ORIGINAL ARTICLE

Kappaphycus alvarezii Supplementation Attenuates Blood Pressure, Blood Cholesterol, ACE and Antioxidant Activities in Hypertensive and Hypercholesteroleamic Rats

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ABSTRACT

Introduction: Kappaphycus alvarezii (*K. alvarezii*) or locally known as red seaweed, was claimed to improve health and well-being. The goal of this study is to investigate how *K. alvarezii* affects blood pressure (BP), total cholesterol (TC), ACE, and antioxidant activities in rats with hyperten-sion and hypercholesterolemia (HTN-HC). **Methods:** Induction of HTN-HC was achieved by feeding rats a high sodium fat diet containing 1% cho-lesterol+7% sodium chloride (NaCl) for 6 weeks except for Normal group. HTN-HC rats were then supplemented with *K. alvarezii* (5 % (w/w) or 10% (w/w) per food intake) or treated with Captopril 30mg/kg/day + Simvastatin 2 mg/kg/day, or on similar diet for another 4 weeks. Before and after induction, as well as after treatment, BP and TC were measured. The rats' TC, ACE, and antioxidant activities were analysed using blood and liver samples. **Results:** The results demonstrate that BP and TC were significantly higher in all groups except the Normal Control after the induction period ($p<0.05$). Drug-treated rats (statin and captopril) and K. al-varezii significantly lowered BP and TC levels when compared to the HTN-HC group ($p<0.05$). Compared to the drug-treated and *K. alvarezii* groups, the HTN-HC group had the highest ACE activity ($p<0.05$). Meanwhile, HTN-HC rats had the lowest FRAP value and the highest CAT activities, while 10% *K. alvarezii* had the opposite reading ($p<0.05$). **Conclusion:** In conclusion, *K. alvarezii* improves blood pressure and total cholesterol levels while decreasing ACE activity in HTN-HC induced rats.

Keywords: ACE, Hypertension, Hypercholesterolemia, *K. alvarezii*, Red seaweed

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INTRODUCTION

Hypertension is currently one of the most concerning medical and public health issues. It affects around one billion people worldwide, making it one of the most severe issues because it increases the risk of cardiovascular disease and atherosclerosis. When blood pressure rises above 140/90 mmHg, this is referred to as hypertension (1). The most significant risk factors for cardiovascular disease which can lead to death, include hypertension, hypercholesterolemia, and diabetes mellitus (2). It has been discovered that dyslipidemia promotes endothelium damage, which disrupts normal vasomotor activity. Hypertension develops as a result of a decrease in nitric oxide release caused by endothelial injury. As a result, some researchers have suggested that the level of plasma lipids such as total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol is directly associated with hypertension development (3). Hypercholesterolemia and hypertension have been linked to an increase in oxidative stress. Oxidative stress is caused by an imbalance between the generation of radicals and the actions of antioxidants, which generates the

possibility of oxidative damage. Hypertension and hyperlipidemia induce oxidative stress in the arterial wall. Thus, supplementation of antioxidants may prevent oxidation in the vessel (4).

Seaweeds, a marine organism, have become a tremendous interest in the research field on their beneficial impacts on human health since they contain many natural bioactive substances such polyphenols, soluble polysaccharides, and carotenoids (5). It is a staple of many Asian diets, and consumption appears to be increasing in Western cultures due to both the influx of Asian cuisine and the health benefits associated with seaweed consumption (6). In the healthcare industry, seaweeds have long been prized for their nutritional benefits for various reasons. Seaweed hydrocolloids, agar, alginate, and carrageenan play essential roles in various industries around the world, from pharmaceuticals and cosmetics to crop production (7). Numerous studies claim that seaweeds are a health-promoting food that can help to improve one's quality of life. According to the study, seaweeds have antiviral, antioxidant, anticoagulant, anti-inflammatory, anti-cancer, and enzyme-inhibiting properties due to the presence of soluble fibres, vitamins, polyunsaturated fatty acids, phytochemicals, minerals, and antioxidants (8-10).

There is growing evidence that *K. alvarezii* has pharmacological and therapeutic effects against oxidative stress and cardiovascular disease. *K. alvarezii*, a red seaweed, have nutraceutical properties such as antioxidant, anticoagulant, anti-cancer, and antibacterial activity and is regarded as a promising source of marine organisms (8,11,12). It is one of the most cultivated seaweed species globally, especially in countries such as the Philippines, Indonesia, Tanzania, and Malaysia (13). Previous studies have found that 5% *K. alvarezii* effectively lowers plasma Total Cholesterol (TC), Low-Density Lipoprotein (LDL), and increases high-density lipoprotein (HDL) in rats fed high cholesterol diets (14,15). In addition, K.alvarezii from Fiji successfully normalised body weight, plasma lipids, and lowered systolic blood pressure in obese rats (16). Although many studies on the pharmacological potential of *K. alvarezii* have been published, and there has yet to be a study on this local seaweed that has combined hypertension and hypercholesterolemia to mimic the human metabolic syndrome. The understanding of the potential of this seaweed remains limited. Thus this study was conducted to investigate the effect of *K. alvarezii* on the blood pressure, total cholesterol, ACE and antioxidant activity in hypertension and hypercholesterolemia induced rats.

MATERIALS AND METHODS

Seaweed

The *K. alvarezii* was identified and analysed by ALS Technichem, Malaysia, under the reference number ALSM22933103284. The seaweed was processed into powder form by the Food Sciences Research Technology Centre, Malaysian Agricultural and Research Development Institute (MARDI), Serdang, Malaysia.

Animals

In this study, thirty male Sprague Dawley rats (8 weeks old) weighing approximately 180-200g were acquired from Chenur Enterprise (Selangor, Malaysia). They were separated into groups and housed in well-ventilated animal houses in standard propylene cages. Throughout the experiment, a room temperature of 25°C ± 2°C was maintained with a light-dark cycle of 12:12h. The rats maintained and acclimatised for one week before the induction of hypertension and hypercholes-terolemia. The rats were randomised into five groups; normal (normal rat chow), negative group (HTN-HC), positive group (HTN-HC + Cap-Sim) captopril 30 mg/kg (17) and simvastatin 1.8 mg/kg, (12) 5% *K. alvarezii* (HTN-HC + 5% K.a (w/w)) and 10% *K. alvarezii* (HTN-HC + 10% K.a (w/w)). All procedures followed the guidelines set forth by the Animal Ethics Com-mittee of the Laboratory of Animal Facility and Management (LAFAM), UiTM Puncak Alam, Selangor, with the reference number UiTM Care: 109/2015.

Pellet preparation and Induction of HTN-HC

The rat pellet was prepared freshly according to Dousip et al.(12) with slight modification. The pellet consisted of 62.5% commercial rat chow, 24% egg yolk 1.5% pure cholesterol, 7% NaCl and 5% corn starch. The ingredients were combined with ground commercial rat chow and formed into a dough. The dough was then sliced into small pieces and baked at 45oC for 24 hours before being stored in airtight containers. For the induction of HTN-HC, the rats were fed the prepared pellet according to their daily food intake for 6 weeks.

Treatment

Diets supplemented with 5% w/w and 10% w/w *K. alvarezii* were prepared in a manner similar to that of the HTN-HC. *K. alvarezii* powder was added to the rats' daily food intake and fed for 4 weeks. Meanwhile, for the control positive group (HTN-HC+ Cap-Sim), the rats were given Captopril -Simvastatin with a dose of 30 mg/kg/day and 2 mg/kg/day (12, 17) in early morning via oral gavage. The given dose was calculated based on human equivalent

dose (HED). The rats' blood pressure, body weight, and total cholesterol were measured at weeks 0, 6, and 10. The CODA non-invasive blood pressure system was used to assess the onset and progression of hypertension using the tail-cuff method (Kent Scientific Corporation).

The rat's blood pressure was measured, with ten pressure readings recorded for each measurement (8). For total cholesterol evaluation, 1 mL of blood was obtained via the orbital sinus. The blood samples were kept in a heparinized tube and centrifuged at 1500 rpm, for 15 min at 4 C to obtain the plasma. The plasma cholesterol analysis was performed at the Hematology and Biochemistry Clinical Laboratory of the Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). The rats were euthanised with ketamine (100 mg/ml) and xylazine (20 mg/ml) at week 10, and blood and liver were collected. The collected liver was then homogenised according to Katalinic's (19) method for antioxidant assay.

Antioxidant Assay

Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP assay was performed according to the method of Benzie (20) with slight modification. In brief, the working FRAP reagent was freshly prepared by mixing 10 volumes (250 ml) of 300 mM acetate buffer, pH 3.6, with 1 volume (25 ml) of 10 mM TPTZ in 40 mM HCl and 1 volume (25 ml) of 20 mM FeCl3, freshly prepared and warmed to 37 C. Approximately 100 µl of liver homogenate was mixed with 300 µl of distilled water and 3 ml of FRAP reagent in the test tube. The sample blank was prepared by mixing 100 µl of liver homogenate with 3.3 ml of distilled water, while the reagent blank was prepared by mixing 3 ml FRAP reagent with 300 µl distilled water. The sample was then incubated for 10 min. The absorbance of the blue coloured complex was analysed against the reagent blank using UV-VIS spectrophotometer at 593 nm. A set of standard solutions of ferrous (II) ions with various concentrations (0.1–2.0 mM) was prepared from ferrous (II) sulphate in distilled water (FeSO4.7H2O). The standard curve obtained is used for the calibration. The FRAP level of the tissue sample was expressed as mmol of Fe3+ reduced to Fe2+ per g of sample (mmol/g sample).

Catalase Assay

The assay was performed according to Aebi.21 In brief, 50 mM, pH 7.0 phosphate buffer and the 30mM hydrogen peroxide (H2O2) solution (0.34ml of 30% H2O2 to 100 ml of phosphate buffer) were freshly prepared prior to the experiment. Approximately 50 µl liver homogenate, 1.95 ml phosphate buffer, and 1 ml hydrogen peroxide were added to the cuvette, and the absorbance was recorded for 3 min at 240 nm with phosphate buffer served as a blank.

ACE activity

The ACE activity was measured using rat angiotensin-converting enzyme ACE ELISA Kit (Sun-long Biotech, China). The procedure followed the guidelines outlined in the manufacturer's in-struction manual. In summary, 40 µl of plasma was collected and diluted 5 times with the pro-vided dilution buffer before being transferred to the well plate. After 30 minutes of incubation at 37°C, 50 µl of washing buffer was added and discarded after 30 seconds. The washing proce-dure was repeated five times. 50 µl HRP-Conjugate reagent was added to each well except the blank control well and incubated for another 30 min at 370C. The washing process was repeated two times before 50 µl Chromogen Solution A and 50 µl Chromogen Solution B were added to each well, mixed and incubated at 37 C for 15 min in the dark. Finally, 50 µl stop solution was added to each well to terminate the reaction and the absorbance was analysed at 450 nm by using the ELISA reader. Rat ACE Standard and its corresponding OD reading were plotted on the log scale. The concentration of Rat ACE in the sample was determined by plotting the sample's OD on the Y-axis. The initial concentration was calculated by multiplying the dilution factor.

Statistical Analysis

Microsoft Excel 2010 was used to compile all of the measurements. Values were calculated and expressed as group means with Standard Error of Mean (SEM). The statistical analysis was done using IBM SPSS version 23. The compiled data were analysed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test, with $p<0.05$ being considered significant.

RESULTS

Body weight and blood pressure

The changes in body weight and blood pressure over the course of the experiment was shown in Table I. For all groups, there were a significant increase ($p<0.05$) in mean body weight from week 0 to week 6 ($p<0.05$). At week 10, both the *K. alvarezii* supplemented groups and the Cap-Sim group had lost body weight. However, no significant different in body weight reduction were observed between these groups. Only HTN-HC showed an increase in body weight throughout the experiment.

The mean value of systolic (SBP) and diastolic (DBP) blood pressure showed a similar pattern. At week 6, there was a significant increase in SBP and DBP in all induction groups ($p<0.05$). This indicates that the hypertension induction was successful. DBP and SBP were significantly lower ($p<0.01$) in the Cap-Sim and *K. alvarezii* supplemented groups at week 10 compared to week 6. Throughout the experiment, HTN-HC shows an increase in SBP and DBP.

Table I : The effect of supplementation of *K.alvarezii* on the body weight, SBP and DBP of induced rats.

	Groups				
	Normal	HTN-HC	HTN-HC + CAP-SIM	HTN-HC + 5% K.a	HTN-HC + 10% K.a
Body weight (g)					
Baseline (week 0)	196.25 ± 12.78	199.74 ± 8.97	188.15 ± 8.95	195.28 ± 4.65	193.65 ± 8.91
Week 6	343.57 ± 13.16	385.12 ± 8.71^	371.21 ± 13.73^	359.23 ± 14.88^	373.97 ± 10.73^
Week 10	390.15 ± 13.82^	419.28 ± 15.97*	363.64 ± 14.62*,**	356.02 ± 15.84*,**	359.29 ± 12.16 *,**
SBP (mm/Hg)					
Baseline (week 0)	126. 13 ± 3.11	129.51 ± 3.12	128.43 ± 1.07	126.11 ± 2.73	129.19 ± 0.65
Week 6	119.50 ± 1.65**	145.50 ± 1.63*,^	155.67 ± 2.47*,^	149.51 ± 2.58*,^	148.83 ± 2.78*,^
Week 10	124.16 ± 1.87**	159.16 ± 1.19*,#	125.67 ± 3.20**,#	124.00 ± 1.87**,#	127.33 ± 2.39**,#
DBP (mm/Hg)					
Baseline (week 0)	86.22 ± 2.09	88.63 ± 3.99	89.01 ± 0.76	87.31 ± 2.53	86.19 ± 3.04
Week 6	84.71 ± 3.86	101.67 ± 0.78*,^	98.50 ± 4.91*,^	100.04 ± 2.22*,^	99.86 ± 3.23*,^
Week 10	88.23 ± 4.51	113.83 ± 2.21*,^	89.00 ± 2.95**,#	87.50 ± 2.10**,#	85.83 ± 2.08**,#

The table shows the measurement of body weight, SBP and DBP at three intervals, week 0, week 6 and week 10. *: significant when compared with Normal at $p < 0.05$; **: significant when compared HTN-HC at $p < 0.01$; ##: significant when HTN-HC+Sim-Cap at $p < 0.05$; ^ significant when compared to Week 0 at $p < 0.05$; #: significant when compared with week 6 at $p < 0.01$.

Total cholesterol

Figure 1 represented the changes in total cholesterol (TC). At week 0, there was no significant difference in TC between the groups. Except for the Normal group, a significant increase in TC was observed at week 6 ($p<0.05$). This indicates that hypertension and hyper cholesterol induction were successful. At week 10, the drug-treated group, Cap-Sim, and K.alvarezii supplemented group showed a significant reduction in mean TC compared to week 6 ($p<0.05$). Negative control group (HTN-HC) has a significant increase in TC value throughout the experimental period compared to all other groups. The lowest mean TC level was observed in 10% *K. alvarezii*; however, there was no significant difference compared to 5% *K. alvarezii* and the Cap-Sim group.

ACE activity

Figure 2 illustrates the plasma ACE levels in induced rats. Based on the findings, *K. alvarezii* significantly lower the ACE levels at the end of the experiment compared to the HTN-HC group ($p<0.05$).

Antioxidant

FRAP

Figure 3 illustrates the outcome of the FRAP analysis. The graph revealed that the 10% *K. alvarezii* group had the highest mean FRAP value (0.77 mmol/g), followed by the 5% *K. alvarezii*, Cap-Sim, and Normal groups. The HTN-HC group, on the other hand, had the lowest mean FRAP value of 0.18 mmol/g. The antioxidant capacity of the 10% *K. alvarezii* group was substantially higher at $p<0.05$ when compared to the other groups.

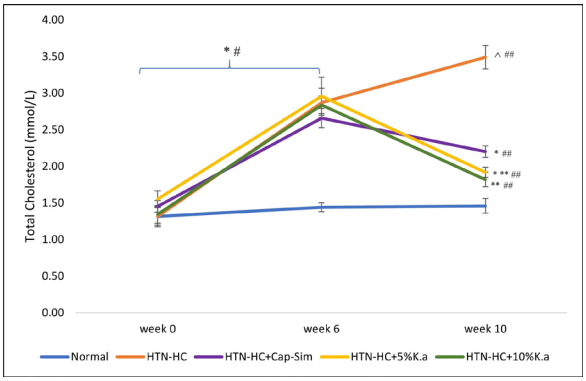

Figure 1 : Effect of *K.alvarezii* on the TC of the HTN-HC induced rats. Improvement of total cholesterol was observed after 4 week supplementation of *K.alvarezii*. Data are presented as means ± SEM (n = 6)* Significant different when compared to Normal at $p<0.01$; ** significant different when compared to HTN-HC+Cap-Sim at $p<0.01$; # significant different when compared to Week 0 at $p<0.05$; ^ significant different when compared to all groups at $p<0.01$; ##significant different when compared Week 6 at $p<0.05$

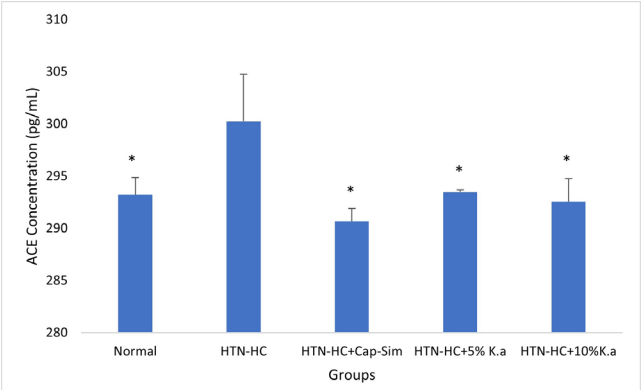

Figure 2 : Effect of *K. alvarezii* on the ACE activity of the HTN-HC induced rats. K.alvarezii showed a lower ACE activity in induce rats (HTN-HC) at the end of research experiment ($p<0.05$). Data are given as means ± SEM (n = 6)

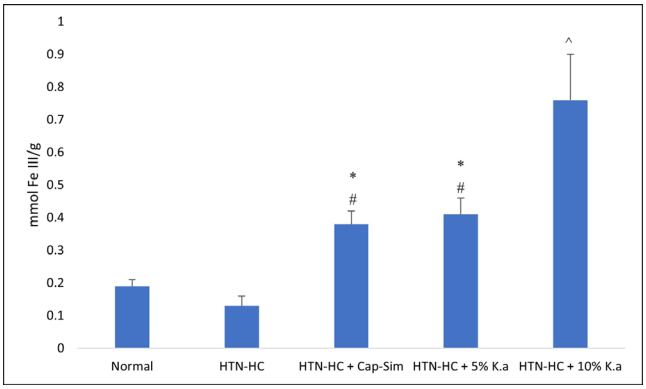

Figure 3 : Effect of *K. alvarezii* on the FRAP analysis of HTN-HC induced rats. 10% K.alvarezii supplementation showed the highest antioxidant activity in the liver. Data are given as means ± SEM (n = 6)*: significant compared with Normal at $p<0.05$, #: significant compared with HTN-HC and ^ significant when compared with all groups at $p<0.05$

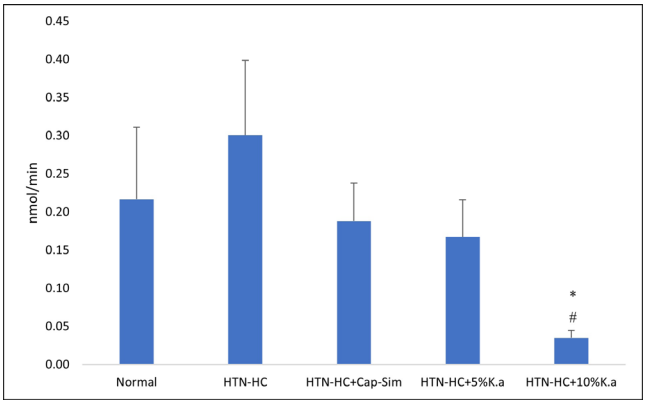

Figure 4 : Effect of *K.alvarezii* on the Catalase test of the HTN-HC induced rats. Lowest catalase activity was observed in 10% *K.alvarezii* suplementation group Data are given as means ± SEM (n = 6)*: significant compared with Normal at $p<0.05$, #:significant with HTN-HC at $p<0.01$

CAT

In the catalase test analysis, the contradictory pattern was observed when compared to FRAP result. Figure 4 illustrated the catalase test of HTN-HC rats with the least effect of *K. alvarezii* supplementation and drug-treated groups. The HTN-HC group had the highest mean catalase value of 0.18 nmol/min compared to all other groups, with a significant difference of $p<0.001$ between them. Compared to all other groups, 10% *K. alvarezii* has the lowest mean CAT value of 0.01 nmol/min

DISCUSSION

The consumption of a high-sodium, high-cholesterol diet appeared to promote body weight gain. According to Naquiah Awang, adding egg yolk to the diet significantly increased the weight of rats because it contains about 32 percent to 60 percent fat calories (22,23). It has been reported that dietary fat consumption does not promote fat oxidation or energy expenditure, resulting in fat accumulation and weight gain (24). In this study, groups given a high sodium-cholesterol diet gained significant body weight. This finding is in accordance with Matanjun's (15) findings, which showed that rats fed a high-fat diet gained weight after four weeks. In addition, supplementing with 10% *K. alvarezii* reduces weight gain. Although no significant reduction in weight gain was observed, Dousip's et al., (12) reported a similar pattern. According to Balasubramaniam et al., (25), seaweed promotes pancreatic lipase inhibition, which inhibits dietary fat absorption. Incorporating soluble dietary fibre in the diet restricts the physical contact between fat and protein at the intestinal villi, leading to reduced digestible energy intake, thus reducing weight gain (26). Flucoxanthin, found in *K. alvarezii*, is thought to reduce abdominal adipose tissue and weight by promoting fatty acid oxidation (26-28). This is attributed to an increase in Uncoupling Protein 1 (UCP1) expression in the mitochondria of abdominal adipose tissue, which leads to more efficient fat burning.

According to Brook et al.,(29) long-term consumption of a high-fat diet in rats can cause oxidative stress, which inactivates nitric oxide and leads to hypertension. Furthermore, after 10 weeks, rats with diet-induced obesity developed hypertension, according to a study. 30 The amount of dietary sodium chloride (NaCl) in the rat's diet is crucial in causing hypertension. A typical rat's diet contains only 0.1 percent NaCl, whereas rat chow contains between 0.3 and 0.4 % in the ingredients. In the current study, hypertensive and hypercholesterolemic rats were successfully induced. The combination of cholesterol and NaCl promoted the development of HTN-HC. After 6 weeks of induction, rats fed a high sodium-cholesterol diet significantly increased SBP and DBP. A previous study claimed that hypertension could be successfully induced in rats fed a high salt diet containing 8% NaCl for 12 weeks (31). At the end of the experiment, groups supplemented with *K. alvarezii* exhibited the same reduction in SBP and DBP as a drug-treated group. According to Jaspars (32), a

renin inhibitory peptide found in Palmaria palmate, red seaweed, and most seaweeds can lower blood pressure in hypertension-induced rats. However, there has been little research on the link between this peptide and its mechanism; thus, further research on the detection of this peptide in *K. alvarezii* is warranted in the future. Vitamin A, B1, B2, B3, B6, and C are abundant in *K. alvarezii* (33). In a study, vitamin C was effective in lowering blood pressure in salt-induced hypertensive rats and protecting the liver and kidney functions (34).

Hypercholesterolemia is thought to develop as a result of consuming large amounts of cholesterol and saturated fats on a regular basis (35). The induction of hypercholesterolemia was successfully established based on the results of the study, which showed that groups fed a high sodium and cholesterol diet had a significant increase in cholesterol levels. At the end of the study, the groups supplemented with *K. alvarezii* had significantly lower total cholesterol levels. This could be due to the presence of polysaccharide, which subsequently increases cholesterol excretion in the faeces (36). The study discovered that the dietary fibre in seaweeds binds to bile salt and reduces the time it takes for lipid and carbohydrate absorption in the intestine. This causes a decrease in hepatic lipogenesis, which results in lower hepatic and plasma triglyceride levels. According to Matanjun (15), *K. alvarezii* contains more carrageenan than *S. polycystum,* which contains alginates and has a better anti-hyperlipidemic effect.

The increase in oxidative stress has been associated with hypertension and hyperlipidemia. Oxidative stress is an imbalance between the radical formation and antioxidants activities which creates a potential for oxidative damage. Since hypertension and hyperlipidemia cause oxidative stress in the arterial wall, antioxidant supplementation may help prevent blood vessel oxidation (4). Catalase is an important intracellular antioxidant enzyme that is primarily found in the liver (37). By scavenging hydrogen peroxide, catalase prevents the production of reactive OH radicals. Due to compensatory mechanisms, as oxidative stress increased, catalase activity increased; thus, lower oxidative stress in the treatments group results in lower catalase activity. *K.alvarezii* was found to be high in antioxidants in this study, contributing to a high FRAP value (38) in the liver. Catalase activity was comparable, with rats supplemented with 10% *K. alvarezii* having the lowest value compared to the other groups. Some of the components found in *K. alvarezii* that are believed to contribute to antioxidant activity include carrageenan, tannins, flavonoids, and phenolic acids (8). This confirmed the findings of Dousip's et al., (12).

Renin-angiotensin-aldosterone system (RAAS) inhibitors and statins are two common types of drugs used to treat HTN and HC respectively. The combination of both drugs claimed to be most effective in preventing cardiovascular risk hence, reducing morbidity and mortality (39). Combining both drugs is perhaps the most effective in reducing morbidity and mortality by reducing cardiovascular risk (39). RAAS activation causes Angiotensin-Converting Enzyme (ACE) to convert Ang I to Ang II, leading to vasoconstriction and eventually an increase in blood pressure. In this study, supplementation with *K. alvarezii* was found to be as effective as Cap-Sim in lowering blood pressure and total cholesterol levels. According to Umar et al., (40) captopril, an ACE inhibitor, reduced blood pressure significantly in hypertensive Wistar rats, similar to the current study. Measuring the concentration of ACE in hypertensive rats may therefore reveal ACE expression activity.

In comparison to the other groups, HTN-HC had the highest ACE concentration. This is in line with a previous study that found increased ACE activities in untreated rats linked to higher plasma Ang II concentrations (41). The decrease in ACE expression in *K. alvarezii* could indicate that this seaweed has ACE inhibitory properties. According to Makkar and Chakraborty (42) the inhibitory activities of the polygalactans from *K. alvarezii* (Doty) Doty Ex Silva and captopril were almost comparable. Furthermore, seaweed peptides and phenolic compounds have been shown to have potent ACE inhibitory activity in studies (43). Furthermore, Ecklonia cava, a brown seaweed, has been shown to have anti-hypertensive properties. The phlorotannin compounds in Ecklonia cava form covalent bonds with the ACE molecule, inhibiting ACE activity (44). *K. alvarezii* was found to contain a similar compound, which could be contributing to the same effect as the previous study.

CONCLUSION

In conclusion, this research suggests that *K. alvarezii* may reduce the risk of cardiovascular dis-ease in hypertension and hypercholesterolemia-induced rats by improving blood pressure and blood cholesterol and inhibiting ACE activity due to antioxidant activities. Identification of ac-tive compounds in *K. alvarezii* and gene expression should be pursued in the future to develop a better understanding of the mechanism at work.

ACKNOWLEDGEMENT

The authors would like to acknowledge UiTM, MARDI, and CUCMS, Malaysia for financial support (100-RMI/GOV 16/6//2(12/2014) and (CRG/04/07/2016), and the Department of Pharmaceutical Sciences, Faculty of Pharmacy, UiTM, CUCMS and other individuals who were directly or indirectly involved for their kind assistance in this research.

ABBREVIATIONS

HTN-HC: hypertension-hypercholesterolemia; Cap-Sim: captopril-simvastatin; BP: blood pressure; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TC: total cholesterol; ACE: Angiotensin-converting enzyme; HDL: high-density lipoprotein; LDL: low-density lipoprotein; FRAP: ferric reducing assay power; CAT: catalase test

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