Iran. J. Ichthyol. (Special Issue 2023): 112-117

P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

Research Article

The liver and renal function test in experimentally immunized rabbits with sonicated antigens of *Klebsiella pneumoniae*

Khalid Mahmood HAMMADI*
Department of Biology, College of Education, Al-Iraqia University, Iraq.
Email: drkhalidmh0@gmail.com

Abstract: This study aimed to investigate the effect of immunization with sonicated *Klebsiella pneumonia* on some blood enzymes in rabbits. Using 20 breed rabbits divided into two equal groups (10 each). 1000 µg/ml S/C of sonicated *K. Pneumonia* antigen was administered to the first group (immunized). The second group was the control which was given 1ml of phosphate buffer saline (pH = 7.2) S/C. According to the results, in the case of liver enzyme, the immunized group had significant increases ($P \leq 0.01$) in all blood enzymes (AST, ALT and ALP) at 2, 10, and 20 days compared to the control group. In addition, the renal test (*B. urea* and creatinine) in the immunized group showed a significant increase in 2 and 10 days compared with zero time or with control, but the results did not show an increase at 20 days.

Keywords: Liver, Renal, Rabbits, Sonicated antigens, *Klebsiella pneumoniae*.
Citation: Hammadi, K.M. 2023. The liver and renal function test in experimentally immunized rabbits with sonicated antigens of *Klebsiella pneumoniae*. Iranian Journal of Ichthyology (Special Issue 1): 112-117.

Introduction

The genus *Klebsiella* was included under the Enterobacteriaceae and their majority are facultative anaerobes (Moore 2015). *Klebsiella pneumoniae* is an opportunistic pathogen causing a number of illnesses. Its most crucial virulence factors are lipopolysaccharide O side chain (O antigen) and opulent capsular polysaccharide (K antigen) (Jagnow & Clegg 2010). They guard it against host phagocytosis keeping the bacterium alive. In addition, *in vivo* biofilm formation shields the pathogen from the host immune system and antibiotic attacks (Chung 2011). *Klebsiella pneumonia* opportunistically infects a range of mucosal sites, including the lower respiratory tract and genitourinary tract (AL-Zorri 2009).

It is critical to understand that an enzyme's activity may be high in a particular organ or tissue - or even unique to that tissue -and it may not vary considerably in the blood when that tissue is injured. However, elevated activity has been linked to hepatocellular disorders (hepatitis) (Coles 1986). Large-scale hemorrhagic necrotizing consolidation of the lung can be caused by *K. pneumonia*. With localized lesions, it causes bacteremia and urinary tract infection. This pathogen was causing disease in the digestive, urinary, and respiratory tract of humans and can cause septic infection (Brooks et al. 2010).

When liver illnesses cause cellular degeneration or destruction, alanine aminotransferase (ALT) levels rise in serum (Coles 1986). The heart muscle has the highest aspartate aminotransferase (AST) activity, followed by the liver and skeletal muscle. The AST test can be used to detect tissue damage in a wide range of tissues. Alkaline phosphatase (AP) is broadly distributed throughout the body and is particularly abundant in the liver, placenta, intestinal mucosa, renal tubule cells, and bone (osteoblasts). These tissues have clearly separate isoenzymes of AP. Corticosteroids and perhaps other medications can trigger the creation of an isoenzyme (Coles 1986). While urea and creatinine were indicators of kidney impairment (Nasiru 2017). Based on the above-mentioned background, this work aimed to

investigate the effect of immunization with sonicated *Klebsiella pneumonia* on some blood enzymes in rabbits.

Materials and Methods

Specific pathogen-free twenty rabbits, aged 6-8 weeks were housed at the animal house in the College of Veterinary Medicine, Baghdad University, where they were provided with food and water *ad libitum*. They were divided randomly into two groups (10 each) as follows (a) the first group received 1000µg/ml of sonicated *K. Pneumonia* antigen S/C, and (b) the second group received 1ml of pH-7.2 phosphate buffer saline S/C as the control.

Serum collection: Following cardiac puncture under anesthesia, serum was prepared. The clotted blood was centrifuged at 1000 g for 20 minutes at 20°C. The sera were combined, and aliquots were kept at 70°C until their examination (Watson 1996).

Bacterial isolation: According to Radostitis et al. (2007), milk samples were collected from clinical mastitis of cows in sterile tubes under septic precaution after discarding the fore milk and transporting immediately to the laboratory by a cooling box. *Klebsiella pneumoniae* was isolated from the milk samples of clinical mastitis, and used for experimental infection in Rabbits. The bacteria were diagnosed by microscopic examination and biochemical and VITEK_2 tests. According to Mitov et al. (1992), dead whole-cell sonicated antigen was prepared. The total protein of both antigens was quantified using the Biuret method (Henry et al. 1974).

Liver function test: Liver function tests of AST and ALT were measured according to the manufacturer Manuel using kits (Bio system). For this purpose, we take 800ml of R1 and add 200µl of R2 into it, then add into the working solution 50µl of the serum and left for 5minin a water bath at a temperature of 37°C. The reading was done using a spectrophotometer at a wavelength of 350nm twice. The first reading was done after one minute and the second reading after two minutes. By subtracting the second reading from the first one (A1-A2), then dividing the result of the sample into Stander, AST and ALT were calculated. ALP test is also done by mixing 800µl of A solution and 200µl from B solution, then adding to the working solution of 20µl (serum), leaving for 4min, and the reading in a spectrophotometer (BTS350).

Kidney function test: A test for kidney function (blood urea) was performed according to the manufacturer's manual (Bio system). We mixed the A2 agent with A1, and then a blood sample was added to it in a gel tube and centrifuged for obtaining serum. Afterward, 1ml of solution A1 and 10µl of the serum were added and left for 5 min in a water bath. Then 1ml of B agent was added to solution A1, mixed well, and by adding the serum, it was left for 5min in a water bath. The solution was read using a spectrophotometer at a wavelength of 600nm. We make a stander for urea by taking 1ml working solution A1, adding 10µl of Stander, and leaving for 5min in a water bath at a temperature 54°C. It was then read with a spectrophotometer at a wavelength of 600nm. The final result was calculated by dividing the result of the sample by Stander and multiplying by 52. For the result of *S. criatinin*, the result of urea was divided by 40 and its normal percentage (0-1).

Statistical analysis: The data were subjected to statistical analysis using ANOVA in the Sigma Sat for Windows software. In all analyses, statistical significance was taken to be $P<0.01$. Values are reported as mean and standard error (mean±SE) (Joda 2008).

Ethical approval: The ethics and scientific committee of the University of Baghdad's veterinary medicine college approved this work.

Results

Liver function test: The results revealed a significant increase ($P<0.01$) in the blood enzymes of AST, ALT, and ALP of the immunized group (81.21±2.7, 88.33±2.7, and 79.22±2.7, respectively) compared to the control one (49.33±2.7, 45.35± 1.7, and 40.32±3.7, respectively) (Table 1). The results of blood enzyme (AST, ALT, and ALP) after 10 days

Table 1. The levels of blood enzymes following 2 days of immunization with Sonicated antigen of *K. pneumoniae* in rabbits.

Groups \ Enzymes	AST (ug/L)	ALT (ug/L)	ALP (ug/L)
Immunized group	81.21±2.7A	88.33±2.7A	79.22±2.7A
Control	49.33±2.7B	45.35±1.7B	40.32±3.7B

The various vertical letters show the groups' significant (P<0.01) difference.

Table 2. Blood enzyme concentrations in rabbits after 10 days of vaccination with *K. pneumoniae* sonicated antigen.

Groups \ Enzymes	AST (ug/L)	ALT (ug/L)	ALP (ug/L)
Immunized group	90.11± 2.1A	98.23±2.2A	87.21±2.5A
Control	50.23±2.6B	44.15±1.4B	40.22±3.1B

The various vertical letters show the groups' significant (P<0.01) difference.

Table 3. The levels of blood enzymes following 20 days of rabbit immunization with *K. pneumoniae* sonicated antigen.

Groups \ Enzymes	AST (ug/L)	ALT (ug/L)	ALP (ug/L)
Immunized group	61. 51± 3.1A	57.13±2.4A	64.41±1.5A
Control	50.23±2.6B	44.15±1.4B	40.22±3.1B

The various vertical letters show the groups' significant (P<0.01) difference.

Table 4. The concentrations of blood urea after Immunization by Sonicated antigen of *K. pneumoniae* in rabbits.

Groups \ Days	0	2	10	20
Immunized group	32. 51±3.2Aa	69.23±2.1Ab	45.31±1.2Ac	31.11±2.2Aa
Control	31.20±2.6 A	31.11±1.4B	30.22±3.1B	30.34±1.1A

Capital letters= differences in the same group, small letters= differences between the different groups, the same letters= no significant differences (P≥0.01), and the different letters= significant differences (P≤0.01).

showed increases significantly (P≤0.01) in the immunized group (90.11±2.1, 98.23±2.2, and 87.21±2.5, respectively) compared to the control group (50.23±2.6, 44.15±1.4, and 40.22±3.1, respectively) (Table 2).

Table 3 shows the blood enzyme levels following a 20-day of immunization. Based on the results, in the immunized group's concentrations of the enzymes of AST, ALT, and ALP were increased significantly (P≤0.01) (61.51±3.1, 57.13±2.4, and 64.41±1.5, respectively) compared to the control group (50.23±2.6, 44.15±1.4, and 40.22±3.1, respectively (Table 3).

Renal function test: The result of blood urea before immunization (zero time) was in the normal range for immunized treatment (30.51±3.2) than that of the control groups (31.20±2.6). The immunized group showed a significant increase (P≤0.01) in 2 and 10 days (69.23±2.1 and 45.31±1.2, respectively, compared with zero time or control one at the same time, but the result did not show an increase at 20 days (Table 4).

In the case of serum creatinine, the results before immunization showed a normal value for the immunized and control groups (1.51±3.2 and 1.20±2.6, respectively). After immunization, the treated group showed a significant increase (P≤0.01) in 2 and 10 days (4.23±3.1 and 3.21±1.2, respectively) compared to the result before immunization or the control group at the same time, but the result did not show any increase at 20 days (Table 5).

Discussion

An opportunistic bacterium, *K. pneumoniae* affects domestic animals and causes pneumonia and

Table 5. The concentrations of Serum Creatinine after Immunization by Sonicated antigen of *K. pneumoniae* in rabbits.

Groups \ Days	0	2	10	20
Immunized group	1.51±3.2Aa	4.23±3.1Ab	3.21±1.0Ac	1.9±2.2Aa
Control	1.20±2.6A	1.11±1.4B	1.22±3.1 B	1.34±1.1A

Capital letters= differences in the same group, small letters= differences between the different groups, the same letters= no significant differences ($P \geq 0.01$), and the different letters= significant differences ($P \leq 0.01$).

suppurative infections in horses, cervicitis and metritis in mares, mastitis in cows, wound infections, urinary tract infections, septicemia, and pneumonia in dogs. It is uncommon for a rabbit to contract *K. pneumoniae*, and this illness usually takes an intestinal form. Previous research details a *K. pneumoniae* outbreak linked to septicemia and respiratory form in a rabbit farm (Sumitha & Sukumar 2014). The signs of *K. pneumoniae* include a lethal presentation with a rapid onset, a high temperature, and hemoptysis. *Klebsiella pneumoniae* opportunistically infects a variety of mucosal surfaces, including the respiratory tract and genitourinary tract. It has been thought of as a pathogen that causes mastitis (Peto et al. 2014). Our study revealed increases in liver enzymes (AST, ALT, and ALP) after immunization with the sonicated antigen of *K. Pneumonia*. This result was in agreement with the study of Alobaidy (2022). Al-Shamaa et al. (2000) that the levels of the liver function enzymes AST and ALT and alkaline phosphate (ALP) have a significant rise. These findings agree with those of the current study. Hepatitis and myopathy are both clinical conditions that are typically identified by ALP, AST, and ALT that are all shown to be high in the disorder of hepatitis and muscle (Mody et al. 1989).

The activity of enzymes varies substantially between tissues and animals. It is crucial to understand that even while an enzyme may have high activity in a certain organ or tissue or even be specialized to that tissue if it does not alter considerably in the blood when that tissue is destroyed. it would not have much of an impact on clinical outcomes. Heart muscle, the liver, and skeletal muscle have the highest AST activity levels. However, elevated activity has been linked to hepatocellular injury (hepatic diseases). According to Ghazal et al. (2016), the liver texture of the New Zealand white rabbit was harmed by single- and multi-walled carbon nanotubes, which resulted in an increase in ALT and AST (Coles 1986). When cellular deterioration or destruction occurs in liver disorders, ALT levels in the serum rise. Since AST is found in all bodily tissues and is not an organ-specific test, it can be used to identify tissue damage in a wide range of tissues. This enzyme can be used to support the diagnosis of muscular degeneration because it is present in extraordinarily high concentrations in both skeletal and cardiac muscle. Although AST levels may rise with liver illnesses in all species, they cannot be used as a screening tool for liver impairment (Coles 1986; AL-Zorri 2009). Typically, blood enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate are used to diagnose both clinical entities, myopathy and hepatitis, LDH, AST, and LDH that are all increased in myopathy and hepatitis, although ALT is more specific to the liver and muscle (Scheig 1996). Increased levels of AST, ALT and ALP, and total and direct serum bilirubin in untreated mice were found to cause damage (Sallie et al. 1991). Our findings are consistent with the findings of this study, as are the structural integrity of hepatocytes and the fact that these enzymes are released into the circulation when cellular damage occurs.

In addition to being widely present throughout the body, alkaline phosphatase (AP) is also highly abundant in the placenta, liver, intestinal mucosa, renal tubule cells, and bone (osteoblasts). These tissues have AP isoenzymes that are uniquely

different. An isoenzyme whose synthesis is induced by corticosteroids and maybe other drugs (Coles 1986). ALP, ALT, AST, and GGT levels in female New Zealand white rabbits considerably rose (AL-bdeery & AL-Zubaidi 2014), and this was attributed to the animals' pregnancy state. According to AL-Zorri (2009), diabetic rabbits exhibited elevated levels of the liver enzymes GOT and GPT.

Our study also revealed an increase in renal function tests as (B.urea) and (S.creatinine), in which the immunized group showed a significant increase in 2 and 10 and this result was in agreement with the study of Alobaidy (2022). Glomerular filtration and renal tubule dysfunctions are linked to creatinine and urea as the primary indices of kidney disease. El-Jakee (2010) discovered that mice given bacterial inoculations had considerably higher ALT, creatinine, and urea levels than control mice. According to Leon et al. (2015), *Salmonella* bacteria can harm the kidneys because urea and creatinine are important indicators of renal injury linked to anomalies in renal tubular structure and glomerular filtration rate showing consistent results with our findings. The findings of the current work agree with those of the findings of El-Jakee (2010), who discovered that the mice who received bacterial injections had significantly higher levels of creatinine and urea. Typhoid patients had higher amounts of urea and creatinine in their blood (Srikaanth & Kumar 2005). In a conclusion, our results showed that the blood enzymes of ALT, AST, ALP, urea, and creatinine increase following immunization with sonicated antigens of *K. pneumonia*, which has been found to cause liver and kidney damage.

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