HAEMATOLOGICAL AND SERUM BIOCHEMICAL CHANGES IN THE RAT MODEL INDUCED WITH *MANNHEIMIA HAEMOLYTICA* AND TREATED WITH SEA CUCUMBER (*STICHOPUS HORRENS*) EXTRACT

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CERTIFICATION

This is to certify that we have read this research paper entitled '**Hematological and Serum Biochemical Changes in the Rat Model Induced with sea cucumber (*Stichopus horrens)* 'extract'** by Muhamad Hidayat bin Saidon, and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course DVT 5436 – Research Project.

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ABSTRACT

The requirement on the course DVT 5436 – Research Project. An abstract of the research paper must be presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan.

Mannheimiosis is one of the important respiratory diseases in ruminants and is still the main challenge to the ruminant industry in tropical countries. Sea cucumber extract has many therapeutic effects, including being an anti-bacterial, antifungal, antitumor, anti-inflammatory and antinociceptive; however, there is no evidence of the preventative and therapeutic effects of sea cucumber (*Stichopus horrens*) extract on pneumonic pasteurellosis in the rat model. Therefore, this study investigates the haematological and serum biochemical changes in rats induced with *Mannheimia haemolytica* and treated with sea cucumber (*Stichopus horrens*) extract. Eight rats were recruited in this study, kept in separate cages and induced with *Mannheimia haemolytica*. Four rats in the treatment control group were given sea cucumber (Stichopus horrens) extract, whereas the remaining four rats in the control group were not.Whole blood and serum were collected on day-28 and analyzed for haematological and serum biochemical changes. A T-test was done to analyze the data obtained. Hematology results were not significant ($p>0.05$) in both treatment and control groups. However, the total white blood cell and differential leucocyte counts were lower in the treatment groups than the negative control. Meanwhile, results on serum biochemical parameters, particularly blood urea nitrogen (BUN), creatinine (CREA) and alanine transaminase (ALT), were significantly ($p<0.05$) lower in the treatment group compared to control group. These findings demonstrated that sea cucumber (Stichopus horrens) extract has the potential in reducing inflammation and organ-specific injury in post-mannheimiosis inoculation.

Keywords: *Mannheimiosis, sea cucumber (Stichopus horrens) extract, haematology, serum biochemical*

ABSTRAK

Keperluan kursus DVT 5436 – Projek Penyelidikan. Abstrak kertas penyelidikan hendaklah dibentangkan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan.

Mannheimiosis adalah salah satu penyakit pernafasan penting dalam ruminan dan masih menjadi cabaran utama kepada industri ruminan di negara tropika. Ekstrak gamat mempunyai banyak kesan terapeutik, termasuk sebagai anti-bakteria, antikulat, antitumor, anti-radang dan antinosiseptif; Walau bagaimanapun, pada masa ini tiada bukti tentang kesan pencegahan dan terapeutik ekstrak gamat (Stichopus horrens) pada pasteurellosis pneumonic dalam model tikus. Kajian ini menyiasat perubahan biokimia hematologi dan serum pada tikus yang disebabkan dengan Mannheimia haemolytica dan dirawat dengan ekstrak gamat (Stichopus horrens). Lapan tikus telah digunakan, disimpan dalam sangkar berasingan dan diinduksi dengan Mannheimia haemolytica. Empat tikus dalam kumpulan kawalan rawatan diberi ekstrak gamat (*Stichopus horrens*), manakala baki empat tikus dalam kumpulan kawalan tidak. Darah dan serum keseluruhan dikumpul pada hari ke-28 dan dianalisis untuk perubahan biokimia hematologi dan serum.. Darah dan serum keseluruhan dikumpul pada hari ke-28 kajian dan dianalisis untuk perubahan biokimia hematologi dan serum. Ujian-T telah dilakukan untuk menganalisis data yang diperolehi. Keputusan hematologi tidak signifikan ($p>0.05$) dalam kedua-dua kumpulan rawatan dan kawalan. Walau bagaimanapun, jumlah sel darah putih dan bilangan leukosit berbeza adalah lebih rendah dalam kumpulan rawatan daripada kawalan negatif. Sementara itu, keputusan pada parameter biokimia serum, terutamanya nitrogen urea darah (BUN), kreatinin (CREA) dan alanine transaminase (ALT), adalah ketara ($p<0.05$) lebih rendah dalam kumpulan rawatan berbanding kawalan negatif. Penemuan ini menunjukkan kesan positif ekstrak gamat (*Stichopus horrens*) dalam mengurangkan keradangan dan kecederaan khusus organ dalam inokulasi selepas mannheimiosis.

Kata kunci: *Mannheimiosis, Ekstrak gamat (Stichopus horrens), haematologi, Biokimia serum*

1.0 INTRODUCTION

Mannheimiosis, also known as pneumonic pasteurellosis, is still the main impediment to domestic animal production in tropical countries. Pneumonia is a respiratory infection that usually affects domestic animals (Oremeyi *et al*., 2013; Faez *et al*., 2015). *Mannheimia haemolytica* is the most common cause of mannheimiosis (Salisi, 2011; Salisi *et al*., 2012). Climate stress, inadequate nutriment, poor husbandry, and transportation stress are predisposing factors that results in immunocompromised animals, leading to pneumonic pasteurellosis, such as in sheep and goats. This will then cause significant financial losses to the farmers (Faez *et al*., 2015).

Mannheimia haemolytica is a coccobacillus-shaped, hemolytic, Gram-negative, non-motile bacterium (Zecchinon *et al*., 2005; Shanthalingam *et al.*, 2009). The bacteriological test, biochemical features, biotyping and serotyping of isolates provide an accurate diagnosis of *M. haemolytica* (Kumar *et al*., 2015). Although some researchers still believe that *Pasteurella multocida* and *M. haemolytica* are both commensals of the upper respiratory tract and that their pathogenic structures are related to severe pulmonary infections in large ruminants. Today, it is now clear that *M. haemolytica*, previously known as *Pasteurella haemolytica (P. haemolytica),* is the causative agent of the pulmonary infection (Radostits *et al*., 2000; Hailu *et al*., 2017).

Holothurians, or sea cucumbers, are marine invertebrates that live in shallow seawater on reef flats and slopes with high hydrodynamic energy (Conand, 1993). There are various Holothuroidea species, and one of them, *Stichopus* spp, is found throughout the tropical

Indo-Pacific region (Conand, 1993). Sea cucumber or alsoknown as "Gamat," in Malay is frequently used orally and topically to cure various ailments, including low back pain and rheumatism (Taiyeb-Ali *et al*., 2003; Bordbar *et al*., 2011). Due to high demand, its use is becoming more common, notably in Malaysia (Baine *et al*., 2012).

Sea cucumbers have a variety of nutrients with significant economic worth. Sea cucumber extract has been proven in trials to have a variety of therapeutic effects, including being an anti-bacterial (Ridzwan *et al*., 1995; Haug *et al*., 2002), antifungal (Shimada, 1969; Shaharuddin, 2006), antitumor (Tong *et al*., 2005; Lu *et al*., 2009), anti anaphylactic (Kim *et al*., 2001), anti-inflammatory (Whitehouse *et al*., 1994; Jassan *et al*., 1997), antinociceptive (Hassan *et al*., 1994), and antioxidant agent (Hawa *et al*., 1999; Althunibat *et al*., 2009). The total number of species on the earth is estimated to be approximately 1135. Only five species were the most sought after because of their high economic and nutritional worth (Geistia 'Fad'ha *et al*., 2017). As a result, the study's goal is to determine whether the sea cucumber (*Stichopus horrens*) extract has a therapeutic effect on *M. haemolytica*-infected rats. Even though there have been previous research on the effects of sea cucumber extract on other organisms, there is limited studies on the effects of sea cucumber on a rat model infected with mannheimiosis.

2.0 RESEARCH PROBLEM

Mannheimia haemolytica causes infection in the lungs by interrupting the innate mucosal defence mechanism, which is the most common cause of acute pulmonary diseases marked as a result of a necrotizing inflammatory reaction (Sabri *et al.*, 2013). Antimicrobial and anti-inflammatory medications are indicated therapy procedures for this condition. Besides that, a vaccine against mannheimiosis was developed to prevent the disease occurrence. Plant and animal-derived natural medicines have long been utilised to treat many human and animal diseases and disorders. Sea cucumber, for example, has been proven to have many pharmacological properties that can assist in lowering the severity and treating a variety of ailments. However, to our knowledge, there is no evidence on the preventative and therapeutic effects of sea cucumber (*Stichopus horrens*) extract on pneumonic pasteurellosis in a rat model. As a result, this study aims to investigate the potential usage of sea cucumber (*Stichopus horrens*) extract as therapeutic and prophylaxis in pneumonic pasteurellosis.

3.0 RESEARCH QUESTION

Does the sea cucumber (*Stichopus horrens*) extract have haematological and serum biochemical changes in the rat model induced with *Mannheimia haemolytica*.

4.0 RESEARCH HYPOTHESIS

Sea cucumber (*Stichopus horrens*) extract has both haematological and serum biochemal changes in a rat model induced with *Mannheimia haemolytica.*

5.0 RESEARCH OBJECTIVE

To investigate the haematological and serum biochemical changes of sea cucumber (*Stichopus horrens) extract* in the rat model induced with *Mannheimia haemolytica.*

6.0 LITERATURE REVIEW

6.1 **Pneumonic pasteurellosis**

Pasteurella multocida and *M. haemolytica* cause pneumonic pasteurellosis, the most frequent respiratory infection in domestic animals (Froner *et al.,* 2019). A complicated combination between bacteria, host immunity, and environmental stress causes disease. Due to expensive treatment costs, decreased daily weight growth, carcass condemnation, and reduced meat and milk output result in significant economic losses (Emikpe *et al.,* 2010). History, clinical indicators, post mortem examination and histological findings, bacteria identification and isolation, and molecular techniques such as polymerase chain reaction are used to diagnose (Froner *et al.,* 2019). For treating pneumonic pasteurellosis, antibiotics such as ampicillin, penicillin, ceftiofur, ceftriaxone, and enrofloxacin are recommended (Rahal *et al.,* 2014). Improvements in living conditions, sanitation and hygiene management, and biosecurity policies are also crucial in preventing the disease from spreading.

6.2 **Virulence factor involved in manheimiosis infection**

The virulence factors, such as capsular structure, fimbriae, endotoxin, and leukotoxin, are usually responsible for boosting the organism's adhesion, colonisation, and proliferation, all of which are important in the mannheimiosis pathogenesis (Marru *et al.,* 2013). Endotoxins are formed due to the microbe's multiplication in the infected lungs that, cause widespread intravascular thrombosis of pulmonary veins, capillaries, leading to ischemic necrosis of the pulmonary parenchyma (Per *et al.,* 2010). Microbial activity is frequently inhibited by substances found in the airways' released serous and mucinous fluids. Lysozyme,

lactoferrin, phospholipase A2, surfactant proteins, peroxidases, secretory leukoprotease inhibitor, lysozyme, bactericidal permeability-inducing factor, and anionic peptides are some of the factors involved (Effendy *et al.*, 1998). The first line of defence against Gram-negative bacteria products, such as *M. haemolytica*, is mammalian bronchial and bronchiolar epithelial cells (Shanthalingam *et al.,* 2014). Other than that, the mucociliary apparatus reduces microbial attachment and multiplication within the respiratory tract's airways (Zamri *et al.,* 2006).

6.3 Pathological changes related to *Mannheimia haemolytica* infection

Pneumonic pasteurellosis is a disease that affects animals that have a compromised pulmonary defence system. A serious respiratory illness with fibrinopurulent bronchopneumonia and septicaemia is commonly recognised (Alemneh *et al.,* 2016). The most visible injury in infected species is confined in one lung area in post-mortem examinations. There is typical 'marbling' of the lung on the liver surface, with lobules ranging from normal to grey to red, where the entire lobule undergoes coagulation necrosis or bleeding. Interlobular septa are filled by fibrin-rich edoema fluid and interlobular lymphatics, which may include fibrin thrombi. There was also an excess of straw-coloured exudate in the thorax, somewhat filled intralobular septa, a red area of consolidation, pleural adhesion, and encapsulated foci containing whitish discharge known as sequestra (Griffin *et al.,* 2010). Similar lesions were observed in the goat infected with pasteurellosis where the presence of exudate in the thoracic cavity (Abdullah *et al.,* 2014).

In the lungs of sick animals, histopathological abnormalities such as bleeding, oedema, necrosis, and white blood cell infiltration were found (Boukahil *et al.,* 2016). Acute cranioventral fibrinous to fibrinopurulent pleuropneumonia is a prominent lesion of *M. haemolytica*-induced pneumonia. Fibrin, oedema red blood cells, and dense clusters of

macrophages and neutrophils were seen in damaged sections of the lung, bronchioles, and alveolar spaces, according to histological examinations (Odugbo *et al*., 2004). The alveoli were flooded with fibrin-rich fluid, macrophage and neutrophil invasion, and typical 'oat cells,' condensed and streaming necrotic macrophages within the injured alveoli. Lobules with coagulation necrosis appear as infarcts with thick neutrophilic infiltrate surrounding the margin. Vasculitis with fibrin thrombi is common, and fibrin-rich exudates are frequently discharged from the bronchioles, spreading from the alveoli (Dassanayake *et al*., 2010).

6.4 Haematological and serum biochemistry findings of mannheimiosis

There is a study where leucocyte counts and subpopulations were taken from the peripheral blood of calves experimentally infected with *M. haemolytica*. The *M. haemolytica* inoculation significantly increased total leucocyte counts and neutrophils, while the lymphocyte count decreased (Rice *et al.*, 2007)).

6.5 Diagnosis of mannheimiosis

According to the protocols, a presumptive diagnosis can be made using data on the enterprise's and region's epizootic well-being, clinical signs, and pathological changes caused by the pathogen. Clinical signs such as coughing, dyspnea, depression are one of the methods in diagnose mannheimiosis. Other than that, laboratory diagnosis by using routine microbiological analysis can be done for the final diagnosis. In cattle, sheep and goats, samples such as heart with ligated blood vessels, spleen, liver, kidneys, lungs on the border of the affected and non-affected tissues, exudates from the chest cavity, and lymph nodes can be taken for laboratory diagnosis. There are also serology and molecular test kits for ELISA diagnosis, and among the commercial PCR diagnostic tools, there is a kit for the detection of *P. multocida* and *M. haemolytica*. The following steps are included in the

bacteriological analysis of pathological and clinical material which are receiving and sending samples for bacteriological research, isolation of a pure culture of the microorganism, and identification and differentiation of selected cultures (Laishevtsev, 2020).

6.6 Treatment of mannheimiosis

Choosing the most efficient anti-bacterial drugs is essential before beginning a treatment course. The most effective antibiotics for treating this disease, according to evidence, are drugs from various groups which are: i) Amoxiclav, ii) danofloxacin, iii) lincomycin, iv) oxytetracycline, v) spectinomycin, vi) tylosin and vii) cephalosporins. In some countries, anti-bacterial medication is used on animals that are newly brought to the herd for the preventive and prophylactic approach to reduce the disease occurrence and mortality rate. The best anti-bacterial activity against the strains of *Mannheimia* was shown by moxifloxacin; this anti-bacterial drug inhibits the growth of 100% of the cultures and about 60% of cultures showed resistance to meropenem and polymyxin B while the other 40% showed resistance to amoxiclav, lincomycin and cefoperazone (Laishevtsev, 2020).

6.7 Biology of sea cucumber

The ossicles of sea cucumbers belonging to the Stichopodidae family are fashioned like C-, S-, and branching rods and have a square or trapezoidal cross-section. The primary approach for species identification is morphological characteristics of the sea cucumber, such as body shape, body colour, and the presence and shape of papillae on both the dorsal and ventral sections of the sea cucumber. There are a few species of sea cucumber in Malaysia which are *Stichopus chloronatus, Stichopus horrens* (Gamat emas), *Stichopus ocellatus,* and *Stichopus vastus* (Kamarudin *et al.,* 2009). In the genus of *Stichopus,* the body is flat and may be covered with podia (Moriarty, 1982).

6.8 Sea cucumber industry in Malaysia

Sea cucumbers are commonly utilised in traditional medicine in Malaysia and other regions of Asia due to their health advantages and functional features. Sea cucumbers belong to the Holothurioidea and Stichopodidae families of marine animals. They are echinoderms with leathery skin and a long, flexible, gelatinous body (Sabuan, 2016). Sea cucumbers are nutrient-dense, including essential nutrients, minerals, fatty acids, bioactive peptides, and other useful elements. These advantages could be attributed to numerous functional chemicals in sea cucumbers (Bordbar *et al*., 2011). Sea cucumbers belonging to the *Stichopus* genus are frequently processed into medicinal and health goods such as oil, lotion, cream, pills, and soap (Kamarudin *et al*., 2009). Sea cucumbers or Gamat-based products are utilised for Malaysia's wound healing and overall wellness. Mothers traditionally used Gamat products after childbirth for their therapeutic effects. Gamat-based products are common in Malaysian traditional medicine, and they can even be combined with other herbs to make cough medicine or medicinal oil (Sabuan, 2016). Chen (2003)

claims that sulphated glycosaminoglycans (GAGs) are responsible for the sea cucumber's therapeutic properties. Glycosaminoglycans, or mucopolysaccharides, are large complex carbohydrate molecules that interact with various proteins involved in physiological and pathological processes (Masre *et al*., 2012).

6.9 Pharmacologic properties of *Stichopus horrens*

From Malaysia to the Society Islands, around French Polynesia, and from southern Japan and Hawaii to New Caledonia, *Stichopus horrens* can be found in the Pacific Ocean. *Stichopus horrens* has a grey-brown body with irregular grey-white markings (Massin *et al*., 2002). Sea cucumbers have been studied for their pharmacological effects, and it has been discovered that they have anti-bacterial, anti-inflammatory, anti-fungal, anti-cancer, and wound healing qualities. Previous study discovered that the sea cucumber *S. horrens* possesses anti-bacterial properties against *Streptococcus mutans* (Ibrahim *el al.*, 2018). *Stichopus horrens* contains promising amounts of antioxidant and cytotoxic natural compounds that could be employed for cancer prevention and treatment. According to findings from Althunibat *et al*., 2013. Moelyono et al (2018) discovered that an ethanolic extract of *S. horrens* possesses anti-inflammatory properties. Compared to the standard (Povidone iodine ointment) and the control, *S. horrens* has wound healing activity, as it showed a higher percentage of wound contraction (Subramaniam *et al*., 2013).

7.0 MATERIALS AND METHODS

7.1 Isolation and Identification of field isolates

Field and local isolates of *M. haemolytica* have been collected. Blood and MacConkey's agar plates were used to culture the bacteria and were incubated at 37°C for 48 hours. For additional confirmation of the bacteria, biochemical tests (catalase test, nitrate reduction test, urease, triple sugar iron), ornithine decarboxylase and O-nitrophenyl B, D-galactosidase (ONPG) disc test were performed.

7.2 Sea cucumber extract collection and contamination evaluation

Sea cucumber extract was bought from a local provider. The sterility of the extract was determined by inoculating 1 ml of it in 1 ml of sterile nutrient broth. The incubation period was 24 hours at 37 °C. After the incubation period, there will be no turbidity. The extract will be further evaluated using agar well diffusion in order to determine the antimicrobial properties.

7.3 Inoculum Preparation and Colony Forming Unit Counts

A few colonies from the blood agar plate were injected into brain-heart infusion (BHI) broth and cultured for 48 hours in an incubator shaker set at 37°C and 150 rpm. The BHI broth was diluted in steps (10-fold). One millilitre of the 107 dilutions was utilised as an inoculum and plated onto a blood agar plate for colony-forming unit (CFU) counting. Appendix A 1 demonstrates the preparation of Mannheimia hemolytica inoculum

7.4 Animal management & experimental design

The study was conducted according to the Animal Care and Use Ethics Committee (ACUC) of the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan. This study purchased eight rats from the Animal Research and Service Centre (ARASC), Universiti Sains Malaysia. The rats were housed in separate cages and fed rat pellets purchased from a pet store. Drinking water was available at all times. Before the trial, the rats were acclimatised for one week. Two groups of rats were formed (Groups 1 and 2). Intraorally, all rats were inoculated with 10^7 CFU/1mL of *M. haemolytica*. Group 1 was used as a control group and did not receive any therapy. Rats in group 2 received sea cucumber extract during acclimatization and post inoculated orally every day at a dose of 5ml/day for 14 days of post-inoculation.

7.5 Daily clinical observation, blood sampling and post mortem evaluation

All rats were checked for clinical indications every day, including mucopurulent discharge, lethargy, and inappetence. The breathing effort, as well as the behaviour, were also monitored. On the 14th day of post-inoculation, all rats were sacrificed using carbon dioxide. Blood samples were taken for further analysis. The serum samples were kept at -20°C until the serum analyses were completed. Appendix A 2 shows rats post inoculated with Mannheimia hemolytica.

7.6 Haematological and biochemical analyses

The total number of white blood cells (WBC), red blood cells (RBC), platelets, and haemoglobin concentration were determined using an automatic haematology analyser (Cell Dyn, 3700, Abbot, USA). Using a microhematocrit tube, the packed cell volume (PCV), icteric index, and plasma protein content were manually measured. On a blood smear, Wright's staining was used to determine the WBC differential count and checked for toxic alterations in the leucocytes. The concentrations of alanine transaminase (ALT), total protein, albumin, creatinine, and urea were determined in serum samples using an automatic biochemistry analyser (TRX 7070, Biorex, Germany).

7.7 Statistical analysis

The Statistical Package for Social Science (SPSS) software version 23 was used to analyse the data statistically. The values are mean and standard deviation (SD) for various parameters. To compare the differences in data between and within groups, a sample T-Test was used. The level of statistical significance, which was established at $p < 0.05$, was determined using post hoc analysis using the Duncan test.

8.0 Results

i) Clinical signs observation

All rats were closely monitored for the first 2 hours post-inoculation and daily observation was made for the next 14 days after the extract was given for any clinical signs shown (Table 1). During the period of observation, prominent signs of depression and sneezing were observed in the control group than in the treatment group.

Table 1: Clinical signs shown in rats of control and treatment groups

	Control				Treatment			
Clinical sign	1	2	3	4	1	2	3	4
Dull and depressed	/	/	/	/	-	/	-	/
Sneezing	/	/	/	/	-	/	-	/
Nasal discharge	-	-	-	-	-	-	-	-
Dyspnea	-	-	-	-	-	-	-	-
Anorexia	-	-	-	-	-	-	-	-

ii) Hematological analysis

Table 2 shows there is no significant difference (p>0.05) of haematological changes between control and treatment groups. Figure 1 shows the bar graph of red blood cell parameters (mean ± standard deviation) between negative and treatment control groups.

Table 2: The haemogram (mean ± standard deviation) in rats induced with *Mannheimia hemolytica* and treated with sea cucumber extract (*Stichopus horrens*)

Parameters/Group	Control	Treatment	***p***
RBC (10^6/µl)	3.80 ± 0.48	4.03 ± 0.54	0.55
HGB (g/dl)	6.88 ± 0.85	6.88 ± 1.37	1.00
HCT (%)	11.45 ± 0.98	10.40 ± 1.43	0.27
MCV (µm^3)	40.53 ± 19.57	41.75 ± 24.59	0.94
MCH (g/dl)	61.05 ± 2.77	60.13 ± 2.60	0.64
MCHC (g/dl)	51.18 ± 1.58	53.55 ± 6.12	0.48

p: Significant value, p<0.05

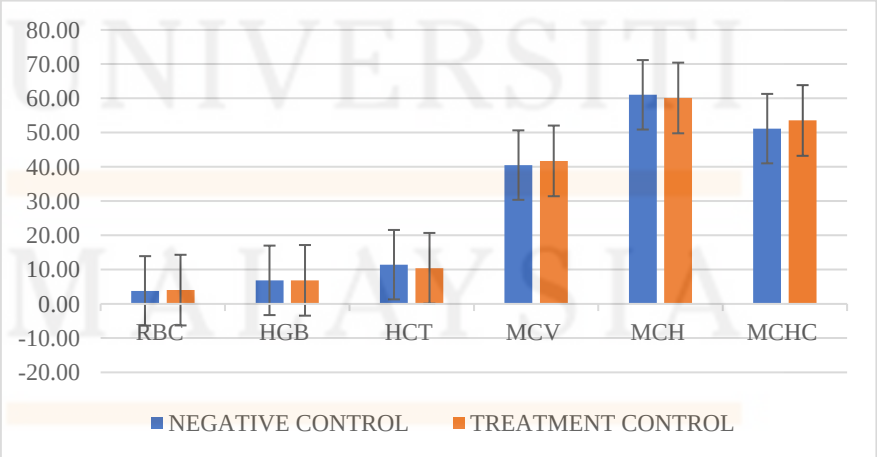

Figure 1: Red blood cell parameters (mean ± standard deviation) between negative and treatment control groups.

There is no significant difference (p>0.05) in the total white blood cell counts and differential leukocyte counts parameters in both groups (Table 3). However, the values were lower in the treatment group compared to the control group. These findings suggested an anti-inflammatory effect of the extract post 28 days of administration. Figure 2 shows the bar graph of white blood cell parameters (mean ± standard deviation) between negative and treatment control groups.

Table 3 Total white blood cells and differential leukocytes count (mean) in the rats induced with *Mannheimia hemolytica* and treated with sea cucumber extract (*Stichopus horrens*).

Parameters/Group	Control	Treatment	p
WBC (10^3/µl)	3.48 ± 0.96	2.40 ± 0.39	0.83
Lymphocytes (10^3/µl)	1.45 ± 0.70	1.05 ± 0.21	0.31
Monocytes (10^3/µl)	0.83 ± 0.40	0.55 ± 0.29	0.31
Granulocytes (10^3/µl)	1.20 ± 0.28	1.05 ± 0.45	0.59

p: Significant value, p<0.05

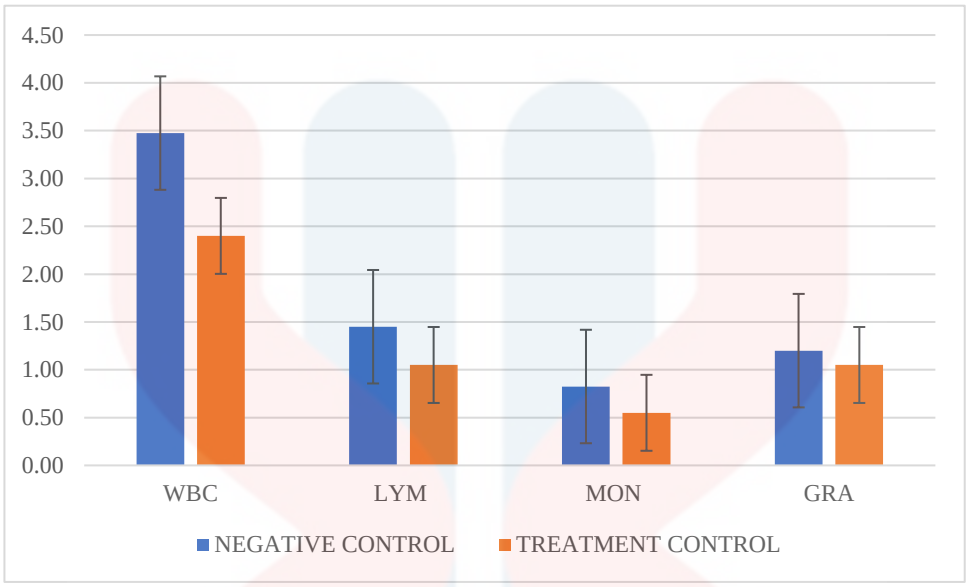

Figure 3: White blood cell parameters (mean ± standard deviation) between negative and treatment control groups.

iii) Serum biochemistry analysis

Table 4 shows the blood urea nitrogen (BUN), creatinine and alanine transaminase (ALT) of rats treated with sea cucumber extract was significantly lower than the negative control group. These findings suggested lesser renal and hepatic injuries in the group of rats that received the extract administration. Figure 4 shows the bar graph of blood urea nitrogen (BUN) and alanine transaminase (ALT) parameters (mean ± standard deviation) between negative and treatment control groups while figure 4 shows the bar graph of creatine values (mean ± standard deviation) between negative and treatment control groups.

Table 4 Renal and hepatic evaluation (mean ± standard deviation) in the rat model induced with *Mannheimia hemolytica* and treated with sea cucumber extract (*Stichopus horrens*).

Parameters/Group	Control	Treatment	***p***
Blood Urea Nitrogen (mg/dl)	23.65 ± 1.44	16.35 ± 1.26	0.01
Creatinine (mg/dl)	0.78 ± 0.10	0.34 ± 0.07	0.01
ALT (U/L)	64.50 ± 4.60	49.45 ± 7.51	0.01

p: Significant value, p<0.05

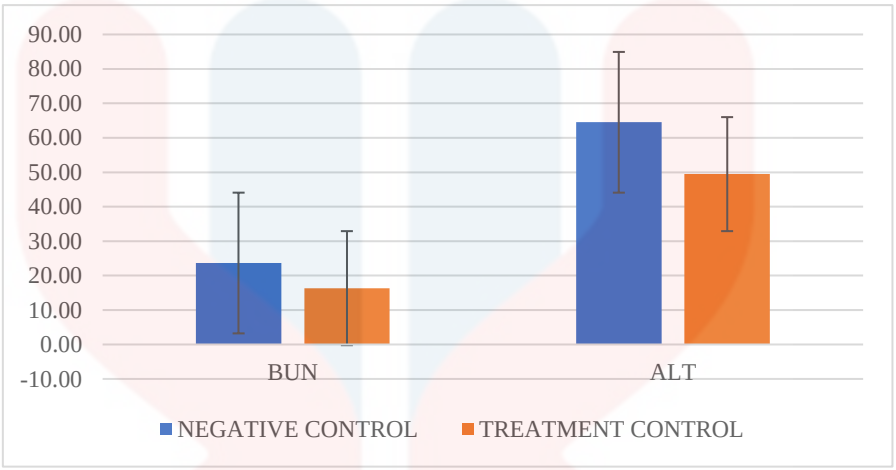

Figure 5: Blood urea nitrogen (BUN) and alanine transaminase (ALT) parameters (mean ± standard deviation) between negative and treatment control groups.

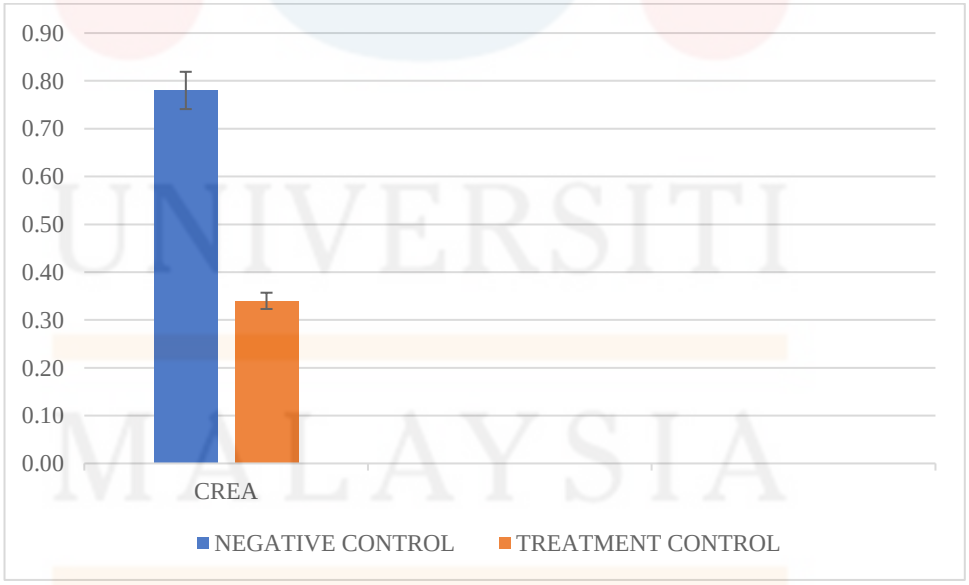

Figure 4: Creatine values (mean ± standard deviation) between negative and treatment control groups.

For the protein evaluation, rats who received sea cucumber extract administration revealed significantly ($p<0.05$) lower total protein and albumin values compared to the negative control group (Table 5). Slightly higher globulin was observed in the treatment group revealing a positive response of the rats toward the Mannheimiosis infection through immunoglobulin production.

Table 5: Protein evaluation (mean ± standard deviation) in the rats induced with *Mannheimia hemolytica* and treated with sea cucumber extract (*Stichopus horrens*).

Parametes/Group	Control	Treatment	*p*
Total protein	7.35 ± 0.38	6.20 ± 0.08	0.01
Albumin	4.48 ± 0.26	3.08 ± 0.29	0.01
Globulin	2.86 ± 0.64	3.13 ± 0.22	0.45

p: Significant value, p<0.05

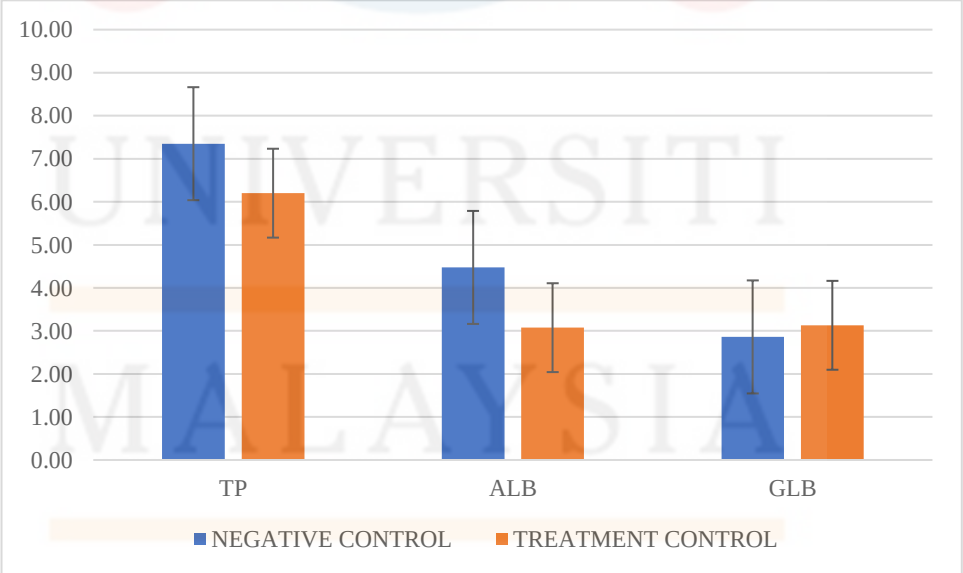

Figure 5: Protein evaluation parameters (mean ± standard deviation) between negative and treatment control groups.

9.0 Discussion

In tropical countries, mannheimiosis, also referred as pneumonic pasteurellosis, is still the most common challenge to domestic animal production. Pneumonia is a respiratory ailment that often generally affect animals (Oremeyi *et al.,* 2013; Faez Firdaus Abdullah *et al.,* 2015). Climate stress, inadequate nutrition, poor husbandry, and transportation stress are all predisposing factors for immunosuppression, which contributes to the development of pneumonic pasteurellosis, which causes major economic losses in sheep and goats (Faez Firdaus Abdullah *et al.,* 2015). Sea cucumbers, interestingly, have a variety of nutrients with significant economic value. Sea cucumber extract has been shown in clinical trials to be antibacterial (Ridzwan *et al.,* 1995; Haug *et al.,* 2002), antifungal (Shimada, 1969; Shaharuddin, 2006), antitumor (Tong *et al.,* 2005; Lu *et al.,* 2009), antianaphylactic (Kim *et al.,* 2001), anti-inflammatory (Whitehouse *et al.,* 1994; Jas (Hawa *et al.,* 1999; Althunibat *et al.,* 2009). This study is to investigate the therapeutic and prophylactic effects of Sea cucumber extract (Stichopus horrens) in rats infected with *Mannheimia haemolytica*.

The health status of animals can be observed through the clinical signs shown. Animals infected with *M. hemolytica* show respiratory and behavioural changes such as dullness and depression, dyspnea and coughing (Rice *et al.,* 2007). In this study, daily monitoring of the rats inoculated with *M. hemolytica* revealed severe dull, depressed and sneezing in the control group compared to the group of rats treated with *S. horrens*. This finding suggested the therapeutic effect of the extract in reducing the severity of infection. In addition, there were no mortalities observed in rats in both treatment and control groups.

Haematological and serum biochemical parameters play an important role in monitoring the animal body's response to the infection (Fulton & Confer, 2012). The presence of the bacteria within the body revealed an increased leucocytes and neutrophils count and a decreased level of lymphocyte, which demonstrate an inflammatory leukogram (Odugbo *et al*., 2004). The present study demonstrated no significant differences (p>0.05) for the haematological parameters in both treatment and control groups. However, the total white blood and leucocyte counts were lower in the treatment group than in the control group on day 28th of the study. Following bacteria inoculation, these findings confirmed the extract has anti-inflammatory and anti-bacterial properties. Even though no study showed the effectiveness of *S. horrens* against *M. hemolytica*, other studies revealed the potential of sea cucumber extracts against other bacteria (Geistia *et al*., 2017).

A serum biochemistry test is crucial to determine the severity of infection through the degree of organ damage. Higher expression of serum organ parameters is associated with persistent organ injury due to infectious agents (Reduan *et al.*, 2020). Among the parameters, hepatic and renal systems are commonly evaluated in an animal study (Farah *et al*., 2011). The present study revealed significantly (p<0.05) lower expression of blood urea nitrogen (BUN), creatinine and alanine transaminase (ALT) in rats treated with sea cucumber than in the control group. These findings suggested lesser renal and hepatic injuries in the group of rats that received the extract administration. For the protein analysis, rats given sea cucumber extract revealed significantly (p<0.05) lower total protein and albumin

levels than the control group. Slightly higher globulin was observed in the treatment group demonstrating a positive response of the rats toward the mannheimiosis infection through immunoglobulin production. The results are evidence that recommends the potential of sea cucumber extract (*Stichopus horrens*) as a therapeutic agent by reducing the severity of organ injury in post bacterial infection.

10.0 Conclusion

In conclusion, changes in haematological and serum biochemical findings suggest the potential of sea cucumber (*Stichopus horrens*) extract as a therapeutic and prophylactic agent against *M. hemolytica* infection by reducing inflammation and organ injury.

11.0 Recommendations and future work

It is recommended to use other species of animals such as rabbits for future experimentation because rats are difficult to restrain and they have smaller diameter of the oral cavity making it difficult to give the extract. Besides, the study can be improved by increasing the number of animals and groups of treatment because more number of samples can give better results. Further study using ruminant models such as goat and sheep shall be conducted to observe the potential of sea cucumber extract as a therapeutic and prophylactic agent against mannheimisois because mannheimiosis in ruminants is the main challenge in the tropical countries therefore using goats and sheep model needs to be conducted. Further analysis can be done, such as histopathology, acute phase proteins and immunohistochemistry analyses to investigate the effect of the sea cucumber extract administration at the tissue and molecular levels because with more diagnostic analysis, the study can be more effective.

Appendix A

Appendix A 3 Preparation of Mannheimia hemolytica inoculum.

Appendix A 4 Rats post inoculated with Mannheimia hemolytica.

Fakulti Perubatan Veterinar

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RUJ. KAMI *(Our Ref.)* : UMK/FPV/ACUE/FYP/012/2022
TARIKH *(Date)* : ٢٥ FEBRUARY 2022

DR. MOHD FARHAN HANIF BIN REDUAN
Main Supervisor
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan

Dear Dr,

APPROVAL OF INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) TO CONDUCT RESEARCH INVOLVING ANIMALS

We are pleased to inform you that your application for approval to conduct research from Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Malaysia Kelantan has been approved. Please refer the table below for approval code:

APPROVAL CODE	*UMK/FPV/ACUE/FYP/012/2022*
TITLE	*HAEMATOLOGICAL AND SERUM BIOCHEMICAL CHANGES IN THE RAT MODEL INDUCED WITH MANNHEIMIA HAEMOLYTICA AND TREATED WITH SEA CUCUMBER (Stichopus horrens) EXTRACT.*

2. Please be noted for the Final Year Project, you are responsible to supervise your student to conduct all animal-related procedures as stated during ethic application. The co-supervisor(s) for the project are encouraged to help with the procedures as well.

3. You are advised to always follow "3R" (REDUCE, REFINE, & REPLACE) and all animal ethics and animal welfare principles to reduce suffering in animal.

Thank you.

"RAJA BERDAULAT, RAKYAT MUAFAKAT, NEGERI BERKAT"
"WAWASAN KEMAKMURAN BERSAMA 2030"
"BERKHIDMAT UNTUK NEGARA"

Yours sincerely,

DR. NOR FADHILAH BINTI KAMARUZZAMAN
Chairman
Institutional Animal Care and Use Committee
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan

UNIVERSITI MALAYSIA KELANTAN اونيۏرسيتي مليسيا كلنتن | Karung Berkunci *(Locked Bag)* 36, Pengkalan Chepa, 16100 Kota Bharu, KELANTAN, MALAYSIA | Tel :609 771 7277 Fax :609 771 7282

Appendix A 5 Approval form from Final Year Research Committee and Animal Ethics Committee 2022

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