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Evaluation the Effect of Cimetidine, Estradiol and Vitamin E on Myoglobinuric Renal Toxicity in Rats

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Abstract: Iron from injured muscles can produce acute renal failure. Cytochrome P 450 is one enzyme that involved in iron-related acute renal failure. Inhibition this enzyme may decrease rhabdomyolysis- induced myoglobinuric nephrotoxicity. The aim of this study was comparison effect of cimetidine, estradiol and vitamin E on myoglobinuric renal toxicity in rats. This study was conducted in 6 groups. Normal saline was administrated in group 1(as control). Glycerol 50% was injected in groups 2 -6 and then the vitamin E, estradiol and cimetidine were administrated in these groups. Twenty-four hours after glycerol injection, serum of rats was isolated. Serum concentrations of BUN, creatinine, uric acid, sodium, phosphor and potassium were determined. Kidneys were removed. Renal tissue changes were examined by Hematoxiline and Eosine coloring and with light microscope. Injection of glycerol caused muscular injury and rabdomyolysis and acute renal failure. Because, serum concentration of BUN, cereatinine, sodium, phosphor and uric acid was significantly changed in group 2 compared with group 1. Serum concentration of BUN and cereatinine was significantly decreased in group 3, 5 and 6. Serum concentration of sodium was decreased in group 2 and 4, but increased in group 5. Serum concentration of potassium was significantly increased in group 5 compared to group 2 and 4. The serum level of phosphor and uric acid was significantly decreased in group 3, 5 and 6. The histological changes confirmed biochemical findings. This study shows cimetidine can have protective effect against myoglobinuric renal failure, while estradiol aggravates it.

Key words: Cimetidine · Estradiol · Vitamin E · Myoglobinuric renal failure · Rats

INTRODUCTION

Acute renal failure (ARF) is a syndrome characterized by an acute loss of renal function. Despite the reversibility of the loss of renal function in most patients who survive, the mortality of ARF remains high (over 50%) [1]. Acute tubular necrosis is the most common form of ARF of renal origin. Rhabdomyolysis-induced myoglobinuric acute renal failure accounts for about 10-40% of all cases of acute renal failure [2].

Iron has been implicated to play an important role in several models of tissue injury, including myoglobinuric acute renal failure [3]. In this model, myoglobin released from the injured muscle is generally accepted as a source of iron [4, 5]. Recent *in vivo* studies suggest that heme Fe causes proximal tubular lipid peroxidation and cytotoxicity, thereby contributing to the pathogenesis of myoglobinuric acute renal failure [6]. The glutathione has important role in glycerol-induced acute renal failure [7, 8]. Molsidomine, a nitric oxide donor and l-arginine protects against rhabdomyolysis-induced myoglobinuric acute renal failure [2].

Cytochrome P 450 is one enzyme that involved in iron-related acute renal failure. Inhibition of this enzyme may decrease rhabdomyolysis- induced myoglobinuric nephrotoxicity [9, 10]. It seems that oxidative stress has

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key role at this pathogenesis [11-16]. In present study, effect of cimetidine (as an effective inhibitor of Cytochrome P 450) and vitamin E (as natural antioxidant) was compared with estradiol (had protective effect on renal failure in some studies) on myoglobinuric renal toxicity in rats.

MATERIALS AND METHODS

Adult male Wistar rats, 10 to 11 weeks old and weighing 225 to 300 g, were obtained from Jundishapour Laboratory Animal Center (Ahvaz-Iran). Animals were allowed to acclimatize in our facility for at least 7 days before treatment. The animal room was maintained at 22°C and fluorescent lighting was controlled with an automatic timer (8:00 a.m. on/10:00 p.m. off). The animals were housed in polycarbonate cages contain additive-free corncob bedding and were allowed free access to Laboratory Rodent Diet Shooshtar Co.

This study was done in 6 groups (6 rats in each group): Normal saline was administrated in group 1(as control). Rats received an intramuscular injection of 50% glycerol into a hind limb at 8ml/kg body weight in groups 2-6. Vitamin E (Osveh Co. Iran), estradiol (Aboureyhan Co. Iran) and cimetidine (Kimiadaru Co. Iran) by two dosages (100 and 150 mg/kg) were administrated [5] in groups 3, 4, 5 and 6, respectively. Rats were injected with glycerol after overnight water deprivation and sacrificed 24 hours later.

The rats were anesthetized by ketamine at dose 100mg/kg b. wt. to facilitate collection of blood samples. The serum of rats was isolated. Urea nitrogen and creatinine were determined using commercial reagents (obtained from Parsazmoon Co. Iran). Phosphorus was measured by commercial kit (Zistshimi Co. Iran). Sodium and potassium were measured with flame photometry apparatus (Cornig 410c, England). The serum uric acid was measured by Spectrophotometer (Unicam-919, England).

Sections from the kidney of each animal were fixed in phosphate-buffered formaldehyde, embedded in paraffin and 5-ìm thick sections were prepared. Sections were stained with Hematoxylin and Eosin for evaluation of kidney tissue. The changes in tissue were examined by light microscope and numbers of necrotic cells were counted in proximal tubules by scaled lens with light microscope.

Statistical Analysis: Data were expressed as mean ± SE. Group variance was analyzed by one-way analysis of variation (ANOVA) and Fisher least significant difference test (LSD) was tested for significant differences between groups. $P = 0.05$ was considered statistically significant.

RESULTS

The injection of glycerol (50%) caused muscular injury and rabdomyolysis and acute renal failure (ARF) in group 2. The rats with glycerol-induced ARF had much higher urea nitrogen levels than normal rats (Fig. 1). The elevation of serum concentration of BUN was significantly decreased in groups 3, 5 and 6 compared with group 2, but not in group 4 (Fig. 1). The serum creatinine was increased in group 2 by glycerol injection with comparison to group 1. Also, this elevation was ameliorated in groups 3, 5 and 6 (Fig. 2). In other hands, vitamin E and cimetidine protected renal function but estradiol did not.

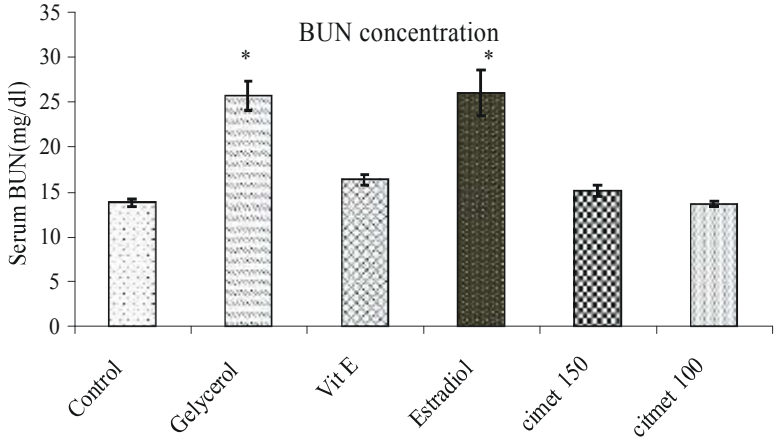

Fig. 1: The mean ± SE of serum BUN concentration of rats: * shows significant difference with control group.

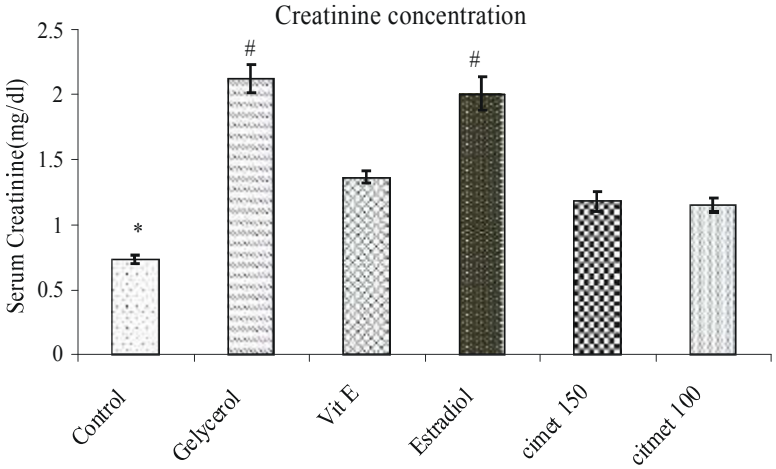

Fig. 2: The mean ± SE of serum creatinine concentration of rats: * shows difference between control group and other groups; and # shows significant difference with other groups.

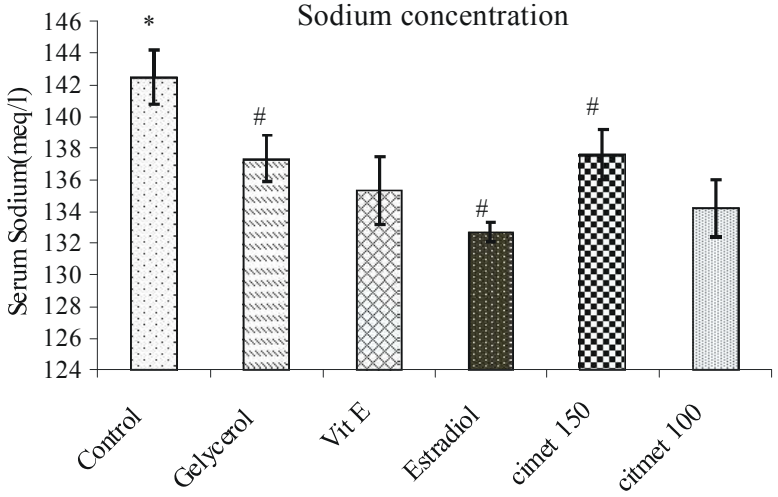

Fig. 3: The mean ± SE of serum sodium concentration of rats: * shows difference between control group and other groups; and # show significant difference with other groups.

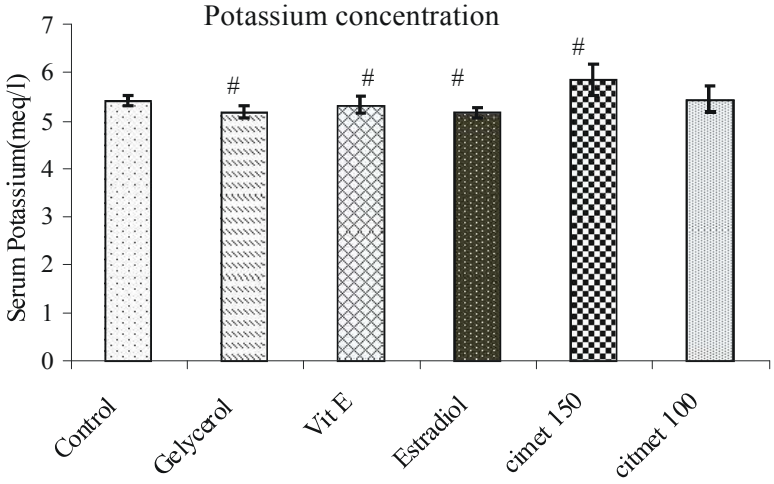

Fig. 4: The mean ± SE of serum potassium concentration of rats: # shows significant difference with other groups.

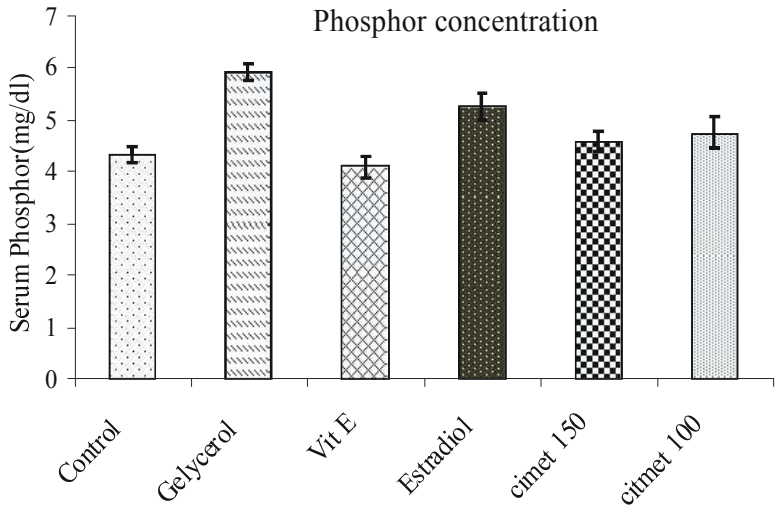

Fig. 5: The mean ± SE of serum phosphorus concentration of rats.

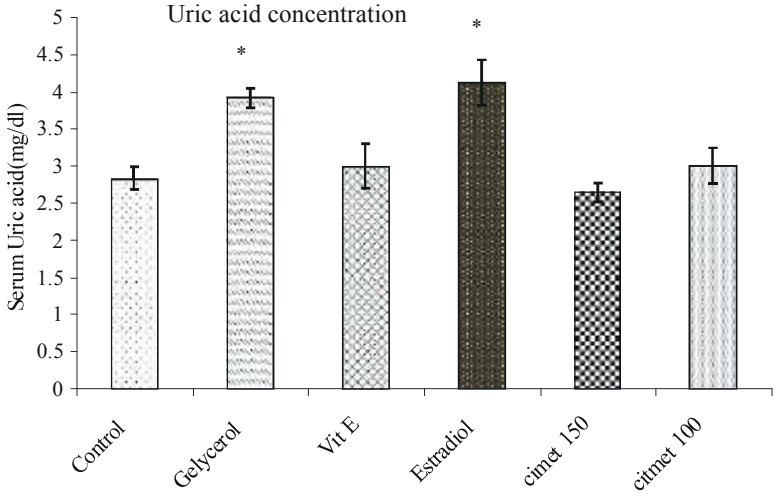

Fig. 6: The mean ± SE of serum uric acid concentration of rats: * shows significant difference with control group.

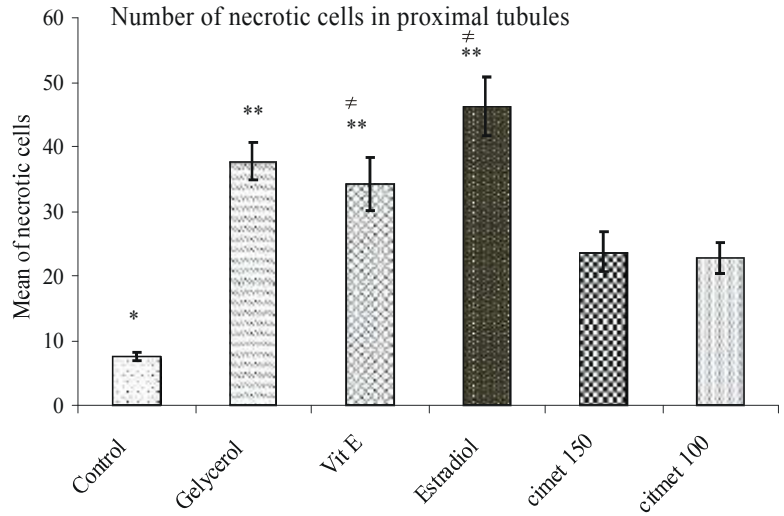

Fig. 7: The mean ± SE of necrotic cells in proximal tubules of kidney of rats: * shows difference between control group and other groups; and # shows significant difference with other groups.

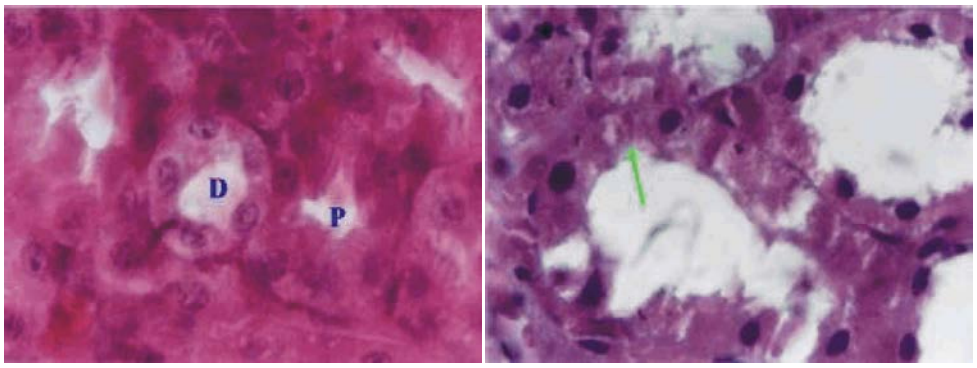

Fig. 8: Histological illustration of kidney (HandE × 1200). The right figure presents normal view (D: distal tubule and P: proximal tubule of nephron section. The left figure shows changes in proximal tubule after glycerol administration (arrow shows cell injury and necrosis)

The serum concentration of sodium was decreased in group 2 with comparison to group1 (Fig. 3). This decrease was lesser in group 5 significantly; thus cimetidine had more protective at dose level of 150mg/kg b. wt. The estradiol potentiated sodium lowering. The serum concentration of potassium was significantly increased in group 5 with comparison to groups 2, 3 and 4 (Fig. 4). The serum concentration of phosphorus was increased by glycerol injection. This elevation was prevented by vitamin E, but not estradiol and cimetidine (Fig. 5). The serum concentration of uric acid was increased in groups 2 and 4 with comparison to group1 (Fig. 6). This elevation was prevented by vitamin E and cimetidine.

The mean number of renal necrotic cells at proximal tubule was significantly increased in group 2 with comparison to group1 (Fig. 7). These histological changes were significantly reduced by vitamin E and cimetidine, but not estradiol. The cimetidine had better protective effect than vitamin E, but estradiol potentiated glycerol-induced lesions (Fig. 7). The histological examination by light microscope shows the proximal tubules were destroyed by glycerol injection (Fig. 8).

DISCUSSION

In the rat model of glycerol-induced ARF, it is generally considered that the degree of renal failure [17, 18]. For more direct evaluation of the effect of cimetidine, vitamin E and estradiol on renal function in ARF, we also measured blood urea nitrogen and Cr levels, two important pathological parameters of renal failure. In the rats with ARF, both parameters were considerably increased, indicating pathological impairment of renal function by glycerol-induced ARF [19, 20]. However, this situation was notably ameliorated in the groups treated with cimetidine and vitamin E. ARF is characterized by rapid decline of the glomerular filtration rate and retention of nitrogenous waste products [21, 22]. Serum urea nitrogen and creatinine increased rapidly, associated with a marked reduction in glomerular filtration rate after glycerol administration [23-26].

At one study was done by Baliga *et al.*[4], there was a marked and a specific increase in the iron content accompanied by a marked decrease in the cytochrome P-450 content in the kidneys of glycerol treated rats. They observed that cimetidine, but not ranitidine, significantly prevented the increase of bleomycin-detectable iron in the kidneys of glycerol-treated rats. The loss of cytochrome P-450 content was substantially blocked by cimetidine, but not by ranitidine. Both the inhibitors of cytochrome P-450 provided functional (as measured by BUN and creatinine) and histological protection against glycerol-induced acute renal failure [4]. The result of our study is similar to this research; we observed protective effect of cimetidine. The cytochrome P-450 was inhibited by both low (100 mg/kg) and high dose (150mg/kg).

The protective effect of vitamin E is related to its antioxidative function. Because the oxidative stress is important factor for myoglobinuric ARF [27, 28] and renal malondialdehyde (MDA) levels, reduced glutathione levels and by enzymatic activity of catalase, glutathione reductase and superoxide dismutase was determined by Chander *et al.* [9]. The other antioxidant such as sodium benzoate (a second hydroxyl radical scavenger), Melatonin, n-aringin (a bioflavonoid with antioxidant potential), quercetin (a bioflavonoid), carvedilol (an antihypertensive drug) and deferoxamine (an iron chelator) had protective effect [29-32].

We observed that estradiol did`nt only had protective property but also oxidant and increased renal toxicity. This effect of estradiol was reported in other studies. It was observed the estradiol injection caused significant increases in both malondialdehyde levels and glutathione peroxidase activity in liver [32-35].

CONCLUSION

Results of this study show vitamin E and cimetidine can have protective effect against myoglobinuric renal failure, while estradiol aggravates it.

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