# Different Expression of Caspase-3 in the Spleen and Liver of Rattus norvegicus Infected with Enterobacter cloacae and Proteus mirabilis as a Septic Model

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Keywords: Apoptosis, Caspase-3, Eanterobacter cloacae, Proteus mirabilis

Abstract: Enterobacter cloacae and Proteus mirabilis are opportunistic pathogenic bacteria that are the main causes of high morbidity and mortality in humans from nosocomial infections in hospitals. Caspase-3 is a death protease that acts as an apoptotic execution on an intrinsic pathway. Caspase-3 plays an important role in the process of cell death that can divide various dead substrates often causing morphological and biochemical changes in cells. The increase in caspase-3 expression indicates the severity of the disease, DNA fragmentation, and condensation of apoptotic chromatin in the examined organs. This research type is true experimental using a post-test in only the control group. The aims of this study are to determine the expression of caspase-3 in the spleen and liver organs of experimental animals Rattus norvegicus, infected with E. cloacae and Proteus mirabilis. E. cloacae and P. mirabilis bacteria were injected through the rats' peritoneum and then observed for 24 hours. The rats' spleens and livers were observed for caspase-3 expression with immunohistochemistry. Caspase-3 expression increases more in the liver than the spleen. The liver serves as a detoxification of infection by spending and inducing cleansing of bacterial infections. Excessive inflammatory responses to the liver can cause harmful effects on the host, with the cell cycle developing cellular stress, causing cell death through caspase.



## 1 INTRODUCTION

Nosocomial infection is an infection that occurs in patients who perform treatment in hospitals or other health facilities. The most common cause (90%) of nosocomial infection is from bacterial infection of Proteus mirabilis, Enterobacter cloacae, Escherichia coli, Staphylococcus aureus and other causes protozoa, fungi, and viruses account for only 10% (Khan et al., 2015). Nosocomial infections in developing countries are a major cause of high morbidity and mortality in hospitals (Mohammed et al., 2014). Data from the WHO indicates that the largest infection site is sepsis (WHO 2018). In the case of gram-negative bacterial infections, the incidence of high sepsis can trigger multiple organ failure and death.

E. cloacae and P. mirabilis are pathogenic gram-negative bacteria that tend to increase morbidity and increase mortality in treatment and hospitalization cases. Both these gram-negative bacteria infect various organs and may undergo tissue death (apoptosis). The second cell wall of the bacteria can produce endotoxins, which can lead to cellular response and impact on endothel damage that can trigger multiple organ damage. Sepsis may also induce apoptosis in innate and adaptive immune cells.

Several studies have shown that apoptosis occurs in many organs during sepsis. In animal models with sepsis, apoptosis is found in the thymus organ, patch peyer, liver, kidney, lung, intestine, and skeletal muscle. In patients with sepsis, apoptosis occurs in the spleen, colon and ileum (Aziz et al., 2014). The spleen plays an important role in the modulation of the immune system and in the maintenance of peripheral tolerance through the clearance of apoptotic cells, differentiation and activation of T and B cells (Vincenzo and Mikael, 2013).

Lipopolysaccharides (LPS) of intestinal gram-negative bacteria was taken periodically to the liver



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through the portal vein then stored by Kupffer cells (KCs) and macrophages that are stunned at the liver. The function of the liver is detoxification, the first barrier against pathogen infection. Kupffer cells and macrophages are the product of the liver, and can take bacterial endotoxin and phagocytosis brought through the portal vein; they play a major role in the clearance of systemic bacterial infections (Mencin et al., 2009).

Executor caspases are an important part of the cell death process during apoptosis. Executor caspases are necessary for apoptosis, and also in the absence of cell death (Khalil et al., 2012). Caspase-3 plays an important role in the process of cell death because of its function that can cleave dead substrates, ultimately leading to morphological and biochemical changes in apoptotic cells (Walsh et al., 2008). Apoptotic regulation is an important aspect in host cell response to stress and bacterial infections of E.cloacae and P.mirabilis. The study on the expression of caspase-3 in the spleen and liver organs that occur in sepsis due to bacterial infection of E. cloacae and P.mirabilis needs to be conducted to determine the cleavage of various dead substrates, which can cause changes to morphology and biochemistry seen in apoptotic cells and can be prevented before multiple organ dysfunction syndrome (MODS) occurs.

## 2 MATERIALS AND METHOD

### 2.1 Animal model

The experimental animals used in this study were Rattus novergicus, the wistar strain, with criteria of male, body weight 150–200 grama, healthy, and aged three months.

### 2.2 Bacteria

Enterobacter cloacae and Proteus mirabilis from the Installation of Clinical Microbiology RSUD Dr. Soetomo, Surabaya. The concentration wild type was105 CFU bacteria with Physiologic Zur (Nacl 0.9%).

### 2.3 Method

The healthy rats were chosen randomly and separated into three groups. Each group was injected intraperitonially with 1ml of PZ, suspension E. cloacae, P. mirabilis, respectively. The rats were observed for 24 hours. Surgery was performed for the removal of the spleen and liver organ. The spleen organ tissue and liver were fixed onto the formalin buffer and the tissue was prepared for paraffin embedded and immunohistochemistry using a rabbit and mouse caspase-3 reagent.

## 3 RESULT

### 3.1 Spleen Organ

The results expression of caspase-3 lymphocyte cells in the spleen of the treatment group with the E. cloacae infection were higher than the treatment group with the P. mirabilis infection (Figure 1).

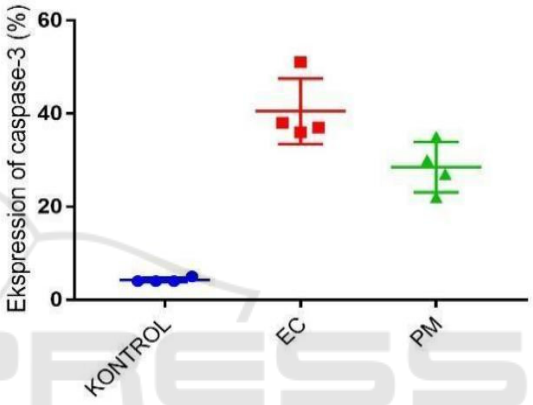


Figure 1: Box-plot caspase-3 expression of lymphocyte cells in the spleen

The results of the highest median values infected with E. cloacae (37.5%) infected with P. mirabilis (28.5%) and control group (4%). The caspase-3 expression of lymphocytes cell was observed with a x100 objective lens magnification light microscope at five viewing fields (Figure 1).

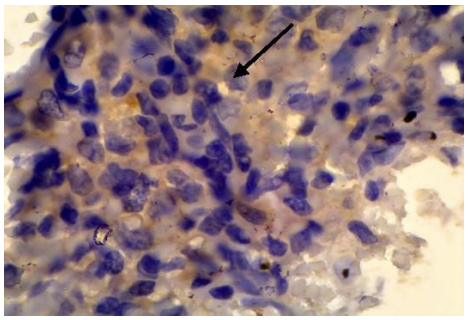

Figure 2: Caspase-3 expression of blue lymphocyte cells in the spleen (Rattus norvegicus) x1000 magnification of the control group.

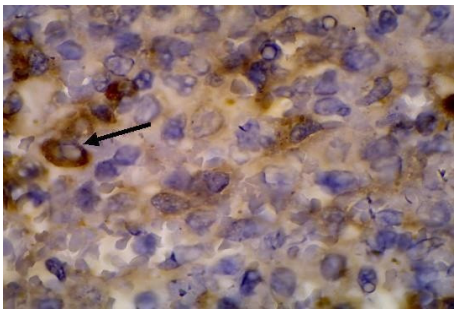

Figure 3: Caspase-3 expressions in the spleen of Ratus norvegicus infected with E. cloacae are shown by brown stained lymphocyte cells (x1000 magnification

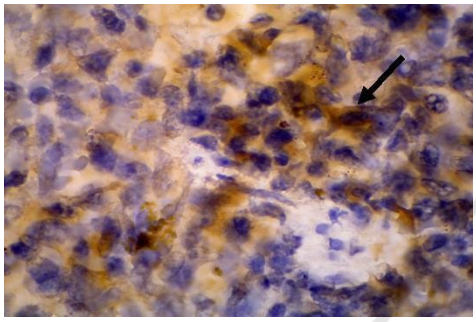

Figure 4: Caspase-3 expressions in the spleen of Ratus norvegicus infected with P. mirabilis are shown by brown stained lymphocyte cells (x1000 magnification).

## 3.2 Liver Organs

The result showed that the expression of caspase--3 hepatocyte cells in liver organ, treatment group infected by E. Cloacae was higher than the treatment group P. mirabilis (Figure 5).

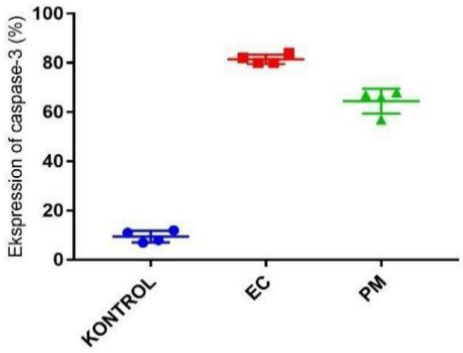


Figure 5: Box-plot of caspase-3 expression of lymphocyte cells in the liver.

The results of the highest median values with E.cloacae (81%) were infected with P. mirabilis (66.5%) and the control group (9.5%). The caspase expression of three hepatocyte cells was observed in a five field of view with x100 magnification (Figure 5).

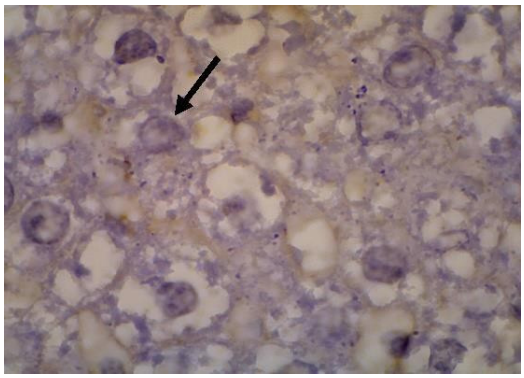

Figure 6: Caspase 3 expressions of hepatocyte cell in liver Rattus norvegicus 1000x magnification control group.

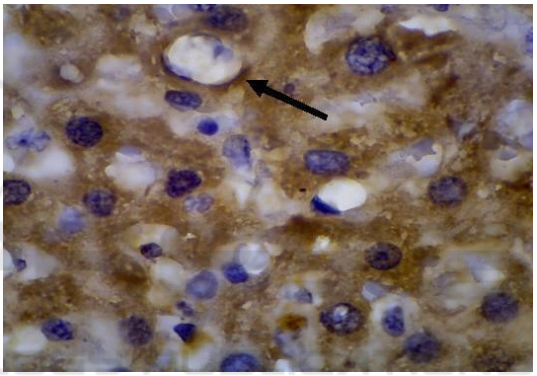

Figure 7: Caspase-3 expressions in the liver of Rattus norvegicus infected with E. cloacae are shown by brown stained hepatocyte cells (x1000 magnification).

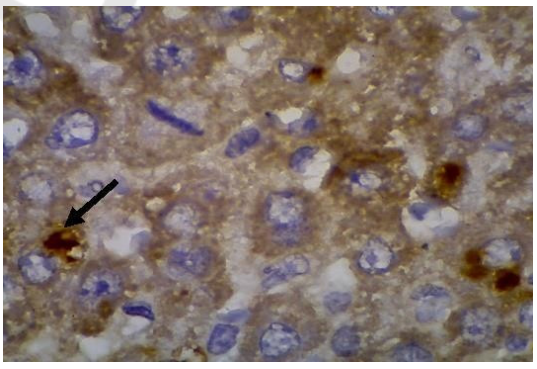

Figure 8: Caspase-3 expressions in the liver of Rattus norvegicus infected with P.mirabilis are shown by brown stained hepatocyte cells (x1000 magnification).

# 4 DISCUSSION

Based on the result, two rats in E.cloacae group died, while the Proteus mirabilis infection group and the control group survived. Those that were alive could maintain homeostasis in the inflammatory process due to gram-negative bacterial infection.

The endotoxins content in the E. cloacae bacteria consisted of glycolipids, LPS macromolecules that make up about 75% of LPS structures comprise the domain of hydrophobic lipids A, oligosaccharide nuclei, and O antigen polysaccharides, which are the outermost and outer membranes of gram-negative bacteria involved in sepsis and pathogenesis causing lethal shock (Nephrol Contrib, 2010). The virulent factor of pathogenic E.colacae bacteria such as lipopolysaccharides, type III secretion, exopolysaccharide and enterotoxin, hemolisyn, thiol activated toxin, and shiga toxin type II were capable to colonize in the hosts body, which can result in organ failure (Krzyminska et al., 2009).

P. mirabilis can differentiate into swarming motility that contribute to the formation of infection (Morgenstein et al., 2010). P. mirabilis produces urease enzymes to improve nutrients in urine, fimbriae or pili serve as adhesin and avoid host immune response (Norsworthy et al., 2017). The spleen is an important role in the modulation of the immune system and in the maintenance of peripheral tolerance through clearance of apoptotic cells, differentiation and activation of T and B cells (Vincenzo and Mikael., 2013).The spleen acts as a filter against the antigenic response carried through the blood by the lymph glands. The antigen carried by the blood is captured and is concentrated by dendritic cells as well as macrophages in the spleen. The spleen contains many phagocyte cells that play a role in eating and destroying microbes in the blood (Abbas et al., 2015).

Apoptosis in hepatocytes may be the most important event in the molecular mechanism of liver failure; apoptosis is the first liver cell response to bacterial toxins, including LPS, the caspase pathway that plays a role in apoptosis including the initiator and caspase execution. Two main caspase initiators, caspase-8 and caspase-9, signal death. Caspase-8 is activated by a signaling of death that binds to a death receptor on the cell surface. Caspase-9 is activated by the cytochrome released by the mitochondria. The caspase initiator's proapoptotic pathway activates the caspases of the executor, caspase-3. Caspase-3 activation is characterized by protein substrate division of DNA molecule breakdown and apoptosis.

In gram-negative bacterial infections endotoxin are 80% injected intravenously in animals to detect in the liver within 20–30 minutes. LPS begins to enter from the intestine, periodically taken to the liver through the portal vein and is then stored by the Kupffer cells and the macrophages are stunned in the liver. The first function of the liver is detoxification.

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The process of apoptosis plays an important role for the development of lymphocytes; the immune response to homeostasis is due to infection and maintains tolerance to self-antigen and cell death infected by pathogenic bacteria (Hardiono., 2016).

Activation of caspase-3 during bacterial infection involves bacterial invasion, which triggers stress on host cells associated with cell intracellular replication. Gram-negative bacterial toxins that have a large molecular weight can cause cell cycle changes to cellular stress with cell death through apoptotic caspase (Wall and McCormick, 2014).

# 5 CONCLUSION

There was an increased difference in caspase-3 expression in the liver and spleen of the R. norvegicus group infected with E.cloacae, which was higher than that infected with P.mirabilis. Increased caspase-3 expression in bacterial infections may increase cell stress in the host and increase organ mortality.

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