clearance using the following equation:

$$\text{Creatinine clearance} = \frac{u}{p} \times v$$

where u = urinary concentration of creatinine (mg/100 ml); p = plasma concentration of creatinine (mg/100 ml); v = urine volume (ml/min).

Serum urea was estimated by QuantiChromTM Urea Assay kit (DIUR-500) [14]. Serum Na and K were estimated by Sodium and Potassium Enzymatic Assay Kit (Liquid Stable) [15,16]. Serum creatine kinase was estimated by Enzy ChromTM Creatine Kinase Assay Kit (ECPK-100) [17]. Nitric oxide was determined in serum according to the method of Miranda et al. [18]. Glutathione was determined in serum according to the method of Beutler et al. [19]. Cytochrome p450 in kidney tissues was measured by ELISA kit supplied by Uscn Life Science Inc.

Histological examination

The histological studies were done at the Histology Department, Faculty of Medicine, Cairo University. Sections were taken from the kidneys of the different groups' rats and fixed in 10% formalin buffered saline solution. Paraffin wax tissue blocks were prepared for sectioning at 5–7 μm using Leica rotator microtome (Germany). The obtained tissue sections were stained by Hematoxylin and Eosin stains for histological examination through the light microscope [20].

To evaluate renal damage, counting the affected glomeruli and the affected tubules was done in 10 serial non overlapping fields (at magnification of ×200) for each specimen for all the experimental groups. Values were calculated as percent of total of 100 glomeruli, or 200 cortical tubules for each specimen. Results were tabulated and statistically analyzed.

Glomeruli were counted as affected according to presence of: segmentation of capillary tuft, narrowing or obliteration of Bowman's space, mesangial expansion, proliferation of parietal layer of Bowman's capsule, distortion, or shrinkage of glomerulus.

Tubules were counted as affected according to presence of: abnormal staining or cytoplasmic vacuolation of lining epithelium, darkened nuclei, detached epithelium, or presence of casts inside lumen.

Statistical methods

Data were coded and entered using the statistical package SPSS version 15. Data was summarised using mean, standard deviation and range for the quantitative variable. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables while non-parametrical Kruskal–Wallis test and Mann–Whitney test were used for non-normally distributed quantitative variables [21]. *P*-values less than 0.05 were considered as statistically significant.

Results

Study of Renal functions in the experimental groups

Intra muscular hypertonic glycerol injection induced a deterioration of glomerular and tubular kidney functions as compared to

Table 1
Comparison between mean ± SD of serum creatinine (mg/dl), BUN (mg/dl), Na (mg/dl), K (mg/dl), GFR (ml/min) and urine volume (ml/24 h) levels in all studied groups.

Parameter	Group (I)	Group (II)	Group (III)	Group (IV)	Group (V)
Serum creatinine (mg/dl)	0.1 ± 0.1	1.3 ± 0.33*	0.66 ± 0.13*#	0.62 ± 0.16*#	0.36 ± 0.09#@
BUN (mg/dl)	28.35 ± 4.05	74.17 ± 8.02*	48.22 ± 4.03*#	46.7 ± 5.41*#	36.62 ± 5.62#@$
Na (mg/dl)	128.02 ± 3.2	168.51 ± 10.99*	145.79 ± 6.41*#	146.05 ± 8.41*#	131.11 ± 5.77#@$
K (mg/dl)	3.53 ± 0.28	5.7 ± 0.87*	4.81 ± 0.29*	4.46 ± 0.77#	3.84 ± 0.64#@
GFR (ml/min)	16.07 ± 1.68	0.87 ± 0.22*	2.55 ± 0.54*	3.35 ± 0.86*#	5.58 ± 1.99*#@
Urine volume (ml/24 h)	4.54 ± 0.35	1.66 ± 0.41*	2.8 ± 0.51*#	3.15 ± 0.44*#	3.66 ± 0.73*#@

Values are represented as mean ± SD.
* Statistically significant compared to corresponding value in group (I) ($P < 0.05$).
# Statistically significant compared to corresponding value in group (II) ($P < 0.05$).
@ Statistically significant compared to corresponding value in group (III) ($P < 0.05$).
$ Statistically significant compared to corresponding value in group (IV) ($P < 0.05$).

control group. Table 1 demonstrates that the values of serum creatinine, BUN, Na and K significantly increased while GFR and urine volume significantly decreased in ARF group (group II) as compared to the control group (group I).

Treatment of ARF rats with either L-carnitine or cimetidine induced a significant decrease in the values of serum creatinine, BUN and Na and a significant increase in urine volume as compared to the ARF group (group II); however, the values of these parameters still exhibited significant changes when compared to the control animals.

The serum K showed a significant decrease and GFR showed a significant increase in ARF + cimetidine group (group IV) as compared to the ARF group (group II), while GFR and serum K were not significantly different in ARF + L-carnitine group (group III) as compared to the same group.

The values of serum creatinine, BUN, Na and K significantly decreased, while GFR and urine volume significantly increased in ARF + L-carnitine + cimetidine group (group V) as compared to the ARF group (group II). The mean values of these parameters, except for GFR and urine volume, showed no significant differences in ARF + L-carnitine + cimetidine group (group V) as compared to the control group (group I).

Also, Table 1 shows that there was no significant change in the levels of the serum creatinine, BUN, Na, K, GFR and urine volume in ARF + cimetidine group (group IV) as compared to the ARF + L-carnitine group (group III), but the value of these parameters were significantly improved in ARF + L-carnitine + cimetidine group (group V) as compared to the ARF + L-carnitine group (group III).

The values of serum creatinine, K, GFR and urine volume showed no significant difference, while the level of BUN and serum Na significantly decreased in ARF + L-carnitine + cimetidine group (group V) as compared to the ARF + cimetidine group (group IV).

Study of SBP in the experimental groups

Table 2 shows that SBP value was significantly increased in ARF group (group II), as compared to the control group (group I). Administration of L-carnitine with hypertonic glycerol (group III) induced a significant decrease in SBP as compared to the ARF group (group II) and to the control group (group I).

SBP was significantly decreased in ARF + cimetidine group (group IV) as compared to the ARF group (group II), but remained significantly higher as compared to the control group (group I).

ARF + L-carnitine + cimetidine group (group V) showed a significant decrease in SBP as compared to the ARF group (group II) and as compared to the control group (group I).

Study of muscle injury in the experimental groups

Table 3 demonstrates that the level of serum creatine kinase was significantly increased in ARF group (group II) as compared to the control group (group I), and significantly decreased in ARF + L-carnitine group (group III) and in ARF + cimetidine group (group IV) as compared to the ARF group (group II). On administration of both agents together in group V, the level of serum creatine kinase was significantly decreased as compared to the ARF group (group II) and

Table 2
Comparison between mean ± SD of systolic blood pressure (mmHg) in all studied groups.

Parameter	Group (I)	Group (II)	Group (III)	Group (IV)	Group (V)
Systolic blood pressure (mmHg)	91.71 ± 3.94	170.68 ± 3.64*	83.42 ± 4.22*#$	99.23 ± 3.06*#@	86.1 ± 2.31*#$

Values are represented as mean ± SD.
* Statistically significant compared to corresponding value in group (I) ($P < 0.05$).
# Statistically significant compared to corresponding value in group (II) ($P < 0.05$).
@ Statistically significant compared to corresponding value in group (III) ($P < 0.05$).
$ Statistically significant compared to corresponding value in group (IV) ($P < 0.05$).

Table 3
Comparison between mean ± SD of serum creatine kinase (μ/l) in all studied groups.

Parameter	Group (I)	Group (II)	Group (III)	Group (IV)	Group (V)
creatine kinase (μ/l)	127.81 ± 3.42	801.15 ± 180.86*	502.99 ± 68.39*#	606.3 ± 124.24*#	404.89 ± 104.66*#$

Values are represented as mean ± SD.
* Statistically significant compared to corresponding value in group (I) ($P < 0.05$).
# Statistically significant compared to corresponding value in group (II) ($P < 0.05$).
@ Statistically significant compared to corresponding value in group (III) ($P < 0.05$).
$ Statistically significant compared to corresponding value in group (IV) ($P < 0.05$).

Table 4
Comparison between mean ± SD of serum NO (μmol/ml), glutathione level (mmol/ml) and cytochrome P450 content in the kidney tissue (nmol/mgptn) in all studied groups.

Parameter	Group (I)	Group (II)	Group (III)	Group (IV)	Group (V)
serum NO (μmol/ml)	0.18 ± 0.03	1.01 ± 0.21*	0.64 ± 0.1*#	0.63 ± 0.13*#	0.38 ± 0.09*#@$
glutathione level (mmol/ml)	53.29 ± 2.51	22.74 ± 5.09*	34.27 ± 3.46*#	35.82 ± 4.82*#	37.46 ± 5.88*#
cytochrome P450 content in the kidney tissue (nmol/mgptn)	1.35 ± 0.27	0.24 ± 0.06*	0.47 ± 0.05*#	0.58 ± 0.06*#	0.74 ± 0.14*#@

Values are represented as mean ± SD.
* Statistically significant compared to corresponding value in group (I) ($P < 0.05$).
# Statistically significant compared to corresponding value in group (II) ($P < 0.05$).
@ Statistically significant compared to corresponding value in group (III) ($P < 0.05$).
$ Statistically significant compared to corresponding value in group (IV) ($P < 0.05$).

as compared to the ARF + cimetidine group (group IV), but was still significantly higher as compared to the control group (group I).

Study of oxidative stress in the experimental groups

Table 4 presents that the value of serum NO level showed a significant increase while glutathione level and cytochrome p450 content in the kidney tissue significantly decreased in ARF group (group II) as compared to the control group (group I). The value of serum NO level showed a significant decrease, while glutathione level and cytochrome p450 content in the kidney tissue were significantly increased in the three treated groups as compared to the ARF group (group II), while the values of these parameters remained deviated significantly as compared to the control group (group I).

Also Table 4 displays that the values of serum NO level, glutathione level and cytochrome p450 content in the kidney tissue showed no significant difference in ARF + cimetidine group (group IV) as compared to the ARF + L-carnitine group (group III).

The value of serum NO level showed a significant decrease and cytochrome p450 content in the kidney tissue showed a significant increase, while glutathione level showed no significant difference in ARF + L-carnitine + cimetidine group (group V) as compared to the ARF + L-carnitine group (group III).

The value of serum NO level showed a significant decrease, while glutathione level and cytochrome p450 content in the kidney tissue showed no significant difference in ARF + L-carnitine + cimetidine group (group V) as compared to the ARF + cimetidine group (group IV).

Histological study

Table 5 clarifies that ARF group showed a significant increase in the percentages of glomerular and tubular affection as compared to the control group. All treated groups (ARF + L-carnitine group, ARF + cimetidine group and ARF + L-carnitine + cimetidine group) demonstrated a significant decrease in the percentages of glomerular and tubular affection as compared to the ARF group. The percentages of glomerular and tubular affection were significantly lower in ARF + L-carnitine + cimetidine group as compared to the ARF + L-carnitine group and as compared to the ARF + cimetidine group. The percentage of tubular affection showed no significant change in ARF + cimetidine group as compared to the ARF + L-carnitine group, while the percentage of glomerular affection was significantly lower in ARF + cimetidine group as compared to the ARF + L-carnitine group.

Sections of renal cortex illustrate the normal pattern of glomerular capillary tuft and Bowman's space in control group (Fig. 1a). Sections in renal cortex of mARF group show partial destruction of the glomeruli with obliterated space, complete lysis of others and tubular casts (Fig. 1b). ARF + L-carnitine group shows partial preserved glomeruli with congestion, and patchy affection of tubules (Fig. 1c). ARF + cimetidine group shows better preservation of architecture, with congestion (Fig. 1 d). ARF + L-carnitine + cimetidine shows near normal appearance with normal glomeruli, Bowman's space and surrounding tubules (Fig. 1e).

Sections of renal medulla of control group illustrates normal tubular staining, diameter, & uniform epithelium (Fig. 2a). Sections in medulla of mARF group shows disruption of lumen and of lining epithelium of many tubules (Fig. 2b). ARF + L-carnitine group shows uniform diameter and staining with localised exudates (Fig. 2c). ARF + cimetidine group also shows preserved tubules but with marked congestion (Fig. 2d). ARF + L-carnitine + cimetidine group shows normal tubules with healthy lining epithelium. (Fig. 2e)

Discussion

Rhabdomyolysis is a well-known clinical syndrome of muscle injury associated with myoglobinuria, electrolyte abnormalities, and often acute kidney injury (AKI). Myoglobin has been identified as the primary muscle constituent contributing to renal damage in rhabdomyolysis. The diagnosis is confirmed by elevated creatine kinase levels, but additional testing is needed to evaluate the potential causes, electrolyte abnormalities, and AKI [22].

Renal functions and SBP

Induction of mARF in the rats received i.m. glycerol was demonstrated by the severe deterioration of the renal functions as compared to the control group. A significant increase in the serum creatinine, blood urea nitrogen, sodium and potassium levels and a significant reduction in urine volume and GFR were noticed as compared to the control group. Histological findings confirmed the renal injury. Also, a significant increase in the systolic blood pressure was recorded.

Table 5
Comparison between mean ± SD of percentage of glomerular affection (%) and percentage of tubular affection (%) in all studied groups.

Parameter	Group (I)	Group (II)	Group (III)	Group (IV)	Group (V)
Glomerular affection (%)	13.17 ± 1.94	63 ± 1.79*	56 ± 2.37*#	46.33 ± 4.03*#@	37.5 ± 2.26*#@$
Tubular affection (%)	7.9 ± 2.02	46.3 ± 4.19*	27.1 ± 6.62*#	22.4 ± 4.2*#	13.6 ± 2.12*#@$

* Statistically significant compared to corresponding value in group (I) ($P < 0.05$).
# Statistically significant compared to corresponding value in group (II) ($P < 0.05$).
@ Statistically significant compared to corresponding value in group (III) ($P < 0.05$).
$ Statistically significant compared to corresponding value in group (IV) ($P < 0.05$).

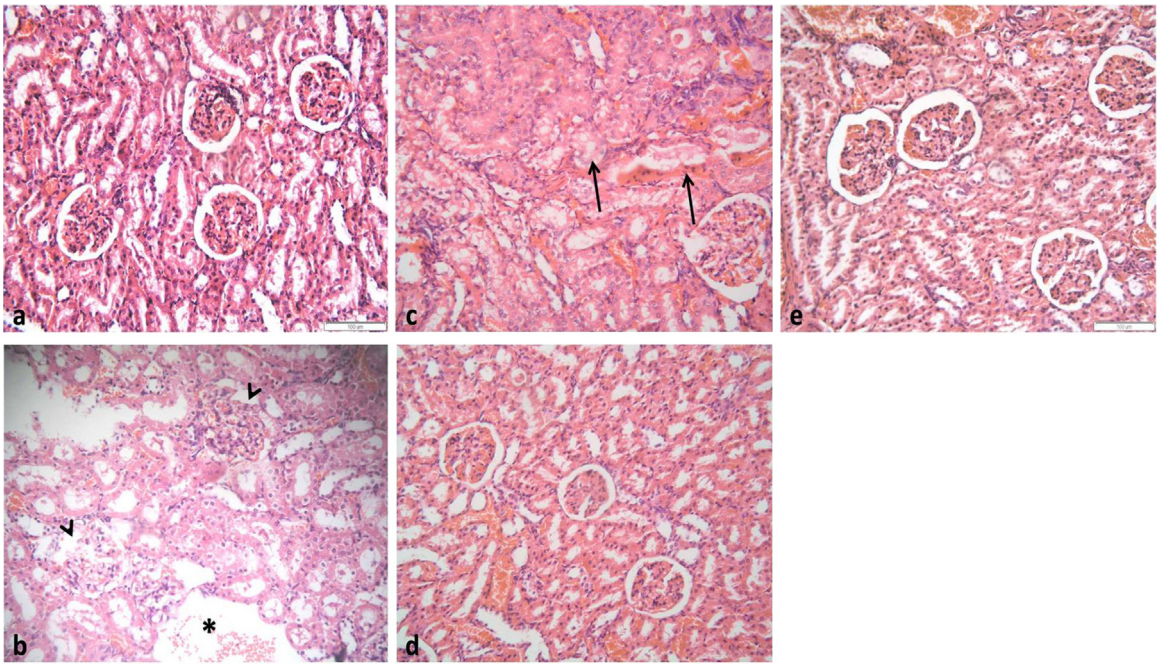

Fig. 1. *Sections in renal cortex show*: (a): Control group shows normal pattern of glomerular capillary tuft and Bowman's space. (b) mARF group shows partial destruction (^) of glomeruli with obliterated space, and complete lysis of others (*), while tubules show casts. (c): ARF + L-carnitine group shows partial preserved glomerulus with congestion, and patchy affection of tubules (arrows). (d) ARF + Cimetidine group shows better preservation of architecture, with congestion. (e) ARF + L-carnitine + Cimetidine group shows near normal appearance (Normal glomeruli, Bowman's space and surrounding tubules) (H&E ×200).

In accordance with our results, Singh et al. and Kaya et al., reported an increase in blood urea and creatinine levels in ARF [23,24]. Also the GFR was found to be decreased [25,26]. Kaya et al. reported a significant increase in serum K level [24]. The increase in blood pressure was also demonstrated by studying the same animal model [27].

The noticed hypernatremia can be explained by low sodium excretion occurring in some types of acute tubular necrosis (ATN), including mARF [28]. Also rhabdomyolysis may aggravate hypernatremia because of the intracellular breakdown of macromolecules into smaller molecules which promote the shift of water from extracellular fluid (ECF) into the muscle cell [29]. On the other hand, elevated K+ is known to be a result of decreased urine output in ARF and severe muscle damage [30].

However, contrary to our study, many studies on glycerol induced myoglobinuric ARF showed a decrease in serum Na levels [7,24,31].

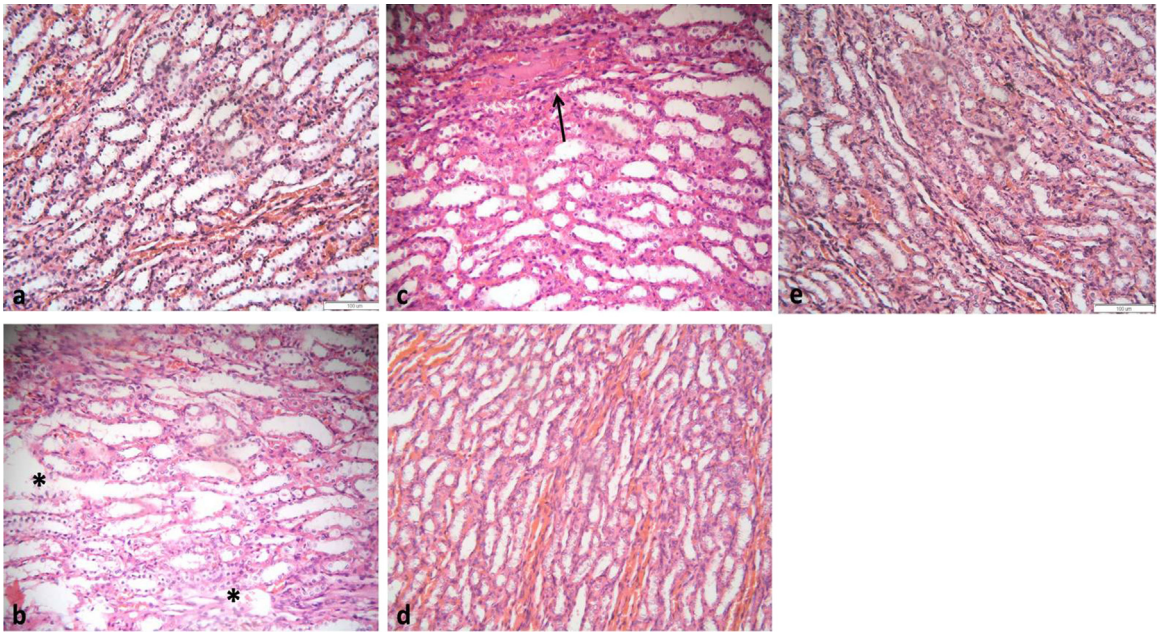

Fig. 2. Sections in medulla show: (a) Control group shows normal tubular staining, diameter, & uniform epithelium. (b) mARF group shows disruption of lumen and of lining epithelium of many tubules (*); (c): ARF + L-carnitine group shows uniform diameter and staining with localised exudates (arrow).; (d) ARF + Cimetidine group also shows preserved tubules but with marked congestion; (e) ARF + L-carnitine + Cimetidine group shows normal tubules with healthy lining epithelium (H&E ×200).

Glycerol-induced ARF and the associated increase in the systolic blood pressure may be attributed to increased angiotensin II (Ang II) or thomboxane A2 (TXA2), or may be due to vasoconstriction induced by down-regulation of peroxisome proliferator-activated receptor gamma (PPAR-γ) expression/activity, probably via an increased free radical generation [32].

On studying the effect of L-carnitine on renal functions, we found that ARF + L-carnitine group (group III) showed a partial improvement in BUN, serum creatinine and Na with a significant increase in urine volume but no significant change in the level of serum K and GFR as compared to ARF group. These results were confirmed by the histological examination which showed a significant decrease in the percentage of affected glomeruli and tubules in ARF + L-carnitine group (group III) as compared to ARF group, while it remained significantly higher as compared to control group (group I).

In addition, rat tail SBP measurements were significantly decreased in ARF + L-carnitine group (group III) as compared to ARF group (group II).

Almost equally to the L-carnitine treatment, the cimetidine treatment in ARF + cimetidine group (group IV) resulted in an improvement of all measured renal function parameters as compared to ARF group (group II). Additionally, the histological examination showed a significant decrease in the percentage of affected glomeruli and tubules in ARF + cimetidine group (group IV) as compared to ARF group, while the percentage remained significantly higher as compared to the control group (group I).

Similarly, a study by Najafzadeh et al. showed a protective effect of cimetidine on mARF and the improvement of renal functions in the same rat model [7].

Muscle injury

The untreated mARF group (group II) showed a significant increase in the level of creatine kinase as compared to the control group (group I). Creatine phosphokinase (CK) is an enzyme, released into the blood stream on muscle cell disintegration. The development of renal failure in the presence of rhabdomyolysis with significantly elevated CK is well described [33–35].

One mechanism by which L-carnitine and cimetidine induced their protective effect is through decreasing muscle injury. In this study, a significant decrease in the level of creatine kinase was observed in the ARF rats received L-carnitine or cimetidine. Similar results regarding L-carnitine were reported by Aydogdu et al. [6], therefore, L-carnitine and cimetidine presumably have a protective role against muscle damage and hence their role in protecting the kidney tissue.

Oxidative stress

Additionally our study demonstrated that cytochrome p450 in kidney tissue was significantly decreased in mARF group as compared to the control group. Baliga et al. added a support by demonstrating a marked decrease in the cytochrome p450 content in a similar animal model [4]. Rhabdomyolysis resulted in an acute tubular necrosis and increased labile iron levels in the kidney of rats [4]. A concomitant reduction in cytochrome p450 enzymes in the kidneys led the investigators to conclude that, degradation of these heme-containing mono-oxygenases might have contributed to increased labile iron levels in the kidney [36].

Also, rats of the myoglobinuric acute renal failure group showed a significant decrease in serum GSH. This was in accordance with studies conducted by Chander et al. [37] and Aydogdu et al. [6]. However, no significant decrease in GSH level was noted by Kaya et al. studying on the same animal model [24].

GSH acts as a radical scavenger by itself, and as a detoxicant by eliminating different electrophilic toxic compounds [38]. GSH concentration closely correlated with the degree of renal failure [39]. Depletion in GSH could contribute to the progression of uremia and leads to an acute renal failure [40].

In addition, our study demonstrated that there was a significant increase in the serum NO level as compared to the control group. Other studies showed no significant difference in NO levels [24]. Gök found a decrease in renal NO levels studying the same animal model [41].

Various animal studies have demonstrated that induction of inducible nitric oxide synthase (iNOS) leads to excessive production of nitric oxide (NO) and subsequent formation of peroxynitrite that causes renal injury [42–44].

Our study demonstrated that administration of L-carnitine in group III, or cimetidine in group IV induced a significant decrease in NO, and a significant increase in serum GSH and renal cytochrome p450 as compared to untreated mARF group (group II).

It is well accepted that oxidative stress is one of the mechanisms involved in the pathogenesis of acute renal injury and arterial hypertension [45]. L-Carnitine has been shown to have anti-oxidant and protective effects against oxidative damage in different organs or tissues, including the kidney [46–48], and to modulate PPAR-γ expression [49].

Cimetidine has imidazole and cyano groups that inhibit cytochrome p450 by interacting with the heme moiety [50], which represents an important source for iron during cell and tissue injury [4]. The effect of cimetidine is specific for cytochrome P-450 as it does not interact with other heme enzymes [51].

In previous studies, it was observed that cimetidine, but not ranitidine, significantly prevented the increase of bleomycin-detectable iron in the kidneys of glycerol-treated rats [4], and that cytochrome p450 was inhibited by both low (100 mg/kg) and high dose (150 mg/kg) of cimetidine [7].

Furthermore, H2 blockers have antioxidant effects that have been reported in the blood of patients with peptic ulcers and in gastric mucosa after ischemia–reperfusion [52]. Ching et al. showed that H2 blockers were also very powerful hydroxyl radical scavengers [53]. The methylated imidazole ring of cimetidine, with a side chain containing sulfur and an amino group, is thought to be a powerful hydroxyl radical scavenger [53]. Another study showed that H2 blockers had powerful scavenging effects on hypochlorous acid and monochloramine, which arise from inflammatory cells such as neutrophils [54]. According to these studies, H2 blockers could potentially be used for the treatment of diseases that are characterised by free radical-mediated oxidative stress in vivo [55].

Accordingly, up to our knowledge, this study was the first to report an increase in the serum level of the antioxidative enzyme GSH in mARF rats treated with cimetidine; however, this also may be due to the inhibition of cytochrome p450 and the subsequent decrease of the labile iron protecting the antioxidant enzyme through restriction of the oxidative injury.

Moreover, our study showed a better renal protection on treating rats with L-carnitine and cimetidine simultaneously. Although each of them has nearly similar protective effect, simultaneous use of both drugs induced a better renal protection. It appears that their action is through different mechanisms that may be synergistic to the action of the other drug.

Conclusion

Cimetidine and L-carnitine have protective effects – almost equally – against myoglobinuric renal failure. Using both agents together made the renal injury minimal.

The simultaneous use of cimetidine and L-carnitine in the treatment of rhabdomyolysis could be a good protective treatment against the development of acute kidney injury which is considered as the most important complication and the most common cause of death in rhabdomyolysis patients.

Future studies are recommended to compare the effect of the simultaneous use of cimetidine and L-carnitine against the effect of the nowadays used guidelines and to explore if inducing these drugs to the guidelines would add benefit. Also, transitional studies on human are recommended.

Conflict of interest

All authors of this paper certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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