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The effect of Krill oil supplementation on blood glucose levels in alloxan-induced hyperglycemic rats

El efecto de la suplementación con aceite de Krill sobre los niveles de glucosa en sangre en ratas hiperglicémicas inducida por aloxano

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SUMMARY

Introduction: *Hyperglycemia is a condition associated with long-term complications and damage of multiple susceptible organs, including the eye, kidney, nerve, and heart. Krill oil contains astaxanthin, which is an antioxidant that acts to fight free radicals. The study aims to determine the effect of krill oil supplementation on blood glucose levels of alloxan-induced hyperglycemic rats.* **Methods:** *This was an experimental pre- and post-control group. Twenty-four hyperglycemic rats were randomly assigned into four groups. Each group received a different intervention (placebo, glibenclamide, fish oil, and krill oil) for 14 days. Blood glucose measurements were carried out four times (after acclimatization, after alloxan induction, day 7 of intervention, and day 14 of intervention).* **Results:** *The average level of blood glucose levels in each group decreased after 14 days of intervention were as follows: placebo group (222.16 mg/dL), glibenclamide group (99.33 mg/dL), fish oil group (114.33 mg/dL), and krill oil group (99 mg/dL). There was a statistically significant difference in the mean reduction of blood glucose levels between groups after seven days of intervention ($F(3.20) = 4.513$, $p=0.014$) and 14 days of supplementation ($F(3.20) = 19.794$, $p<0.001$). Post-hoc comparisons indicated that the mean reduction of blood glucose levels for the krill oil group was significantly different from all other groups after 7 days and 14 days of intervention.* **Conclusion:** *Supplementation of krill oil for 7–14 days can significantly reduce blood glucose levels in hyperglycemic rats.*

Keywords: *Krill oil, blood glucose, hyperglycemia.*

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RESUMEN

Introducción: *La hiperglicemia es una afección asociada con complicaciones a largo plazo y daño de múltiples órganos susceptibles, incluidos el ojo, el riñón, el nervio y el corazón. El aceite de Krill contiene astaxantina, que es un antioxidante que actúa para combatir los radicales libres. El estudio tiene como objetivo determinar el efecto de la suplementación con aceite de Krill sobre los niveles de glucosa en sangre de ratas hiperglicémicas inducidas por aloxano.* **Métodos:** *Este fue un verdadero grupo experimental previo y posterior al control. Se asignaron al azar veinticuatro ratas hiperglicémicas en cuatro grupos. A cada grupo se le administró una intervención diferente (placebo, glibenclamida, aceite de pescado y aceite de Krill) durante 14 días. Las mediciones de glucosa en sangre se realizaron cuatro veces (después de la aclimatación, después de la inducción con aloxano, el día 7 de la intervención y el día 14 de la intervención).* **Resultados:** *El nivel promedio de glicemia en cada grupo disminuyó después de 14 días de intervención fue el siguiente: grupo placebo (222,16 mg/dL), grupo glibenclamida (99,33 mg / dL), grupo aceite de pescado (114,33 mg/dL), y grupo de aceite de Krill (99 mg/dL). Hubo una diferencia estadísticamente significativa en la reducción media de los niveles de glucosa en sangre entre los grupos después de siete días de intervención ($F (3,20) = 4,513$, $p = 0,014$) y 14 días de suplementación ($F (3,20) = 19,794$, $p <0,001$). Las comparaciones post hoc indicaron que la reducción media de los niveles de glucosa en sangre para el grupo de aceite de Krill fue significativamente diferente de todos los demás grupos después de 7 días y 14 días de intervención.* **Conclusión:** *La suplementación con aceite de Krill durante 7 a 14 días puede reducir significativamente los niveles de glucosa en sangre de ratas hiperglicémicas.*

Palabras clave: *Aceite de krill, glucosa en sangre, hiperglucemia.*

INTRODUCTION

Diabetes mellitus (DM) is a term that refers to a collection of metabolic illnesses that are characterized by hyperglycemia in the body (1). Hyperglycemia is a medical term for circumstances where blood sugar levels are higher than normal, and most often it is caused by DM (2,3). People with DM tend to have an increased risk of having severe health problems, reduced quality of life, and mortality.

Based on International Diabetes Federation (IDF), there were 151 million people with DM worldwide in 2000. This number increased almost threefold in 2015 and is expected to increase to 693 million by 2045 (4). Diabetes is a worldwide epidemic. In 2017, an estimated 425 million persons aged 20-79 years had DM. Approximately 79 % of them reside in low- and middle-income nations. In high-income nations, the prevalence of DM peaked in the 75-79 age range; in middle-income countries, it peaked in the 60-74 age range. The prevalence of DM in the 55-64 age range in low-income countries (4).

Long-term hyperglycemia is associated with the development of DM complications (5). Hyperglycemia triggers several signaling pathways that cause the production of reactive oxygen species (ROS), inflammation, cytokine secretion, cellular death, and damage of multiple susceptible organs, leading to DM complications. As a result, inhibiting ROS formation may be critical in preventing diabetes complications (6,7).

Krill oil, which is extracted from shrimp-like crustaceans named Krill (*Euphausia superba*), is a natural and pure source of long-chain omega-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and phospholipids (8,9). Astaxanthin Krill oil also contains antioxidants such as astaxanthin and vitamins A and E and is known to protect cells against free radicals (10). Numerous studies on astaxanthin's effect on oxidative stress concluded that it suppresses oxygen species by interfering with enzymes and their pathways. Additionally, astaxanthin improves the antioxidant defense system's activation (11).

Omega-3 fatty acids, particularly EPA and DHA, are required in the human diet. They exert anti-inflammatory and antioxidant actions via binding to cell membrane receptors, affecting downstream mediators and altering gene expression (12). Supplementation of EPA and DHA on hyperglycemic and dyslipidemic rats has been shown to decrease serum markers of oxidative stress and inflammation (13).

This study aims to determine whether krill oil supplementation affects blood glucose levels. Astaxanthin and EPA DHA contained in krill oil

are expected to reduce ROS production, which leads to a decrease in blood sugar levels and glucose homeostasis. Thus, it would slow the progression of DM complications.

METHODS

Experimental animals

Male Wistar rats weighing 150-200 g were kept in a 12-hour light/12-hour dark cycle at room temperature of 24 ± 5°C and relative humidity of 30 %-55 %. For a one-week acclimation phase, the animals were fed a regular pellet diet and free access to water. The animals were handled in conformity with accepted laboratory animal care practices. The study was approved by the ethical committee of Universitas Muslim Indonesia and Ibnu Sina Hospital (Register Number: UMI011912426).

Experimental induction of hyperglycemia

The rats received two intraperitoneal injections of alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body weight at 5-day intervals. On the second day following the second induction, animals having a fasting glucose level ≥ 215 mg/dL were declared hyperglycemic and included in the study.

Experimental procedure

We utilized a total of 24 rats. The rats were divided into four six-rat groups. Each group received a different intervention for 14 days. Group 1: Rats received NA-CMC 1% solution (placebo). Group 2: Rats received glibenclamide 3.25 mg/kg body weight. Group 3: Rats received fish oil 0.3 mL/kg body weight (*Sea Quill*). Group 4: Rats received krill oil 0.2 mL/kg body weight (*Nature's Health*). All interventions in groups 2, 3, and 4 were given using an intragastric tube. The rats had free access to tap water and a regular pellet meal during the trial period. To avoid stress after the trial period, rats were decapitated.

Outcome measurement

Blood samples were drawn from the rat vena coccygeal and measured for glucose concentration after acclimatization (Da), after alloxan induction (Di), day 7 of intervention (D7), and day 14 of intervention (D14).

Statistical analysis

Statistical Product and Service Solutions (SPSS) 22.0 software was used for all statistical analyses. Changes in blood glucose levels within the group were analyzed using paired T-test. The One-way ANOVA test was used to examine the mean reductions in blood glucose levels on days 7 and 14 following the intervention. Statistical significance was defined as a $p<0.05$.

RESULTS

The average level of blood glucose levels in each group after induction, day 7 of intervention, and day 14 of intervention (Di; D7; D14) were as follows: placebo group (300 mg/dL; 243.83 mg/dL; 222.16 mg/dL), glibenclamide group (224.5 mg/dL; 170.33 mg/dL; 99.33 mg/dL), fish oil group (214.67 mg/dL; 144.33 mg/dL; 114.33 mg/dL), and krill oil group (279.16 mg/dL; 175.83 mg/dl; 99 mg/dL) (Figure 1).

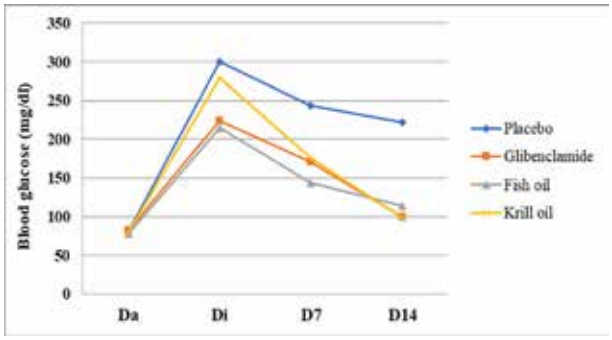

Figure 1. Changes in blood glucose level within-group after acclimatization (Da), after alloxan induction (Di), day 7 of intervention (D7), and day 14 of intervention (D14).

Changes in blood glucose levels after induction–day 7 of intervention (Di–D7) and after induction–day 14 of intervention (Di–D14) in each group were analyzed using paired T-test. The result showed that the blood glucose levels in each group were significantly decreased following the 14-days intervention period (Figure 2).

Mean reduction of blood glucose at day 7 and day 14 after intervention between groups were compared using the One-Way ANOVA test. The result showed that there was a significant difference in the mean reduction of blood glucose level between groups at day 7 after intervention ($F(3.20) = 4.513$, $p=0.014$) and day 14 after intervention ($F(3.20) = 19.794$, $p<0.001$), respectively. Post-hoc comparisons indicated that the mean reduction of blood glucose levels in group 4 (krill oil) was significantly different from all other groups on day 7 and day 14 after the intervention.

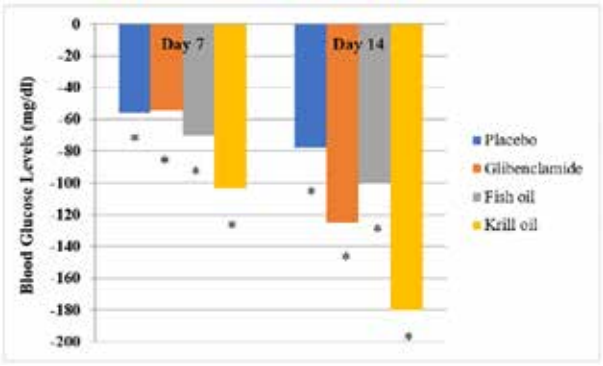

Figure 2. The reduction of blood glucose levels in D7 and D 17 in each group. Changes were compared with the glucose level at Di. *p <0.05)

DISCUSSION

Our study revealed that supplementation of krill oil and fish oil for 7-14 days reduced blood sugar levels significantly, but the greatest decrease occurred in the krill oil group. Krill oil is high in omega-3 phospholipids, making it an excellent supply of omega-3 fatty acids and choline from the phospholipid head group (8). In animal trials, omega-3 PUFA supplementation enhanced insulin sensitization, possibly through higher levels of adiponectin, a newly discovered protective risk factor, and decreased inflammation. Supplementation with omega-3 PUFAs was also hypothesized to improve glycemic control. Although the mechanisms are unknown, animal models have revealed several potential mechanisms, including increased hepatic insulin sensitivity via fatty acid oxidation and lipogenesis reduction, increased adipocytokine production, direct and indirect anti-inflammatory effects, and associated improvements in insulin sensitivity in the liver, muscle, and adipose tissue (14).

Krill oil has a different molecular form from fish oil. Omega-3 fatty acids found in krill oil are phospholipid-bound. Omega-3 fatty acids are triglyceride-bound, regardless of the source (8). DHA and more specifically, EPA had a greater bioavailability when omega-3 fatty acids were given as marine phospholipids, as observed in mice and humans (15,16). This may account for the higher reduction in blood glucose levels observed in the krill oil group compared to the fish oil group.

By scavenging free radicals and limiting lipid peroxidation, antioxidants play a critical role in protecting against molecular oxidative damage. Natural antioxidants are found in a wide variety of natural products, including fruit, vegetables, and seafood (17). Astaxanthin, which is found in krill oil, is presently recognized as the strongest antioxidant carotenoid. Astaxanthin can quench singlet oxygen, scavenge oxygen free radicals directly, inhibit fatty acid chain reactions, and exhibit significant antioxidant activity. It efficiently protects against oxidative stress-induced damage and lipid peroxidation, boosts the body's antioxidant capacity, and inhibits the formation of advanced glycation end products (AGE) (18-20). A previous study has demonstrated that alloxan-induced diabetic rats had an increase in blood glucose levels and a significant decrease of enzymatic and non-enzymatic antioxidants. The injection of astaxanthin to these diabetic rats resulted in a considerable drop in glycemia and a return to a near-normal antioxidant state (17).

In the current study, we used alloxan-induced hyperglycemic rats. The hyperglycemic effect of alloxan occurs in two ways. First, alloxan inhibits glucose-induced insulin release selectively by inhibiting glucokinase, the beta cell's glucose sensor. Second, it induces insulin-dependent diabetes by triggering the generation of ROS, which results in the selective necrosis of beta cells (21).

Chronic hyperglycemia is a key determinant in the pathogenesis of DM. In Type 1 DM (T1DM), hyperglycemia-induced increased ROS generation induces the release of inflammatory cytokines and promotes apoptosis in pancreatic cells, resulting in decreased insulin output. Hyperglycemia-induced ROS generation affects insulin sensitivity and signaling in insulin-responsive tissues in Type 2 DM (T2DM) (22). The development of vascular complications occurs as a result of several mechanisms, including activation of the polyol and hexosamine pathways, activation of protein kinase C (PKC), increased oxidative stress, increased production of AGE, and increased synthesis of growth factors, cytokines, and angiotensin II (23,24). Hyperglycemia increases the development of diacylglycerol (DAG), PKC, and NAD(P) H-oxidase, resulting in the creation of ROS and oxidative stress in diabetes (6,25). Additionally, DM depletes the body's antioxidative defensive ability. Antioxidants, both enzymatic and non-enzymatic, play a critical function in the oxidation process by scavenging or inhibiting the production of ROS (17).

CONCLUSION

Supplementation of Krill oil for 7-14 days can significantly reduce blood glucose levels in hyperglycemic rats. Thus, it has the potential to inhibit the progression of diabetes complications. Further investigation on the effect of Krill oil on ROS production and the significant sample test in a cohort method is needed to confirm this statement.

Conflicts of Interest

The authors declare no conflict of interest.

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