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Acute renal failure due to Rhabdomyolysis

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Abstract : Acute renal failure (ARF) is a common condition with a high risk of death. Rhabdomyolysis is one of the causes of acute renal failure. By knowing the pathological condition in an animal model of ARF can provide an overview on other researchers to manipulate treatment for a particular purpose. This study aims to know the pathological conditions of animal models of ARF due to rhabdomyolysis by using glycerol inject.
28 rats (Rattus norvegicus) were divided into two treatment groups, Group I as the control group, Group II-induced with glycerol is divided into five small groups, Group II -1 hours, Group II-3 hours, Group II-6 hours, Group II-12 hours, and Group II- 24 hours. Urea and creatinine serum were analyzed by spectrophotometer methods and histopathology of the kidney was analyzed by microscope descriptively.
Results of repeated ANOVA analysis showed significant differences between each group ($P < 0.05$). The significance occurred in the urea levels in group 12 and 24 hours. Whereas creatinine levels were known that the treatment group 12 hours and 24 hours showed a significant increase when compared with the control group 0 hours. Based on the research that induction of acute renal failure with 50% glycerol in rats can cause the renal damage seen in renal histopathology images starting at 3 hours but the increasing of urea and creatinine levels above normal were seen at 12 hours after induction.
Keywords: Acute renal failure, Rhabdomyolysis.

Introduction

Rhabdomyolysis is a syndrome that involves damage and breakdown of skeletal muscle, causing myoglobin and other intracellular proteins and electrolytes to leak into the circulation. The development of rhabdomyolysis is associated with a wide variety of diseases, injuries, medications, and toxins Myoglobin is filtered at the level of the glomerulus, and, as a result, when present in excess can lead to acute renal failure[1]. Patients with rhabdomyolysis that is associated with acute kidney injury usually present with a clinical picture of volume depletion that is due to the sequestration of water in injured muscles. Acute renal failure is defined as abnormalities in the function or structure of the kidney causing kidney damage including abnormalities in blood tests, urine or tissue[2]. This condition is associated with retention of creatinine, urea, and products that are normally excreted by kidneys[3]. Rhabdomyolysis-ARF Acute renal failure is a condition with a high risk of death in humans and animals. In humans, between 20-30% of patients in critical condition, with a range of 6% ultimately require a kidney transplant therapy[4,5].

Irrespective of the cause of rhabdomyolysis the mortality rate may still be as high as 8%. According to Curry[6], The pathogenic mechanisms Involved in glycerol-induced renal failure include ischemic injury, tubular

nephrotoxicity the caused by myoglobin, and the renal actions of cytokines released after rhabdomyolysis. Rhabdomyolysis Refers to the breakdown of skeletal muscle that leads to leakage of muscle contents, such as myoglobin and creatinine kinase (CK), into the extracellular fluid. Serum myoglobin is filtered by the glomerulus, leading to myoglobinuria. Which may lead to direct renal tubular injury and a clinical presentation ranging from mild to life-threatening acute renal failure (ARF)[7].

Animal models are pivotal for understanding the characteristics of acute renal failure (ARF) and the development of effective therapy for its optimal management. That is the way this research was conducted to know the pathological conditions of animal models of ARF that can be used as a reference in research on animals models of ARF by using glycerol injection. So that the utilization of animal models of ARF can be used appropriately to support a wide range of other studies.

Experimental

Induction of experimental animals performed in rats (Rattus norvegicus) Wistar strain were divided into two treatment groups, namely Group I as a control group consisting of 4 rats and Group II as a group induced acute renal failure with glycerol consisted of 24 rats. Group II is further divided into five small groups, each consisting of 4 rats which are group II 1 hour-1st, group II hour3rd Group II hour 6th, the group II-hour 12th and group II hour 24. Group II induction of acute renal failure in rats using 50% glycerol single dose of 10 mL / kg was injected intramuscular[8]. Whereas in the group I performed the injection using sterile distilled water with the same dose. Induction treatment of experimental animals is done after 12 hours of fasting.

Blood sampling in group I and group II performed injection of anesthetic ketamine (60 mg / kg) in rats. Blood collected for further examination for renal function tests. Renal function was evaluated using the colorimetric Jaffe method (Creatinine and Urea kits DiaSys diagnostic systems GmbH Germany) to calculate the serum levels of urea and creatinine. The Data were analyzed by SPSS 17 statistical program by using repeated ANOVA with 95% confidence level.

Histopathology examination was performed on kidney tissue of rats after euthanizing. Kidneys are excised and examined with macroscopic quantities. The organ then inserted into 10% buffered formalin for making preparations for histopathology. The histopathology results were analyzed descriptively. The whole animal has been in compliance with the guidelines declared by institutional animal care and use committee with the registration number No. 289/EC/KEPK/04/2015.

Result and Discussion

Urinary Findings

The group of rats was injected glycerol showing discoloration of the urine becomes dark brown, it was different from the control group in which urine was yellow (Figure 1). Dirty-brownish discoloration of the urine as a result of myoglobinuria is typical[1]. The pathogenic mechanisms involved in glycerol-induced renal failure include ischemic injury, tubular nephrotoxicity caused by myoglobin, and the renal actions of cytokines released after rhabdomyolysis[6]. Induction of glycerol has caused rhabdomyolysis. Rhabdomyolysis is the disintegration of striated muscles, which results in the release of myoglobin and other muscular cell contents into the extracellular fluid and the circulation. Myoglobin, a dark-red protein, which is filtered by the glomerulus, will not appear in the urine until the renal threshold is met at 0.5–1.5 mg/dl; injury to as little as 100 g of muscle can lead to serum myoglobin levels in excess of 1.5 mg/dl and subsequent myoglobinuria[9]. Myoglobinuria resolves pigment excretion is limited because of kidney dysfunction. Myoglobin, along with uric acid, can damage the kidney directly by forming casts within the renal tubules leading to their obstruction. This blockage of renal tubules by myoglobin and uric acid can be exacerbated by other physiologic changes commonly associated with the development of rhabdomyolysis (i.e., significant volume depletion or electrolyte abnormalities) by causing myoglobin to form into a gel-like substance leading to the eventual blockage of the distal nephron. Myoglobin and uric acid casts along with the sludging of tubular epithelial cells can cause acute tubular necrosis (ATN)[10]. Myoglobin can also lead to pigment-induced intrarenal vasoconstriction and decreased glomerular filtration rate (GFR) by scavenging the vasodilatation nitrous oxide from the renal microcirculation leading to decreased renal functionm[10].

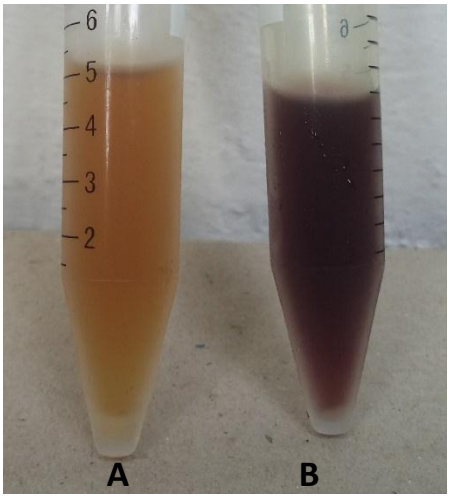

Figure 1. Urine of the rats; A. control group B. treatment group post induction of 50% glycerol

The large numbers of disorders known to cause rhabdomyolysis include intrinsic muscle dysfunction such as burns, intrinsic muscle disease, trauma and excessive physical exertion, metabolic disorders, hypoxia, drugs, toxins, infections, temperature extremes and idiopathic disorders[11]. Twenty to 50% of rhabdomyolysis cases are complicated by AKF[12]. On the other hand, rhabdomyolysis is responsible for 5–20% of all cases of AKF[13]. Myoglobinuria is most often detected by dipstick testing; a positive test can indicate hematuria, myoglobinuria, or hemoglobinuria, which is not helpful in leading to a final diagnosis.

Urea Levels

Urea is a common measurement parameter used to estimate kidney function. Results of the analysis of levels of blood urea rats can be seen in Table 1. Urea level in group I as the control group was showed 69.8 mg/dL, normal urea levels in rats which are (10-58 mg/dL)[14]. Group II (treated) showed significantly increased levels of urea at 12 and 24 hours after induction, compared with control rats ($p<0.05$).

Table 1. Serum urea (mg/dL) levels.

Calculation	**Time (hours)**					
	Control	**1**	**3**	**6**	**12**	**24**
Mean±SD urea (mg/dL)	69.8±24.92	142±15.65	172±11.51	228±66.20	318±38.98	519±36.64

Mean urea levels in Group II gradually increased and peaked at 24 hours with an average serum urea of 519 mg/dL (Figure 2), which is a significant increase when compared with the control ($P <0.05$). Glycerol can cause renal damage characterized by elevated urea levels[15]. Increased levels of urea in the treated animals at 6 hours is probably due to hypovolemia and dehydration as the early post-rhabdomyolysis response. Rhabdomyolysis can lead to a decrease in the body fluid volume[16]. The increase in urea levels can also be affected by animal dehydration status due prior to injection of by glycerol injection, the animals were fasted 12 hours. The increase in urea in the blood in addition to impaired renal function may be affected by hypovolemia, high protein diet, shock, dehydration, and bleeding in the gastrointestinal[17].

Creatinine Levels

Creatinine is the end product of creatinine metabolism. Creatinine is mainly synthesized in the liver, found almost all of skeletal muscle. In skeletal muscle creatinine reversibly bound to phosphate in the form of creatinine phosphate as an energy storage. In certain individuals, the daily amount of creatinine formation tends to remain the same, the amount of creatinine levels proportional to muscle mass[18]. The results of the analysis of blood creatinine levels of rats are showed in Table 2. The mean creatinine levels in the control group was 0.98 mg/dL. Creatinine levels in the control rats were within the normal range 0.20-0.80 mg/dL[14]. The creatinine

level in group II (Treated) gradually increased and peaked at 24 hours with an average serum creatinine of 5.3 mg/dL, which is a significant increase when compared with the control (P <0.05).

Table 2. Serum creatinine (mg/dL) levels.

Calculation	Time (hours)					
	Control	1	3	6	12	24
Mean±SD creatinine (mg/dL)	0.98±0.13	2±0.00	2±0.00	2.2±0.57	3.1±0.65	5.3±0.27

According to Ayvaz[19], creatinine levels in rats can increase up to 7.56 mg/dL 48 hours after injection with 50% glycerol. The increasing of creatinine levels in this study was likely due to rhabdomyolysis and a decreasing of renal function due to glycerol's toxic effects. Creatinine levels due to acute renal failure stands at >3 mg/dL so that the group II at 12 hour indicate the occurrence of the acute renal failure. Histopathology changes showed decreasing renal function evidenced by necrosis and dilatation of tubular renal epithelium at 3 hours. Current standard tests to determine the status of kidney function is by measuring serum creatinine and urine production. However, the initial measurement of serum creatinine may not reflect the extent of the injury as the accumulation of creatinine always lags behind injury events[20].

Kidney histopathology

Microscopically the kidneys from the control group did not exhibit pathological changes whilst the treated group showed histopathological changes in the kidney (Figure 3). In rats, injection of 50% glycerol can cause acute tubular necrosis (ATN) with morphological characteristics such as tubular cell necrosis, tubular lumen dilation, and swelling of the proximal tubular cells with brush border loss[21,22].

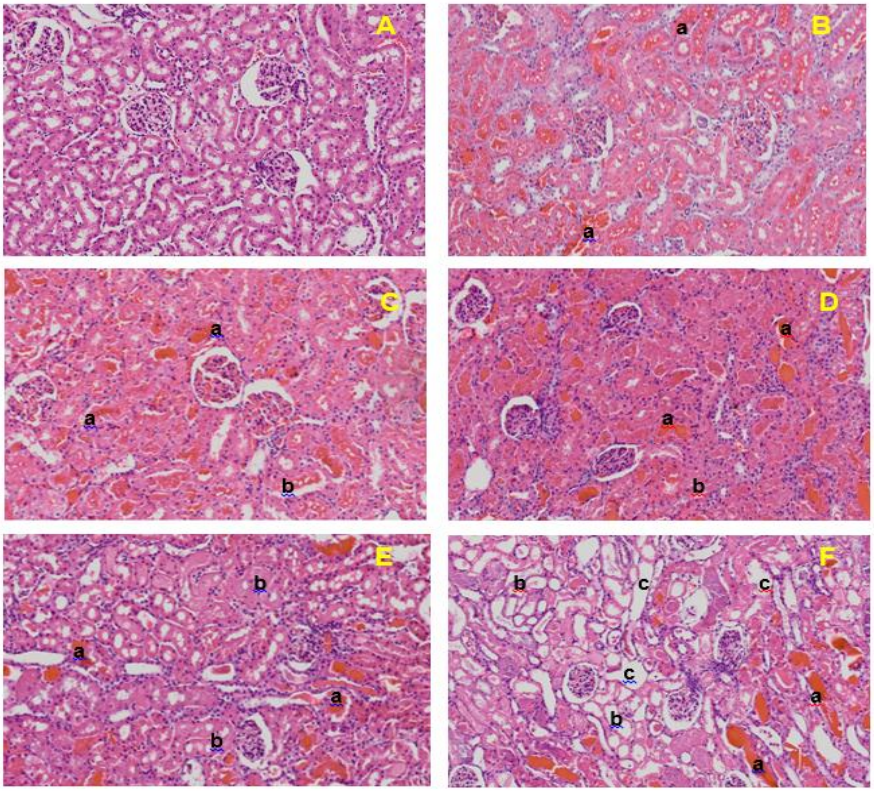

Figure 2. Photomicrograph kidney of rat (A) control group; (B) 1 hour post induced 50% glycerol; (C) 3 hour post induced 50% glycerol; (D) 6 hour post induced 50% glycerol; (E) 12 hour post induced 50% glycerol; (F) 24 hour post induced 50% glycerol. (a) tubular lumen-containing homogeneous eosinophilic mass, (b) tubular epithelial cell necrosis, (c) dilated tubular lumen (H&E staining, 400x).

Our research has shown that in 24 hours the most severe kidney damage with signs of acute tubular necrosis already visible. In this study, ATN was characterized mostly by necrosis of tubular epithelial lumen, some tubular lumen were dilatated and some contained homogeneous eosinophilic (or yellowish red) masses, and there was infiltration of lymphocytes into the interstitial tissue. The acute tubular necrosis can be caused by ischemia and/or toxic agents in the kidney. These agents include aminoglycoside antibiotics, cisplatin chemotherapy agents, heme pigment due to hemolysis and rhabdomyolysis[23].

Acute renal failure caused by glycerol injection can lead to rhabdomyolysis[1,15,24].The number of mechanisms have been proposed to explain the pathophysiology of rhabdomyolysis-induced renal failure, including decreased delivery of blood to the glomerulus, reduced glomerular filtration rate, leakage of filtrate across a damaged tubular epithelium, or tubular obstruction by myoglobin casts[16]. The current consensus is that renal failure is due to the combined effects of hypovolemia, aciduria, and direct cytotoxicity due to accumulation of renal tubular Myoglobin[25].

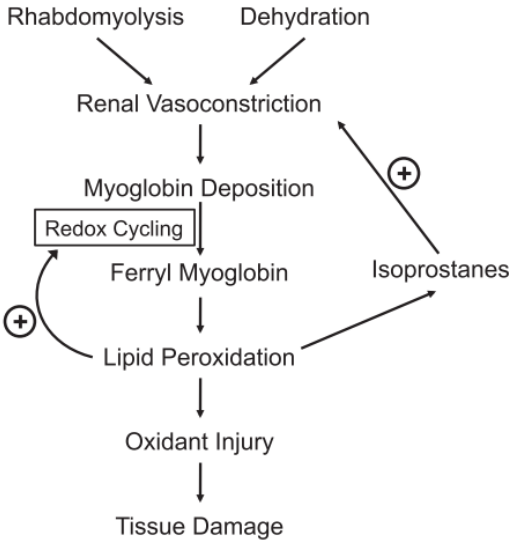

Figure 3. The pathophysiologic mechanism leading to kidney injury after rhabdomyolysis[26]

Rhabdomyolysis can induce renal failure as a result of reduced blood supply to glomeruli, decreased glomerular filtration, tubular epithelial damage, and tubular obstruction by residual myoglobin[26]. Myoglobinuria would look for in case of rhabdomyolysis. Myoglobin is a dark red 17.8-kDa protein that is freely filtered by the glomerulus, enters the tubule epithelial cell through endocytosis, and is metabolized[9]. Myoglobin becomes concentrated along the renal tubules, a process that is enhanced by volume depletion and renal vasoconstriction, and it precipitates when it interacts with the Tamm–Horsfall protein, a process favored by acidic urine[27]. Tubule obstruction occurs principally at the level of the distal tubules, and direct tubule cytotoxicity occurs mainly in the proximal tubules[16].

Myoglobin seems to have no marked nephrotoxic effect in the tubules unless the urine is acidic. Myoglobin is a heme protein; it contains iron, as ferrous oxide (Fe2+), which is necessary for the binding of molecular oxygen. However, molecular oxygen can promote the oxidation of Fe2+ to ferric oxide (Fe3+), thus generating a hydroxyl radical. This oxidative potential is counteracted by effective intracellular antioxidant molecules. However, cellular release of myoglobin leads to uncontrolled leakage of reactive oxygen species, and free radicals cause cellular injury[28]. Iron in myoglobin and hemoglobin released into the bloodstream as a result of myolysis and hemolysis induces the formation of free radicals and lipid peroxidation, therefore, plays a critical role in the pathogenesis of acute renal failure[11,29,30].

Conclusion

Based on the research that the induction of acute renal failure with 50% glycerol in rats caused renal injury seen in renal histopathology images starting at 3 hours but the increasing of urea and creatinine levels above normal were seen at 12 hours after induction.

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