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Enhanced Glucose-6-Phosphate Dehydrogenase Activities in Krill Oil Treated Female Wistar Rats Attenuated Adenine-Induced Cardio-Inflammatory Markers

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Abstract

Background: Adenine is a nucleotide found in diet of fowls, cow lungs, and kidneys of animals with a capacity for deleterious cardiovascular tissue damage. Krill oil is a major omega-3 containing supplement with huge health beneficial effects. This study determines the role of Krill oil in adenine-induced cardiac toxicity of female Wistar rats.

Materials and Method: Twenty female Wistar rats weighing between 120±5g were distributed into 4 groups; Control (CTR), Krill oil (Kh) (3% of diet), Adenine (18mg/kg), and Kh (3% of diet) + Adenine (18mg/kg bw orally). After six weeks, animals were anesthetized by chloroform inhalation and blood collected by cardiac puncture. Plasma and cardiac homogenate were analyzed and data expressed as mean ± SEM; $p < 0.05$ were accepted as significant.

Results: Krill oil increased plasma and tissue glucose-6-phosphate dehydrogenase (G6PDH), Nicotinamide adenine dinucleodtide phosphate (NADPH), Glutathione (GSH) and Glutathione peroxidase (GPx) in normal and Adenine-treated rats and decreased Interleukin 1- beta (IL-β), tumor necrosis factor-alpha (TNF-α), Uric acid (UA) and C reactive protein (CRP). Adenine increased IL-β, TNF-α and CRP when compared with control and Krill oil reduced significantly in both control and the adenine treated group.

Conclusion: The study indicates that Adenine promotes cardiac tissue injury by increasing inflammatory markers and Krill oil elicited a double fold increase in intracellular redox potential and lowering inflammatory cardiac risk markers.

Keywords: Adenine; anti-inflammatory; antioxidant; cardiovascular diseases; Krill oil

Introduction

Cardiovascular disease is a leading cause of death worldwide. It comprises disease that involves the heart and systemic blood vessels. Inflammation is considered to play a key role in both disease initiation and progression. Oxidative stress and inflammation are known contributors to atherosclerosis, endothelial dysfunction, and overall cardiovascular disease risk (1).

Antioxidant levels in patients with inflammatory diseases frequently decline due to inadequate dietary intake or more commonly, increasing demand under conditions of overwhelming generation of reactive oxygen species (ROS) by immunological effector cells, such as macrophages. Antioxidants may advantageously interfere with diseases related to oxidative stress (2).

Adenine is a purine base, a primordial molecule that is a component of essential biomolecules such as nucleic acids and adenine nucleotides also a modulator of various physiological functions (3).

Adenine content is observed in chicken intestine, goat intestine and cow lung (4) and research showed that adenine treated rats increase cardiovascular disease progression (5). Adenine increases systolic blood pressure and the concentrations of troponin I, tumor necrosis factor-α, and interleukin-1β in heart homogenates. The ingestion of adenine in rodents is known to induce inflammation, fibrosis and enhances the induction of cardiovascular injury. It promotes chronic kidney disease associated with cardiac inflammation, oxidative stress, Nrf2 expression, and DNA damage (6).

Krill oil is a phospholipid-rich oil with eicosapentaenoic acid (EPA): docosahexaenoic acid (DHA) ratio of 1.8:1. (7). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two omega-3 fatty acids that have been linked to a number of health advantages, including a decreased risk of cardiovascular disease (CVD). Omega 3 fatty acids, which can be obtained through diet or supplementation, are crucial for cardiovascular health. Krill oil is a viable replacement for omega-3 supplements (8) and exhibits reduced expression of numerous mRNA known to be involved in the cardiac remodeling process (9).

Supplementing with krill oil may enhance endothelial performance. The bioavailability and structure of the omega-3 fatty acids in krill oil, as well as its ability to reduce inflammation or other physiological features, may be responsible for this improvement (1). Inflammation, oxidative stress, and metabolic abnormalities are the major components of the complicated CVDs. In ischemic cardiomyopathy, excessive reactive oxygen species (ROS) generation can exacerbate cardiomyocyte injury and lead to mitochondrial dysfunction. It has also been suggested

that inflammatory signaling networks may contribute to ischemic cardiovascular disease (10).

Krill oil consumption is thought to be beneficial to health because it lowers cardiovascular disease risk factors by influencing plasma triacylglycerols, lipoprotein particles, fatty acid profiles, redox status, and perhaps inflammation. It enhances the antioxidant capacity, double bond index (DBI), and fatty acid anti-inflammatory index (7).

The study aimed therefore to determine the redox action of Krill oil on adenine induced cardiovascular injury in female Wistar rats.

Materials and method

Animals grouping

The ethical approval was given by the University of Ilorin Research Ethical Committee after the recommendation of the Faculty of Basic Medical Sciences Ethical Review Committee, University of Ilorin, Ilorin, Nigeria with ethical approval number (UERC/ASN/2018/357).

Twenty adults female Wistar rats weighing between 120±5g were obtained from the animal house of the College of Health Sciences, University of Ilorin (Ilorin, Nigeria). The rats were fed ad libitum with standard rat chow and had unlimited access to tap water. After a week of acclimatization, the animals were randomly assigned to four groups (n = 5 rats/group). Group 1 (Control) rats was fed with standard chow (CTR), Group 2 was fed with Antarctic krill oil (3% of diet), Group 3 was fed Adenine (18 mg/kg bw orally) and group 4 was fed Antarctic krill (3% of diet) + Adenine (18mg/kg bw orally). Rats were maintained in the animal house under environmental conditions of temperature (22–26 C), relative humidity (50–60%) and 12-h dark/light cycle.

Treatment

Antarctic Krill oil (3% of diet) -Hailisheng Group Co., Ltd Zhoushan Zhejiang, China) and Adenine (18mg/kg bw) was used for the treatment, treatment lasted for six weeks (11,12).

Collection of blood sample

At the end of six-weeks treatment, the animals were anesthetized by exposure to chloroform vapor. Blood was collected by cardiac puncture into heparinized tube and was centrifuged at 3000 rpm for 5 min at room temperature. Plasma was stored frozen until needed for biochemical assay.

Preparation of cardiac tissue homogenates

After dissection, the heart was excised, cleared of adhering connective tissues, blotted, and weighed. After weighing, 100 mg of tissue was carefully removed, homogenized in phosphate-buffered solution (PBS) with a glass homogenizer, and centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant was collected and stored frozen until required for biochemical assays.

Determination of G6PDH and NADPH

Glucose-6-phosphate dehygrogenase (G6PDH) was measured by standardized enzymatic colorimetric methods using assay kit obtained from Randox Laboratory Ltd. (Co. Antrim, UK).

Determination of plasma and cardiac uric acid and TNF-alpha

Plasma and cardiac uric acid were determined by non-enzymatic colorimetric method using assay kits from Randox Laboratory Ltd. (Co. Antrim, UK) and following the manufacturers' procedures. TNF-α was determined by the quantitative standard sadwich ELISA technique using monoclonal antibody specific for this parameter with kits obtained from Elabscience Biotechnology lnc. (Wuhan, China).

Determination of plasma and CRP

Plasma and cardiac c-reactive protein (CRP) were determined via standardized enzymatic colorimetric methods by using specific assay kits as described in the instruction manuals of the manufacturer (Fortress diagnostics, Antrim, UK).

Determination of plasma and cardiac GPx and GSH

Glutathione peroxidase (GPx) and Glutathione (GSH) were measured by the standardized enzymatic colorimetric method using assay kit obtained from Fortress diagnostics (Antrim, United Kingdom).

Determination of plasma IL-1β

Plasma IL-1β was determined by using ELISA kit from assay was done using the Sandwich-ELISA kit purchased from Elabscience®

Statistical analysis

The data was presented as Means ± SEM for all of the variables. SPSS was used to carry out statistical analysis of the data. The mean values of variables were compared using a one-way analysis of variance (ANOVA). Bonferroni's test was used to determine the significance of pairwise comparisons of mean values among the groups. The p-value ($p < 0.05$) was used to determine significance differences.

Results

Effect of Krill oil on G6PDH and NADPH in normal and adenine treated rats

Krill oil administration led to a significant increase in plasma and cardiac G6PDH and NADPH levels while adenine caused a significant reduction when compared with control groups. However, there was an observable increase in both cardiac G6PDH and NADPH levels in Krill+ Adenine groups when compared to the adenine group alone (Figure 1).

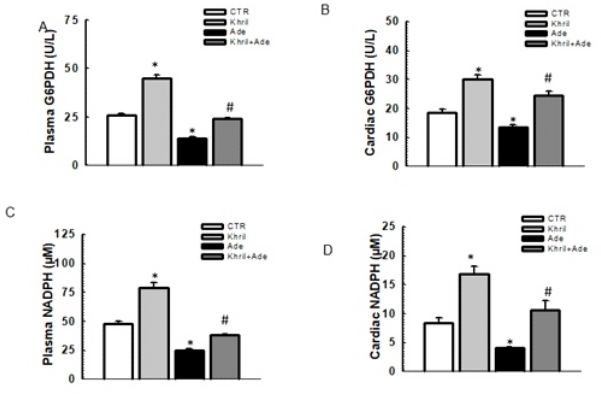

Figure 1: Effect of Krill oil on plasma and cardiac G6PDH and NADPH (*p<0.05 vs CTR, #p<0.05 vsAde,* CTR- control, * Ade- Adenine). Data were analyzed by one-way ANOVA followed by Bonferroni's Post hoc test. Values are expressed as mean ±SEM of 5 rats per group and P < 0.05 was taken as statistically significant.

Effect of Krill oil on GPx and GSH in normal and adenine treated rats

Plasma and cardiac GPx and GSH was elevated in the krill groups when compared to the control and adenine groups. Adenine caused a significant reduction in plasma GSH level when compared to the control group. Furthermore, rats administered Krill+Adenine showed significant increase in cardiac GSH levels when compared to the control and adenine groups (Figure 2).

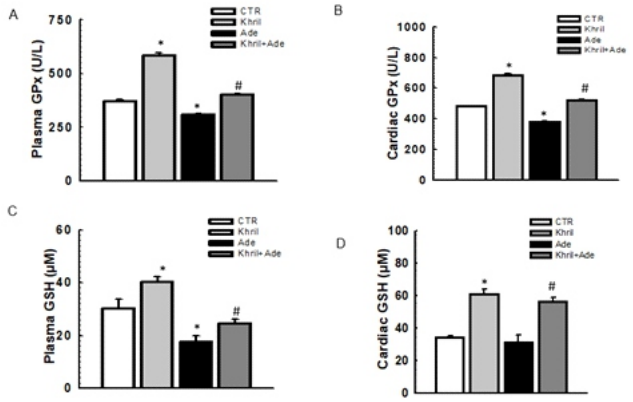

Figure 2: Effect of Krill oil on plasma and cardiac GPx and GSH (*p<0.05 vs CTR, #p<0.05 vs Ade,* CTR- control, * Ade- Adenine). Data were analyzed by one-way ANOVA followed by Bonferroni's Post hoc test. Values are expressed as mean ±SEM of 5 rats per group and P < 0.05 was taken as statistically significant.

Effect of Krill oil on Interleukin 1- Beta (IL-1β) and Tumor necrosis factor alpha (TNF-α) in normal and adenine treated rats

Krill oil reduced plasma and cardiac Interleukin 1-Beta when compared with control group. On the other hand, rats administered adenine showed significant increase in interleukin 1-Beta and TNF-α when compared to control and krill groups. In addition, there was an observable reduction in both interleukin 1-Beta and TNF-α levels in groups administered Krill+Adenine when compared only to the adenine group (Figure 3).

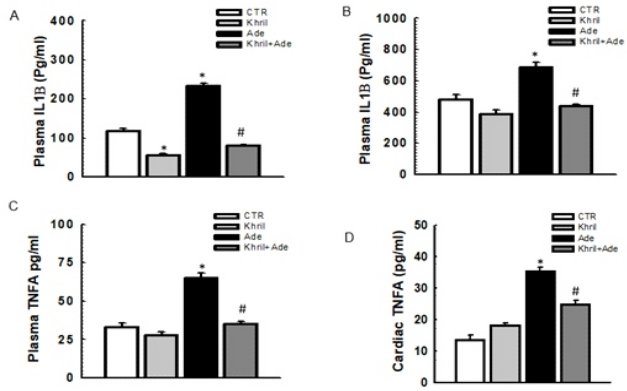

Figure 3: Effect of Krill oil on plasma and cardiac (IL-1β) and TNF-α (*p<0.05 vs CTR, #p<0.05 vsAde,* CTR- control, * Ade- Adenine). Data were analyzed by one-way ANOVA followed by Bonferroni's Post hoc test. Values are expressed as mean ±SEM of 5 rats per group and P < 0.05 was taken as statistically significant.

Effect of Krill oil on C-reactive protein (CRP) and Uric acid in normal and adenine treated rats

Administration of Krill oil showed a significant reduction in plasma and cardiac CRP and Uric acid levels when compared to the control and adenine groups. Adenine administered rats showed significant increase in both plasma and cardiac CRP and Uric acid levels. In addition, Krill+Adenine group showed a significant reduction when compared to control and adenine groups (Figure 4).

Discussion

The result of the present study showed that Krill oil enhanced redox homeostasis by increasing G6PDH levels and was shown to cause a reduction of cardiac inflammatory injury which was however enhanced by Adenine. From the study, krill oil caused a significant increase in plasma and cardiac G6PDH, NADPH, GPx and GSH levels acting as antioxidants which helps in preventing oxidative stress and the accumulation of free radicals. In addition, adenine elevated uric acid, interleukin-1beta which is shown to enhance cardiac

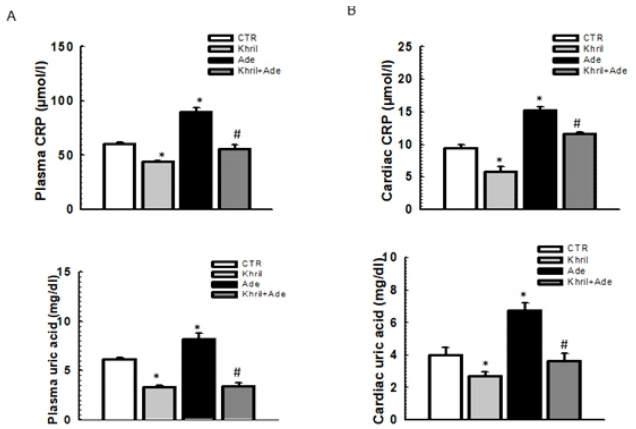

Figure 4: Effect of Krill oil on plasma and cardiac C-reactive protein (CRP) and Uric acid (*p<0.05 vs CTR, #p<0.05 vsAde,* CTR- control, * Ade- Adenine). Data were analyzed by one-way ANOVA followed by Bonferroni's Post hoc test. Values are expressed as mean ±SEM of 5 rats per group and P < 0.05 was taken as statistically significant.

damage by promoting cardiac inflammation which are important risk factors in the development of cardiovascular disease (Figure 5).

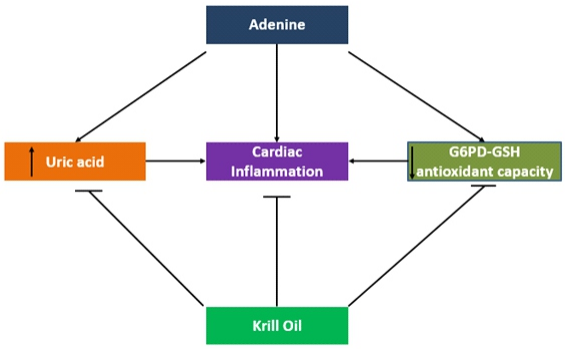

Figure 5: Graphical abstract showing the effect of krill oil and adenine on cardio-inflammatory injury.

Studies has been demonstrated that a deficiency in the redox pathway G6PDH increases cardiovascular risks by up to 70% and causes a dysregulation of reactive oxygen species (13). For cells to maintain redox equilibrium and reductive biosynthesis, glucose-6-phosphate dehydrogenase (G6PDH) must produce reducing equivalent NADPH and it is essential for cellular functions involving redox signaling and fuels the recycling of glutathione which is of great importance in antioxidant defense. Cells are more prone to growth retardation when there is insufficient G6PDH activation (14,15). Krill oil in this study was shown to promote redox potential via tremendous elevation of G6PDH and NADPH.

NADPH is necessary to control lipid metabolism and redox-dependent signaling and deficiency of G6PDH may impair heart performance (16). Krill oil administration from the study increased G6PDH which is vital in reducing NAD to NADPH and helps in cellular oxidative damage. Consequent with studies which has shown that NADPH plays important role in reducing glutathione, it helps to maintain cellular GSH levels which is central to preventing damage to the cardiac tissue due to oxidative stress (17,18). The increase in GSH level is beneficial in cardiovascular function by acting as a potent antioxidant and helps in the restoration of intracellular redox homeostasis (19). From the study adenine was shown to have opposing effect which suppressed antioxidant activities and enhanced the induction of cardiovascular injury.

It was observed from the study that cardiac TNF-α levels was low in the control compared to rats treated with adenine. TNF-α an indicator of inflammation that induces cell hypertrophy and cell enlargement and affects cardiomyocte contractility. Anti-inflammatory effects of krill oil were achieved via lowering cardiac TNF-α and IL-1β concentrations. The reported reduction in TNF-α, UA, IL-1β, and CRP in krill oil may offer a mechanism preventing the development of cardiovascular risks (1,20).

Krill oil from this study was shown to cause a double fold increase in the redox indices G6PDH and NADPH when compared with the control and adenine treated groups. Furthermore, result showed that groups administered Krill and adenine had elevated cardiac GSH and GPx levels. The administration of krill ameliorated the effect of adenine in lowering antioxidant markers which would have promoted oxidative stress and cardiovascular injury.

Inflammation impairs cardiac function directly and is linked to heart failure risk factors and TNF-α is a powerful indicator of heart failure (21). Studies has shown that that krill oil consumption reduces the effect of pro-inflammatory cytokine signaling via suppression of inflammatory pathways which could promote oxidative stress such as those controlled by IL-1β and TNF-α (11). Consequent with our study which showed that the administration of krill oil suppressed the effect caused by Adenine in enhancing oxidative stress and the progression of cardiac tissue damage.

The study also demonstrated that adenine had the impact of raising CRP levels, high CRP level increases the risk of cardiovascular diseases. CRP generation promotes systemic inflammation and development of cardiovascular injury. The correlation between increased CRP levels and practically all significant cardiovascular risk factors, such as metabolic syndrome, hypertension, dyslipidemia, and insulin resistance and diabetes has been demonstrated in previous studies. Additionally, in vitro research has demonstrated that TNF- α enhances the activation of NF-kB in smooth muscle and endothelial cells, which

in turn triggers the production of cytokines and vascular adhesion molecules that cause inflammation and promotes oxidative stress in the cardiovascular system (22).

This study showed that Krill oil enhances the effect of GPX and GSH, the deficiency of cardiac and systemic glutathione relates to heart failure progression and cardiac remodelling in animal models. GPx is essential in protection of cells from cardiovascular diseases. (23,24). According to research, krill oil provides vascular benefits including the avoidance of arrhythmias, potential plaque stabilization, and decreases heart inflammation [8]. A significant amount of data points to a robust correlation between high UA levels and the incidence and progression of atherosclerosis. Inflammatory markers are expressed more frequently when there are high intracellular UA concentrations which increases inflammasome activation. Vascular inflammation due to inflammatory mediators such as CRP promotes the development and progression of cardiovascular injury. Furthermore, this study showed that Krill oil suppresses the action of cardio-inflammatory risk markers, suppressing the circulating levels of UA, TNF-α and IL-1β. Adenine showed a negative effect by promoting the risk markers that enhanced cardiac tissue damage. To keep redox signaling and cell homeostasis in check, krill oil acts as a mediator for G6PDH. Cardiomyocytes need G6PDH to keep cytosolic GSH levels at a healthy level and to protect them from ROS damage. Inhibition of G6PDH depletes GSH levels leading to cardiomyocyte dysfunction (25). Observation from the study showed that Krill oil has cardioprotective roles by increasing the mobilization of GPx, G6PDH, GSH in the circulation.

Conclusion

The results from this study showed that adenine increased cardiac inflammatory risk makers which are capable of causing oxidative stress and promote cardiac tissue injury while Krill oil lowered proinflammatory cardiac risk markers and potentiated intracellular redox activity due to its major oxidant and reductant ability.

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Conflict of Interest

The authors declare no conflict of interest

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