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Evaluation of *Scylla serrata* Protein Extract on Hematological Parameters in Male Wistar Rats

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Abstract: This study aimed to determine the potential of the Mud crab (*Scylla serrata*) as a source to treat blood disorders. Eighteen male Wistar rats were randomly divided into three groups (2 treatment groups and 1 control group) of six rats (n = 6). All groups were kept under similar condition. Mud crab stock solutions were prepared in 7.0 mg mL^{-1} protein/kg and 52 mg mL^{-1} protein/kg doses and orally administered to treatment groups for three weeks. Distilled water was used for the control group. Blood samples were obtained from each rat via cardiac puncture for further analysis of various haematological parameters. The result revealed that the average protein content of the extract was 5.2±0.06 mg mL^{-1} protein/kg. RBC, Hb, HCT and MCH level increased significantly in the treatment groups compared to control group. In conclusion, mud crab contains vital nutrients that boost production of red blood cell indices, hence could serve as an essential food supplement to treat anemia.

Key words: Mud crab (*Scylla serrata*), haematological parameters, analysis, essential food, anemia

INTRODUCTION

Mud crab or scientifically address as *Scylla serrata* is marine, estuarine animals inhabit deep in the mud of salty water along the shoreline covered in mangroves. In Malaysia, mud crabs are found in mangrove areas mainly in Kedah, Perak and Kelantan. Seafood as a natural product, particularly clam and mussel can be an alternative treatment to treat certain blood disorders (Archibong *et al*., 2014a, b). Mud crab is highly commercialized to the tourist market largely due to its size and meat quality (Naylor *et al*., 2002). The mud crab is also an important source of food for coastal inhabitants in the Indo-Pacific region where it is widely distributed (Fratini *et al*., 2010). Mud crab is one of the best sources of protein to marine life and mankind .Previous studies had reported that mud crab contains more protein compared to mutton, chicken, duck and fish (Friedman, 1996; Thorarinsdottir *et al*., 2004). A study conducted by Chiou and Huang (2003) reveals that the levels of crude protein in male and female mud crab were 15.0±20.3% and 15.7±21.4%, respectively. Another study conducted by Zafar *et al*. (2004) showed that mud crab contains 78.50% protein which is higher compared to mussel (63.75%) and oyster (40.14%) . Most of the food that is consumed by human body were digested and absorbed into the blood stream. These foods may give impact to the blood cells in different ways. Numerous studies had been conducted in order to investigate the relation between edible seafood consumption and haematological parameters (Millward *et al*., 2008). The studies reveal that two different seafood species which are clams (*Egeria radiata*) and rock snail (*Thais coronata*) were able to boost blood parameters in rats (Archibong *et al*., 2014a, b). Intention to seek for an alternative treatment for anemia or dengue called for the investigation on, *Scylla serrata* or known as mud crab to be study for the effect on hematological parameter *in vivo*. Protein in seafood is more digestible compared to poultry and meat due to its low connective tissue content (Oehlenschlager, 2012). Transfusion treatment had been applied in the hospital settings especially for anemia or dengue cases. However, the demand of the blood components ever present and growing and sometimes cannot be fulfill by the Hospitals. Moreover, the transfusion therapy also may increase the risk of Transfusion Transmitted Diseases (TTD) among patients. Thus, the search for safe treatment particularly natural product therapy is required as a choice to boost haematological parameters. Currently, much attention has been devoted to edible seafood which believed can increase the haematological parameters as stated earlier.

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Therefore, the effect of consumption of Malaysian edible seafood on the haematological parameters needs to be discovered. The emergence of the alternative medicines is very helpful for treatment of disease, especially for a disease that treatment has not been widely found yet (Kadir *et al*., 2013). Upon this, a scientific proof of the alternative treatment will provide an ethical recommendation on its usage for blood disorder treatment (Dharmarathna *et al*., 2013). This study will demonstrate the new potential source of seafood that can possibly serve as an alternative therapeutic treatment to overcome the issue of deficiency of blood component such as dengue fever case or anemia. This study is done to seek a deeper knowledge about the actual quantity of protein presence in *Scylla serrata* and its impact on haematological parameters in rats.

MATERIALS AND METHODS

Sample preparation: Freshly obtained *Scylla serrata* were washed with tap water and distilled water to remove all debris and materials accumulated to the crab. The crabs then were cooked with moist heat to kill all the bacteria, preventing bacterial contamination to the extraction. Aqueous extraction were prepared by homogenize the meat crab sample with distilled water using the electric grinder and then filtered by using cotton gauze. This procedure was done according to Archibong *et al*. (2014a, b) with slight modification. The sample is dry freeze using Christ ALPHA 2-4 freeze dryer at the Faculty of Applied Sciences of UiTM Shah Alam. The final product is in powder form. By using distilled water as solvent, acquired stock concentration were prepared. (Camargo *et al*., 2011; Nguyen *et al*., 2012).

Protein estimation: The protein content in the mud crab was estimated by using the Bradford assay. In this assay, a commercial set of Pre-diluted Protein Assay Standard Bovine Serum Albumin (BSA) which contain six different concentrations were used as a standard for protein samples.

Experimental design: About 18 male rats of Wistar strain with average body weight 180-240 g were used this study as the model for hematological investigation (n = 6). The animals were purchased from Laboratory Animal Facility and Material (LAFAM), Faculty of Pharmacies, UiTM Puncak Alam and were kept in an animal holding room at Level 6, Faculty of Health Sciences, UiTM Puncak Alam. The rats were caged in standard condition where three per cage and were acclimatized to the laboratory conditions 2 weeks before the experiment. Food and water were available ad libitum. An experiment was conducted according to the guideline approved by the Ethics Committee of Faculty of Pharmacies (UiTM CARE), UiTM Puncak Alam. All (Archibong *et al*., 2014a, b) rats were assigned into three different groups where each group consists six rats (n = 6). Group 1 (control group) was given distilled water via oral administration. Group 2 received low dose (7.0 mg protein/kg) mud crab extract while Group 3 received high dose (52 mg protein/kg) of the mud crab extract also via oral administration. The feeding regimens lasted for 3 week.

Analysis of hematological parameter: Blood samples were collected from the rats after 6 week of treatment. All rats were first anesthetized with 1 m^2 mixture of ketamine and xylazine intramuscularly. Blood samples were drawn through cardiac puncture by using a 5 mL syringe into EDTA capped tube. The tubes were inverted several times to mix the blood with EDTA to avoid any blood clotting to occur. Full Blood Count (FBC) analysis was done using Abbott CELL-DYN Emerald automated hematological parameters at Unit Kesihatan of UiTM Puncak Alam.

Statistical analysis: Statistical Package for Social Science (SPSS) Version 18.0 Software and One-way analysis of variance (ANOVA) and then followed by a post-hoc test to determine significant differences between the 2 groups. ($p<0.05$) were accepted as significant.

RESULTS AND DISCUSSION

Analysis of protein concentration in mud crab extraction by using bovine serum albumin (bsa) protein standard: As shown from Table 1, the protein content in the sample was 0.52 in a solution of 1 mg mL^{-1}. Thus, the final concentration of the extract after multiplying by dilution factor is 5.2 mg mL^{-1}. Mud crab is increasingly realized as a good source of protein which can be utilized for the benefit of human body. The present study was carried out

Table 1: Concentration of protein (mg mL^{-1}) in the mud crab meat sample basedon absorbance reading at 595 nm

Concentration of sample solution (mg mL^{-1})	Absorbance 595 nm	Concentration of protein (mg mL^{-1}) Mean ±2 SD	Concentration of protein (mg mL^{-1}) 10 (dilution factor Mean ±2 SD
10	0.448	0.52±0.006	5.2±0.06

n = Triplicate result is expressed as mean±standard deviation of triplicate measurement

Table 2: Comparison on hematological parameters in different experimental groups, control(n = 6), low dose (n = 6) and high dose (n = 6) groups as mean, standard deviation and p value

Parameters	Unit	Control	Low dose extract	High dose extract	p-value
RBC	$\times 10^6$/UL	6.03±0.40	5.71±0.30	6.25±0.18	**0.023
Hb	gdL^{-1}	13.53±0.80	13.80±0.21	14.61±0.75	***0.026
HCT	%	38.17±1.90	38.80±0.50	41.07±2.24	***0.026
MCV	fl	63.4±2.82	68.07±3.67	65.73±2.95	0.67
MCH	pg	22.48±1.06	24.17±1.25	23.40±0.98	*0.05
MCHC	$g\ dL^{-1}$	35.45±0.37	35.50±0.32	35.60±0.32	0.788
RDW-SD	fl	14.98±0.48	15.17±0.45	15.05±1.10	0.910
	$\times 10^3$/UL	838.83±9	964.50±1	853.61±	0.155
MPV	%	6.35±0.19	6.25±0.21	6.42±0.3	0.537
WBC	$\times 10^3$/UL	9.63±2.90	11.98±1.74	10.03±1.8	0.177
LYM	$\times 10^3$/UL	7.27±2.38	9.92±1.38	8.42±1.5	0.067
MID	$\times 10^3$/UL	1.22±0.42	1.38±0.24	0.97±0.2	0.095
GRA	$\times 10^3$/UL	1.12±0.66	0.68±0.40	0.65±0.2	0.198
LYM%	%	75.68±7.38	82.86±2.02	83.95±1.9	***0.013
MID%	%	12.90±2.20	11.83±2.49	9.58±0.7	**0.030
GRA%	%	11.42±6.27	5.30±2.45	6.47±2.4	0.049

Values are expressed as mean ± SEM, n = 6;* significant increase between control and low dose extract group pair ($p<0.05$); **significant increase between control and high dose extract group pair ($p<0.05$); *** significant increase between control, low dose and high dose extract group pairs ($p<0.05$)

to investigate the level of protein content in mud crab extraction and its consumption effect towards the hematological parameters in rats. The sample extraction had been freeze dried before its protein content were estimated using the Bradford assay (Crossman *et al*., 2000; Bradford, 1976). In the present study, the result obtained showed the protein content of mud crab is 5.2 mg protein/ m^2 which is higher compared to Egeria radiata and Thais coronata (Archibong *et al*., 2014a, b). The values of protein in the present study are in agreement in with other studies (Sudhakar *et al*., 2009). The protein content in the meat of the mud crab can be influenced by numbers of factors including extract preparation method. It has been reported that the freeze dry method are better in preserving protein particularly in seafood.

This might be a possible explanation of the high protein content in the mud crab (Bilgin and Zeliha, 2011; Deng *et al*., 2014). Therefore, the obtained results indicated that mud crab is one of the promising sources of protein for human body. The extract-treated animals showed an increased in the RBC, Haemoglobin (Hb) and Haematocrit (HCT) level. These findings are consistent with previous study that report significant increase for these parameters. The increased in Hb concentration and the RBC count is not surprising, since Hb is an integral component of the RBC. It has been reported that mud crab are excellent source of vitamin A, vitamin B12, vitamin B6, thiamine and iron (Lacombe, 2002). These substances are known as the basic requirement needed for the production of RBC. The vitamins are capable of enhancing erythropoiesis and stimulating the maturation of the erythrocytes.

Analysis of hematological parameter effect after the consumption of protein mud crab in wistar rats: 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; MPV = Mean Platelet Volume WBC = White Blood Cell; LYM = Lymphocytes; MID Minimum Inhibitory Dilution; GRA = Granulocytes; LYM% = Lymphocytes percentage; MID% = MID percentage; GRA% = Granulocytes percentage Therefore, this increase in erythrocyte count could be brought about by the presence of these accessory food substances and protein in the extracts. An increase in the Hb level had proved that the protein in the extract help in boosting the erythropoiesis process by enhancing Hb production (Krantz, 2011; Lacombe, 2002).

Conjugation of erythropoietin and protein will result in free amino acids which have the characteristics to cause mitochondria produce more Hb.Hence, the extract may contain erythropoietin-like agent cannot be disputed. Mean Corpuscular Volume (MCV) relates to the average size of a single RBC. MCV finding showed no difference between treated and untreated animal groups where it is consistent with data obtained from a study conducted by Archibong *et al*. (2014a, b) indicating that the extract leads to production of normocytic cells. In contrast to that, Mean Corpuscular Haemoglobin (MCH) was increased in extract treated rats. The increased are consistent with the increased in Hb level in treated groups. The results are in agreement with those of A. Archibong who said that the seafood extract causes high production in Hb level. Red Cell Distribution Width (RDW) is used to measure the consistency of the size of RBCs (degree of anisocytosis) in the body (Hoffbrand and Moss, 2011). For the current study, RDW value was higher in treated groups, suggesting that the extract may cause the production of RBCs that have more variable in

size. According to Kaslow, high RDW is due to deficiency in B12 or folic acid. Modification in the method of preparation of mud crab extract might contribute to the decrease of B12 level in the extract that causes high RDW value.

CONCLUSION

High protein content in *Scylla serrata* extract has showed potential to boost the RBC, Hb, HCT and MCH level in treated animal groups. Thus, it can be concluded that *Scylla serrata* extract exhibit high protein content and could be recommended as an alternative source to treat anemia. It is recommended to specifically identify the biologically active ingredients of *Scylla serrata* to discover the active ingredient that gave positive outcome for red blood cell indicators.

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