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EVALUATION OF SUB-CHRONIC TOXICITY AND HEAVY METAL TOXICITY OF *KAPPAPHYCUS ALVAREZII IN- VIVO*

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ABSTRACT: Seaweed consumption is becoming more popular in Asian population especially the red seaweed, *Kappaphycus alvarezii* (*K. alvarezii*). However, there are few reported studies on the safety of *K. alvarezii* for consumption in Malaysia. This study focuses on the *in vivo* evaluation of toxicity and heavy metals of *K. alvarezii* performed in albino rats. *K. alvarezii* were sent for heavy metal analysis while methanolic extracts of *K. alvarezii* were administered orally to albino rats (2000mg/kg) daily for a total of 28 days according to Organization for Economic Co-operation and Development (OECD) guidelines. The water level and feed consumption of both groups were observed daily. At day 28th the control and experimental group of rats were sacrificed, blood and organs such as kidney and liver were collected and sent for haematology and biochemical analysis. There was low to no detectable concentration of heavy metals such as arsenic, iron and zinc in *K. alvarezii*. In addition, the low levels of heavy metals in the *K. alvarezii* samples did not cause toxicity in rats due to the results obtained from haematological test and biochemical parameters showed no significant different between experimental and control groups of rats under statistical analysis. It is therefore concluded that *K. alvarezii* which was proven to be rich in nutrients and antioxidants is safe for human consumption.

INTRODUCTION: *Kappaphycus alvarezii* (*K. alvarezii*) are literally multicellular organism that belongings to Rhodophyta, a red algae. It has been shown that *K. alvarezii* is unique with its photosynthetic pigment, phycoerythin that characterized it under group of Rhodophyta [1]. They are marine algae that were found abundantly in Southeast Asia with favorable temperature from 21oC to 24ºC [2].

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Furthermore, *Kappaphycus* are fast growing and abundantly present in the subtidal zone of the reef and sandy-coral areas. In Philipines, *Kappaphycus* are largely cultivated in conjunction with their commercial significance in the market.

Over the past few decades, *K. alvarezii* has been significantly introduced into global markets and widely consumed by people all around the world. The importance of this species is remarkable together with the increasing of health consciousness of people towards the natural therapeutic products. *K. alvarezii* is suggested as healthy and nutraceutical food. It is well known that *K. alvarezii* are antioxidant-rich in addition with high dietary fibres, minerals and vitamins. This specie is attractive for many researchers and

could be exploited for their uses in medicines and also food industry [3]. Since ancient time, seaweed had been introduced to people as food source. On top of that, *K. alvarezii* are reported as the largest group of seaweed consumed by people from all around the world [4]. Unfortunately, systemic studies on sub-chronic toxicity of *K. alvarezii* are not available in Malaysia. Furthermore, rapid industrialization and economic development are reported in Malaysia leading to an increase in water pollution around coastal areas [5]. Moreover, seaweeds have high metal accumulation capacity as reported by Bersada *et al.* [6].

Toxicity study is necessary to investigate the potential risk of *K. alvarezii* upon ingestion. This experiment is able to provide information about the possible hazards to human health after prolonged exposure to this species of seaweed. In the present study, we employed Sparague Dawley rats as experimental model to test for the sub chronic toxicity and heavy metal toxicity of the *K. alvarezii*. Haematological and biochemical parameters were investigated with respect to the significance of *K. alvarezii* toxicity.

MATERIAL AND METHOD:

Assessment of heavy metal in *K. alvarezii*:

K. alvarezii*:*

K. *alvarezii* were drained and soaked in water to remove excess salts. The clean seaweed were placed in 60^{o}C oven for drying. The weights of the seaweed were measure daily until consistent weight achieved. After that, dried samples of *K. alvarezii* were sent to DXN Holding Berhad, Laboratory Department for heavy metal screening.

Preparation of seaweed extraction:

K. alvarezii was purchased and collected from the cultivation site in Sabah, Malaysia. The seaweeds were soaked and washed thoroughly with water to remove excessive salt and contaminant before the extraction began. Initially, the cleaned seaweed was dried until a constant weight achieved. It was continued by grinding the seaweed coarsely into powder form with the aid of liquid nitrogen. 25g of seaweed powder was preweighed and 250 ml of 70% methanol by MERCK KGaA (Darmstadt, Germany) were added into the conical flask. This methanol extraction was placed on a shaker at 1200 rpm at room temperature. After 2 hours, the filtrate of the methanol extraction was concentrated by rotary evaporator at 45^{o}C. The crude extract was kept in falcon tube and stored in -20^{o}C deep freezer for further use.

Animal preparation:

Male and female Sprague Dawley rats, purchased from University Kebangsaan- Malaysia (UKM), were chosen to become experimental model in this study. All the rats were acclimatized in animal holding area for a week under standard environmental condition according to OECD guidelines [7]. Each group of rats consisted of 6 male and female rats respectively. They were housed in stainless steel cage and provided with standard pellet diet and water ad libitium.

Sub-chronic toxicity study:

The rats were randomly picked and grouped as testing and control groups for each male and female category. Weight of rats was taken into account during the preparation of the extract supplement for the rats. 200mg of *K. alvarezii* extract per kg of body weights was prepared and administered orally to the experimental groups. This single dosage administration was performed daily for 28 days. Animals models were handled with care throughout this study period. Control group was fed with standard pellet and water ad libitium meanwhile treating them with identical care as the experimental one. Appearance and daily performance of the rats were tightly monitored throughout the 28 days.

Abnormalities and any other clinical signs or findings such as mortality or distress pattern were observed and recorded daily. In response to the sub-chronic toxicity study, body weight of each rat was assessed before the commencement of the extract, daily before the administration of supplement and on the day of sacrifice with the aid of weighing scale. On day 29th, rats were sacrificed and blood samples were collected using cardiac puncture technique for both experimental and control group of rats. The blood samples mainly for haematological analysis were collected in EDTA tube whereas, the blood for biochemistry tests were contained in plain tube without any anticoagulant.

Haematological parameters:
Haemoglobin, RBC count, WBC count, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were examined by UCSI Path Lab.

Biochemical parameters:
Glucose, Cholesterol, Total protein, Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Urea, Creatinine, Calcium, Phosphorus, Sodium, Potassium and Chloride were assessed by UCSI Path Lab.

Statistical analysis:
The data obtained from haematological and biochemical tests were interpreted and examined to access the sub-chronic and heavy metal toxicity studies. One-Way ANOVA statistical test was performed using SPSS Statistic Software to illuminate the significant difference of the data between groups. Data were accepted as significant different at ((P>0.05))

RESULTS:
Screening of heavy metals in dried sample of *K. alvarezii*: To determine the potential toxicity that caused by supplementation of *K. alvarezii* extract on the experimental group, heavy metal contents in the seaweed were the first attempt. There were three heavy metals which were Arsenic, Iron and Zinc detected with a level of 3.9, 14.9 and 3.0 ppm respectively. On top of that, the concentration of Cadmium, Chromium, Manganese, Lead, Mercury, Nickel and Selenium were lower than 0.1 ppm. Thus, these heavy metals were considered as non-detectable.

Effect of seaweed extract on body weight of rats:
At the beginning of this study, both male and female rats had initial weight ranging from 250-330 g and 190-230 g, respectively. It was found that the body weight of male rats was always higher than the females one among the groups. At the end of the study period, all groups of rats were found to increase in their mean weight. In conjunction with that, mean weight of supplemented groups had discovered to be 372.06 g and 241.70 g which is not significantly ((P>0.05)) similar as compared to control groups. This might be due to the presence of secondary metabolite, caretonoids in *K.alvarezii* which facilitate β-oxidation activity in fat tissues 8. Further investigation should be done for detail explanation. The mean weight of all groups was tabulated in **Table 2.**

TABLE 2: BODY WEIGHT OF RATS FOR EXPERIMENTAL AND CONTROL GROUPS ON 29TH DAY (g)

	Experimental		Control	
	Male	**Female**	**Male**	**Female**
1st week	321.10 ± 11.95	221.14 ± 8.21	382.24 ± 23.45	255.80 ± 22.18
2nd week	341.43 ± 11.51	227.51 ± 10.75	407.79 ± 25.41	261.31 ± 11.71
3rd week	355.45 ± 13.08	233.23 ± 11.96	415.31 ± 27.40	265.06 ± 12.86
4th week	372.06 ± 17.69	241.70 ± 12.89	439.80 ± 26.16	269.03 ±7.96

Values are expressed as mean ± SEM (n=6) in each group, n- number of animals, SEM- Standard error of mean

Effect of seaweed extracts on haematological parameters:
Rats were sacrificed on 29th day and haematological tests were performed by using the withdrawn blood. No significant ((P>0.05)) difference can be observed from the mean values of Haemoglobin (Hb), Red Blood Cell (RBC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) between experimental and control groups. The results for haematological parameters were presented in **Table 3**.

Effect of seaweed extract on Biochemical parameters:
Results for serum biochemical parameters were tabulated in **Table 4.** It was clearly revealed from the table that there was no significant ((P>0.05)) difference for the level of Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), Aspartate Transaminase (AST), glucose, cholesterol, total protein, urea, creatinine, calcium, phosphorus, sodium, potassium and chloride in the serum between the experimental and control groups.

TABLE 3: MEAN VALUES OF HAEMATOLOGICAL PARAMETERS FOR EXPERIMENTAL AND CONTROL GROUPS

Parameters	Male			Female			
	Testing	Control	Reference range	Testing	Control	Reference range	Treatment effect
Haemoglobin (g/dl)	14.23 ± 0.96	14.68 ± 0.96	13.7-17.6	13.58 ± 0.76	14.24 ± 0.78	13.7-16.8	NS
RBC (x 10^{12}/L)	7.88 ± 0.49	8.18 ± 0.51	7.27-9.65	7.36 ± 0.45	7.73 ± 0.51	7.07-9.03	NS
WBC(x 10^{9}/L)	13.80 ± 3.12	10.80 ± 4.22	1.96-8.25	5.94 ± 3.44	4.70 ± 2.30	1.13-7.49	NS
PCV (%)	46.00 ± 3.22	47.17 ± 3.76	39.6-52.5	44.00 ± 3.32	45.80 ± 2.17	37.9-49.9	NS
MCV (fl)	58.50 ± 1.97	57.83 ± 2.48	48.9-57.9	59.80 ± 1.79	59.40 ± 3.13	49.9-58.3	NS
MCH (pg)	18.00 ± 0.63	17.83 ± 0.75	17.1-20.4	18.60 ± 0.55	18.60 ± 0.55	17.8-20.9	NS
MCHC (g/dl)	30.88 ± 1.26	31.08 ± 0.54	32.9-37.5	30.92 ± 1.26	31.12 ± 1.42	33.2-37.9	NS

Values are expressed as mean ± SEM (n=6) in each group, n- number of animals, SEM- Standard error of mean

TABLE 4: MEAN VALUES OF BIOCHEMICAL PARAMETERS FOR EXPERIMENTAL AND CONTROL GROUPS

Parameters	Male			Female			
	Testing	Control	Reference range	Testing	Control	Reference range	Treatment effect
Glucose (mmol/L)	11.30 ±2.90	10.40 ±1.64	3.88-11.54	13.71 ± 5.02	10.77 ±3.15	4.22-9.71	NS
Cholesterol(mmol/L)	1.43 ±0.12	1.77 ±0.30	2.05-4.72	1.76 ±0.21	1.76 ±0.21	1.33-4.05	NS
Total Protein (g/L)	66.07 ±7.32	67.40 ±4.27	52-71	66.20 ± 5.09	72.88 ±2.57	55-77	NS
ALP (IU/L)	194.6 ± 49.32	190.17 ± 48.49	62-230	170.40 ± 20.61	122.00 ± 40.85	26-147	NS
ALT (IU/L)	88.00 ± 31.24	76.00 ± 14.57	18-45	74.60 ± 14.14	66.40 ± 19.50	16-48	NS
AST (IU/L)	186.0 ± 135.73	110.83 ± 24.39	74-143	188.00 ±140.11	199.00 ±203.79	65-203	NS
Urea (mmol/L)	7.33 ±0.72	5.67 ±0.95	0.68-1.36	6.70 ±0.91	5.74 ±0.38	0.73-1.50	NS
Creatinine (µmol/L)	36.00 ±4.52	35.67 ±3.27	17.68-44.2	42.40 ± 5.68	42.8 ±2.39	17.68-53.04	NS
Calcium (mmol/L)	2.62 ±0.21	2.65 ±0.09	0.53-0.63	2.66 ±0.11	2.73 ±0.18	0.53-0.62	NS
Phosphorus(mmol/L)	2.28 ±0.56	2.34 ±0.13	0.31-0.58	2.23 ±0.61	2.34 ±0.59	0.27-0.59	NS
Sodium (mmol/L)	140.1 ± 1.33	143.83 ± 1.33	142-151	139.20 ± 1.30	142.00 ± 1.41	140-150	NS
Potassium (mmol/L)	7.22 ±1.90	5.53 ±0.39	3.82-5.55	6.46 ±1.68	5.43 ±1.87	3.31-4.9	NS
Chloride (mmol/L)	101.6 ± 2.65	101.35 ± 1.07	100-106	101.08 ± 1.32	102.88 ± 1.37	100-107	NS

Values are expressed as mean ± SEM (n=6) in each group, n- number of animals, SEM- Standard error of mean

Effect of seaweed extract on organ weight of rats: Organs like kidney and liver were removed from all rats and weighed. The readings of those weights were illustrated in **Table 5** below. Similarly, there was no significant (P>0.05) difference of organ weight between experimental and control groups.

TABLE 5: MEAN WEIGHT OF ORGAN FOR BOTH EXPERIMENTAL AND CONTROL GROUPS (g)

	Testing		Control		Treatment effect
	Male	Female	Male	Female	NS
Kidney (Left)	1.42 ± 0.14	0.80 ± 0.09	1.32 ± 0.05	0.86 ± 0.14	NS
Kidney (Right)	1.38 ± 0.14	0.80 ± 0.08	1.38 ± 0.07	0.85 ± 0.10	NS
Liver	12.92 ± 0.86	8.65 ± 0.74	14.13 ± 1.43	9.07 ± 0.99	NS

Values are expressed as mean ± SEM (n=6) in each group, n- number of animals, SEM- Standard error of mean

DISCUSSION: In this study, some heavy metals like arsenic, iron and zinc were found present in the seaweed. This result indicated that *K. alvarezii* which originate from marine may cause toxicity due to heavy metal accumulation. It was believed that heavy metal that entered body system tend to bond strongly with biomolecules which eventually lead to various bio-dysfunction [9, 10]. *K. alvarezii* extract was introduced to Sprague Dawley rats for sub-chronic toxicity and heavy metal toxicity. The rats were under observation along with supplementation of *K. alvarezii* extract for 28 days. During this study period, no mortality case was observed and manifestation signs like diarrhea and abnormal stool colour were not occured. In this respect, experimental group tolerated the dosage given in this study.

Throughout the study period, the mean weight of both experimental and control groups increased. Meanwhile, there was no significant (P>0.05) increase in weight of rats which consumed seaweed extract as compared to the control. This may be due to the presence of fucoxanthin, one of the caretonoid produced by *K. alvarezii*. According to Maye [8], fucoxanthin has down-regulating potential of lipogenic enzyme activities and facilitating β-oxidation activity in fat tissues. Due to less

reference of explanation, further studies need to be explored in this matter.

In haematological perspective, no significant (P>0.05) difference was observed between experimental and control group. All values obtained from hematological analysis placed between the standard range for rats except White Blood Cell count for male rats. This might be explained since fighting scenario was observed among the male albino rats during the study period. Cell damage due to injuries might elicit immune system, hence increase the cell count of white blood cell. Thus, *K. alvarezii* did not disclose any toxicity and was proved to be non toxic as observation can be compared with the control group.

By referring to **Table 4**, some parameters such as ALT, AST, urea, calcium, phosphorus and potassium were found out of their standard range. Potassium level revealed from rats serum was explainable because all seaweeds offer high amount of potassium especially to the supplemented group in this study [11]. Moreover, the value obtained for urea was under wise consideration since the source of urea might originate from the diet [12]. Besides, calcium and phosphorus level might be interfered by anesthetic agent used during dissection because this agent tend to obstruct the airway and body electrolyte level [13, 14].

On top of that, the level of ALT and AST exceeded the normal range that they should be present in rat body. In fact, animal models are often under stress during the experiment period. These enzymes were often induced by emotional stress as reported by Olga *et al*. [15]. Nevertheless, it can be deduced that the consumption of seaweed extracts did not elicit any toxicity that might be due to heavy metal and repeated dosage since no significant difference was observed in biochemical analysis.

CONCLUSION: In short, no adverse effect reflected from the experimental group after comparison with the control group. Haematological and biochemical parameters showed no significant difference between treated and control group. Furthermore, organ weight of rats were found compatible between groups indicated that consumption of *K. alvarezii* extract did not alter normal body function. Thus, *K. alvarezii* can be presented as safe for consumption. Further studies on nutraceutical properties of *K. alvarezii* are encouraging to support more in-depth studies on its properties such as anticancer. Mechanism of *K. alvarezii* to defense against heavy metals should be studied to enhance the understanding of society regarding the safety of seaweed consumption.

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REFERENCES:

1. Saranya C. Estimation of major pigment content in seaweeds collected from Pondicherry Coast. The Experiment 2013; 9(1): 522-525.
2. Neish IC. Good agronomy practices for Kappaphycus and Eucheuma: including an overview of basic biology. Siplanet Foundation Monograph 2008; 3: 1-72.
3. Matanjun P, Mohamed S, Mustapha NM, Muhammad K. Nutrient content of tropical edible seaweeds, Eucheuma cottonii, Caulerpa lentillifera and Sargassum polycystum. Journal of Applied Phycology 2009; 21(1):75–80.
4. Senthil A, Mamatha BS, Vishwanath P. Studies on development and storage stability of instant spice adjunct mix from seaweed (Eucheuma). Journal of Food Science Technology 2011; 48(6): 712-717.
5. Ong MC, Kamaruzzaman BY. An assessment of metals (Pb and Cu) contamination in bottom sediment from South China Sea Coastal Waters, Malaysia. American Journal of Applied Sciences 2009; 6(7): 1418-1423.
6. Bersada V, Andrade JM, Schultze F, Gonzalez J. Heavy metals in edible seaweeds commercialized for human consumption. Journal of Marine System 2009; 75: 305-313.
7. OECD. Repeated dose oral toxicity test method. In: OECD Guidelines for testing of chemicals, No 407. Organization for Economic Cooperation and Development, Paris, France 2008.
8. Maye PA. Biosynthesis of fatty acids, in "Harper's Biochemistry", 25th ed, R. K. Murray and H. A. Harper, Ed. Stamford (CT): Appleton and Lange; 2000. p. 230-257.
9. Duruibe JO, Ogwuegbu MOC, Egwurugwu JN. Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences 2007; 2(5): 112-118.
10. Holum JR. Elements of general and biological chemistry. New York: John Wiley and Sons; 1983. p. 324, 326, 353, 469.
11. Chandini SK, Ganesan P, Suresh PV, Bhaskar N. Seaweeds as a source of nutrionally beneficial compounds.

A Review. Journal of Food Science and Technology 2008; 45(1): 1-13.
12. Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AAK, Vernekar SN. Markers of renal function tests. North American Journal of Medical Sciences 2010; 2(4): 170-173.
13. Cheesman KH, Slater TF. An introduction of free radical Biochemistry. British Medical Bulletin 1993; 49(3): 481-493.
14. Walwadkar SD, Suryakar AN, Katkam RV, Kumbar KM, Ankush RD. Oxidative stress and calcium-phosphorus levels in Rheumatoid Arthritis. Indian Journal of Clinical Biochemistry 2006; 21(2): 134-137.
15. Olga S, Anna A, Miguel P, Enric P, Ignasi R, Maria S. Acute stress-induced tissue injury in mice: Differences between emotional and social stress. Cell Stress and Chaperones 2002; 7(1): 36-46.

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