**RESEARCH ARTICLE**

# Effect of consumption of *Perna Viridis* on hematological parameters in rats.

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**Abstract:**

*Perna viridis* has been used as a traditional remedy for a variety of diseases for centuries. It also contains nutrients such as calcium, potassium, zinc, iron, phosphorus and copper that may help to increase hematological parameters a good source of minerals Thus, *Perna viridis* has the potential to be used to boost blood parameters. This study aimed to quantify the amount of protein and to determine the effect of *Perna viridis* on hematological parameters in rats. The sample was processed through the freeze-drying process and the amount of protein was determined using the Bradford assay. Eighteen rats were randomly divided into 3 groups: control (normal diet), low dose (7.0 mg/ml protein / kg) and high dose (52.0 mg/ml protein / kg. Treatment groups were given each dose orally for three weeks. The control group was given distilled water. All groups were kept under similar conditions. Each rat's blood was taken via cardiac puncture and analyzed using the CELL- DYN Abbott Emerald analyser for a variety of hematological markers. The results revealed that the protein content of the extract was 5.5 mg/ml. Hemoglobin concentration showed an increase in the mean for the high dose group and low dose group compared to the control group. As for platelet count, there is a significant increase in the low dose group ($p<0.05$) compared to the high dose group. It has been proven that *Perna viridis* has the ability to increase the platelet count at low doses. It would be a promising source of protein and could be recommended as an effective alternative way to increase platelet transfusion in more natural ways.

**Keywords:** hematological parameter, hemoglobin, *Perna viridis*. platelet count, protein.

## 1. INTRODUCTION

Seafood has been a major source of many essential nutrients for humans. It's not only good in taste but also rich in nutritional value. Protein, lipids, and essential vitamins and minerals are all available to consumer of seafood (Sampels, S. 2014). In marine animals, the largest and most divert groups come from the phylum mullusca. Within mollusca, bivalvia make up the largest class of mollusca, which is made up of around 20,000 species, including invertebrates like clams and mussels (Chapman, A. D. 2009). For mussels, *Mulitus* and *Perna* are the most abundant mussel species. Musselas have been used traditionally. In China, for example, the sauce made from a decoction of *Mylitus edulis* is thought to boost the immune system, improve kidney and liver function, and treat impotence and menoxenia. There are many benefits of mussel and also other shellfish due to their content. Mussels, for example, have a high protein content due to their muscle tissue (Grienke et al., 2014). Bivalve molluscs also contain essential vitamin, essential amino acid, enzyme, peptide and polysaccharides (Karnjanapratum et al., 2013). Recent study shown that Collagen domain, and low complexity regions with abundant certain amino acids, were also identified from *P. viridis* shell. These proteins can be broadly categorized into six groups, and the shell matrix proteins (SMP) -containing domains, such as immunomodulatory, might imply their role in different biological functions, not only in biomineralization (Zhi Liao et al., 2019). There is research that states that shellfish are rich in vitamins A, D, and E (Rittenschober et al., 2013). Not only that, shellfish also contain niacin (vitamin $B_3$), vitamin $B_6$ and vitamin $B_{12}$, which are important for many metabolic processes (Michal, 2014). To produce healthy red blood cell (RBC), our body also needs vitamin $B_{12}$. It is important for RBC production. Vitamin $B_{12}$ deficiency can be associated with megaloblastic anaemia (Vogiatzoglou et al., 2009). Our blood components include platelets, RBC and white blood cells (WBC). In RBC, the functional group is hemoglobin. The structure of hemoglobin consists of a poly-peptide chain and haem that carries iron protoporphyrin (Marengo-rowe A. J., 2006). A lack of iron can cause iron deficiency anemia. To overcome it, iron must be taken in the daily diet, which can provide the

most immediate source of iron (Miller, J. L. 2013). Iron deficiency anemia can be caused by a lack of iron, megaloblastic anemia by a lack of vitamin B12, and folic acid can cause a decrease in hemoglobin and red blood cell concentration (Loh & Khor, 2010). The risk of anemia can be made worse due to the risk of transfusion of red blood cells, which is a chosen treatment for severe anemia (Michael & McEvoy, 2013). Not only does anemia need a blood transfusion, for many years, dengue has been a threat to many lives. It's also causing the deaths of millions of people (Mohd-Zaki et al., 2014). The risk of dengue can be worse due to a sudden drop in platelets (Gamakaranage et al., 2012). In this condition, transfusion of blood components such as platelets is needed (Khan Assir et al., 2013). However, frequent transfusions can result in a variety of serious complications, including Tranfusion Transmitted Diesases (TTD) and transfusion reactions (Michael & McEvoy, 2013). To avoid frequent blood transfusions, alternative ways are needed to overcome the depletion of blood or blood components in people. Shellfish can provide the nutrition needed for healthy blood production. The urge to find a safe alternative treatment has opened up the chances of identifying potential natural sources such as seafood, which can boost hematological parameters naturally. This can help avoid all the potential complications and solve the problem in the safest way. Mussels contain high level of protein due to its large part of muscle. However, very low research publication reporting significant bioactivity exerted high molecular weight protein. Thus, the aim of this experimental study is to determine the quantity of protein in *Pernia viridis* extract and the effect of *Perna viridis* on hematological parameters in rats by administering a *Perna viridis* prepared solution to the rats through force feeding.

This study has the potential to explore alternative ways or treatments to supply the nutrition that is needed for healthy blood production and could reveal a new alternative therapeutic treatment in treating diseases such as anemia and dengue fever. Red cell indices were used to determine the etiology of anemias. The parameter being studied was red blood cell count (RBC), indices include mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) reticulocyte ratio, platelet count, and white blood cell count (WBC) and leukocyte differentiation.

## 2. MATERIALS AND METHODS

### 2.1. Sample Processing

Fresh *Perna viridis* (Figure 1) was obtained from a local market in Selayang, Malaysia, washed, and cooked for 10 minutes over moist heat. *The* soft tissue of *Perna viridis* was collected and the shells were discarded. The soft tissues (100g) were ground with 100mL of distilled water. The ground soft tissues were filtered through cotton gauze to obtain the aqueous extract (Archibong et al., 2014). 60mL of aqueous extract was filled into a 250mL Schott bottle and kept at-80$^{o}$C for freeze drying for one week (Nguyen et al., 2012). The dried sample was in powder form and was grounded to homogenize the powder and stored in a clean falcon tube. The powder sample was kept in a 4$^{o}$C chiller until further use.

Figure 1**:** *Perna viridis*. Known as green lipped mussel due to their green color shell.

### 2.2 Determination of protein

The Bradford assay was used to determine protein in the sample (Bradford, 1976). In this assay, a protein standard curve was prepared using pre-diluted bovine serum albumin (2 µg/ml) and the concentrations used were 250 µg/ml, 500 µg/ml, 750 µg/mL, 1000 µg/mL and 1500 µg/ml. The absorbance readings were taken 3 times with a 3 second time interval at 595 nm after incubation at room temperature for 5 minutes. The mean was obtained from the absorbance and a protein standard curve was plotted using Microsoft Office Excel 2007. As for the sample, 10 mg/ml stock of *Perna viridis extract* was prepared by diluting the powder with distilled water. The standard curve was used to quantify the protein content in the sample.

 

### 2.3 Experimental design

The usage of laboratory animals for this study and study design has been approved by the UiTM Committee on Animal Research and Ethic (UiTM CARE: 73/2015) was approved the use of animals in this study. The handling of rats was carried out according to the guidelines set up by Laboratory Animal Facility and Management (LAFAM) UiTM. In this study, eighteen (18) male Wistar rats with an average body weight of 180-240 g were used as the haematological model. The animals were bought from Laboratory Animal Facility and Material (LAFAM), Faculty of Pharmacies, UiTM Puncak Alam, and were housed in an animal holding area on Level 6 of the Faculty of Health Sciences. The rats were housed in regular settings, three to a cage, and acclimated to laboratory conditions two weeks prior to the experiment. Food and water were available ad libitum. Each of the eighteen (18) rats was divided into three groups, with each group consisting of six rats (n=6). Group 1 (the control group) was given distilled water. Group 2 received a low dose of *Perna viridis* extract (7.0 mg protein/kg), while Group 3 received a high dose of of *Perna viridis* extract (52 mg protein/kg). This procedure was repeated daily via oral administration and the feeding schedules were for three weeks.

### 2.4 Hematological Parameters Analysis

After 3 weeks of treatment, the rats were anaesthetised using 1 ml mixture of ketamine and xylazine intramuscularly. The amount of drug injected depends on the rat's body weight, which only 0.2 ml per 200 g or 0.3 per 300 g (Table I). The blood sample was obtained through a cardiac puncture and collected in a K2 EDTA tube and immediately placed in an ice box. Then, the tube was sent to Unit Kesihatan UiTM on the same day for full blood analysis using the CELL- DYN Abbott Emerald analyser. The parameter being studied was red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte ratio, platelet count, white blood cell count (WBC) and leukocyte differentiation.

Table 1: The preparation of Katamil Xylazin mixture. A stock of drug was prepared using 0.75ml of Katamil added with 0.1mL Xylazin in Falzon tube.

| Chemical | Volume needed(mL) |
|---|---|
| Katamil | 0.75 |
| Xylazin | 0.71 |

### 2.5 Statistical Analysis

Results were expressed as mean ± SEM where n=6. SPSS 18.0 for Windows was used to conduct the analysis (SPSS Inc., Chicago, IL, USA). Before proceeding with the Tukey test, the data will be evaluated using one-way analysis of variance (ANOVA). The data was presented as a mean (x), standard deviation (SD), and standard error of the mean (SEM). The difference between the control group, the low dose group, and the high dose group was significant at a p-value of 0.05.

## 3. RESULT AND DISCUSSION

### 3.1 Analysis of protein content in freeze dried *Perna viridis*

The pre-diluted bovine serum albumin (BSA) standards were tested using the Bradford reagent and the absorbance readings were taken at 595 nm. From the absorbance readings, a standard curve was plotted (Figure 2).

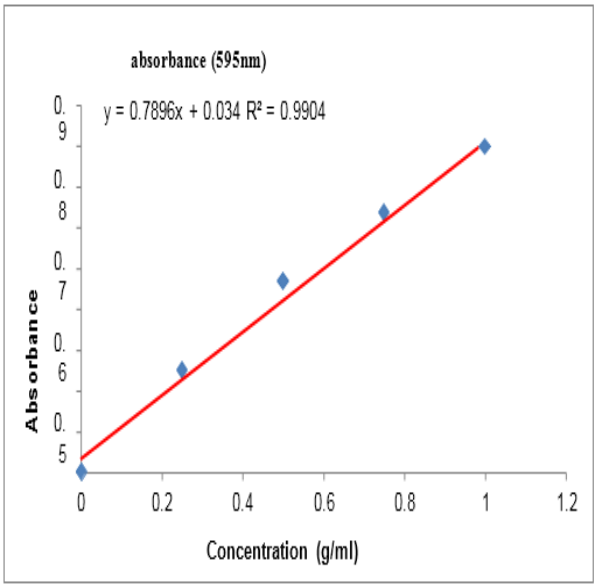


Figure 2: *Perna viridis*. Graph of absorbance at 595nm against BSA protein concentration

 

### 3.2 Hematology Parameters Analysis

**Red blood cells indices**

The RBC indices in the low dose group (10.1±2.45) and high dose groups (11.3±3.35) were both significantly increased ($p<0.05$).There was no significant difference in red blood cell (RBC) count between the control (6.02 x $10^6$/µL) and low dosage groups (6.04 x $10^6$/µL), whereas the high dose group (5.90 x $10^6$/µL) had a lower mean than the control and low dose groups but not significance (Table 2). Hemoglobin (HGB) concentration showed an increase in mean for the high dose group (13.8 g/dL) and low dose group (13.7 g/dL) compared to the control group (13.5 g/dL) but not significant. There was no significant difference in the mean hematocrit percentage, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and cell distribution width (RDW) between the low dose, high dose, and control groups.

Table 2: Comparison of red blood cell indices in different experimental groups, control (n=6), low dose (n=6) and high dose (n=6) groups as mean, standard deviation and P-value.

| | RBC ($10^6$/µL) | HGB g/dL | HCT(%) | MCV (fL) | MCH(pg) | MCHC g/dL | RDW(%) |
|---|---|---|---|---|---|---|---|
| Control | 6.02±0.4 | 13.5±0.8 | 38.7±1.9 | 63.4±2.82 | 22.5±1.06 | 35.5±0.36 | 14.9±0.48 |
| Low dose | 6.04±0.34 | 13.7±0.61 | 38.2±1.68 | 63.4±2.03 | 22.6±0.61 | 35.7±0.23 | 15.6±0.75 |
| High dose | 5.9±0.3 | 13.8±0.43 | 38.9±1.14 | 65.9±3.07 | 23.4±0.56 | 35.5±0.56 | 14.7±1.19 |

Values are expressed as mean ± SEM, n = 6
RBC = red blood cell; Hb = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width.

**Platelet indices**

The platelet and platelet indices of the various experimental groups are shown in Table 3. When compared to platelets count in the control group (838.8 x $10^3$/µL), platelets count in the low dosage group (947.3 x $10^3$/µL) was substantially higher ($p<0.05$). There is a significant decrease ($p<0.05$) in platelets count between the high dose (724.3 x $10^3$/µL) and the low dose groups.

Table 3: Comparison of platelet indices in different experimental groups, control (n=6), low dose (n=6), and high dose (n=6) groups as mean, standard deviation and P-value.

| | PLT($10^3$/µL) | MPV(fL) |
|---|---|---|
| Control | 838.8±96.1 | 6.4±0.19 |
| Low dose | *947.3±157.9 | 6.6±0.54 |
| High dose | *724.3±94.3 | 6.7±0.46 |

p<0.05 values are expressed as mean ± SEM, n = 6
*significant between low dose group and high dose group
MPV = mean platelet volume

**White blood cell indices**

The results of white blood cell parameters assessed using the CELL- DYN Abbott Emerald analyser for the control, low dosage, and high dose groups are shown in Table 4. There are no statistically significant changes in total WBC count between the control, low-dose, and high-dose groups. Between the control group (7.2 x $10^3$/µL), the high dose (9.6 x $10^3$/µL), and the low dose (7.9 x $10^3$/µL), there are increases in lymphocyte count, although not considerably.

Table 4: The result of white blood cells (WBC) for control, low dose and high dose group after 3 weeks of treatment

| | WBC ($10^3$/µL) | LYM ($10^3$/µL) | MID ($10^3$/µL) | GRA ($10^3$/µL) | *LYM(%) | GRA(%) | *MID(%) |
|---|---|---|---|---|---|---|---|
| Control | 9.6±2.9 | 7.2±2.37 | 1.2±2.2 | 1.1±0.66 | *75.7±7.37 | 11.4±6.27 | *12.9±2.2 |
| Low dose | 10.1±2.45 | 7.9±1.71 | 1.1±0.92 | 1.1±0.49 | 79.1±3.77 | 10.1±4.53 | 10.8±0.92 |
| High dose | 11.3±3.35 | 9.6±3.08 | 1.0±1.44 | 0.6±0.33 | *84±3.6 | 5.7±3.6 | *10.3±1.44 |

*p<0.05
between high dose and control group

As for lymphocyte percentage (LYM %), the higher dose extract treatment groups were significantly ($P<0.05$) higher compared with the control group.

The results from this study showed that *Pernia viridis* had a high protein content of 0.55mg/ml in 10 mg/ml. Mussles are high in protein since they contain a lot of muscle thus making it rich in protein (Zhi Liao et al., 2019 & Bradford,1976). Since protein accounts for the major part of mussel meat, it is not surprising that proteins, peptides and

 

amino acids make up the most significant group of bioactive found among mussels and as a good source of protein which is used in many vital processes in the human body (Scotti et al., 2001; Soumady & Asokan, 2011 & Mohammad Zafar et al., 2004). Therefore, the findings suggest that *Perna viridis* is one of the most promising and important sources of protein for the human body, with the potential to improve haematological parameters. The mean RBC concentration did not change statistically; however, it did increase slightly from control to low dose, but declined in the high dose group. The result is vice versa in Archibong et al., whereby the results demonstrate a rise in RBC, HGB, and MCH (Archibong et al., 2014). However, contrary to earlier research, this study was unable to show that the extract increased RBC, HGB, and MCH values. The length of the research varies. The rats were treated for 6 weeks by Archibong et al., but in our study, the therapy was just 3 weeks due to time constraints, therefore the blast cells may presence but not have matured enough to develop normal red cells. This is most likely the cause of the discrepancies in the results. Between the high dose (724.3 x $10^3$/µL) and low-dose groups, there was a significant decreased in platelet count ($p<0.05$). Platelet counts was increased in the low-dose group. This demonstrates that *Perna viridis* has the ability to raise platelets and that the low dose is a suitable dose for it.

However, there is no significant difference in mean platelet volume (MPV) between control groups (6.4 fL) and low-dose (6.6 fL) and high-dose (6.6 fL) groups (6.7 fL). The same result was obtained in a study done by Archibong et al. using *Egeria radiata* (clam). The research stated that the clam extract contains a thrombopoietin-like agent (humoral regulator of platelet production), or other compound that is able to induce the release of thrombopoietin (Archibong et al., 2014). The thrombopoietin-like agent may also be available in *Perna viridis.*

In total WBC count, there was an increased in lymphocyte count, but not significant. Increase in lymphocyte in both treatment groups caused the total WBC count to increase. This study also shown that *Perna viridis* cause decreased in monocytes, eosinophil, basophils and also granulocyte. The finding was supported by a previous study done using *Egeria radiata* (clam) on rats, which shown decrease in eosinophil and monocyte in the treatment groups (Archibong et al., 2014). In the research done using *Egeria radiata* by Archibong et al to see the effect of the seafood on hematological parameter in rats, Archibong et al proven that *Egeria radiata* has the ability to increase lymphocyte count than is expected to result in increased of total WBC. Since invertebrates rely only on innate immunity, including antimicrobial peptides, to battle infectious pathogens, immune effectors from these animals are thought to be effective and quick inhibitors of microbial development. (Sperstad et al., 2011). Mussels contain antimicrobial peptides (AMPs) that have now been tested to become a drug candidate for human against infectious disease. AMPs exhibit antibacterial, antifungal and antiviral properties, (Charlet et al.,1996 and Mitta et al, 2000). As a result, the total WBC count increased in both treatment groups and therefore likely that consumption of *Perna viridis* may boost the immune system.

## 4. CONCLUSION

In the conclusion, *Perna viridis* has a high protein content and has the ability to enhance platelet count at low doses as well as to increase the lymphocyte count.

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