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INCREASED PAROXONASE-3 LEVELS IN RATS FED HIGH CHOLESTEROL FEED WITH INDUCTION OF ETHANOL EXTRACT FROM KENIKIR LEAVES (COSMOS CAUDATUS)

Peningkatan Kadar Paroxonase-3 Pada Tikus yang Diberikan Pakan Tinggi Kolesterol Dengan Induksi Ekstrak Etanol dari Daun Kenikir (Cosmos caudatus)

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ABSTRAK

Ekspresi paraoxonase-3 (PON-3) pada HDL berhubungan dengan fungsi antioksidan HDL. PON3 merupakan protein yang penting dalam melindungi pembuluh darah dari aterosklerosis. Cosmos caudatus merupakan salah satu bahan alami yang berkhasiat meningkatkan kadar HDL. Tujuan penelitian adalah untuk menunjukkan pengaruh ekstrak etanol dari daun kenikir (C.caudatus) terhadap kadar PON-3 serum pada tikus yang diberi diet tinggi kolesterol. Penelitian dilakukan di Laboratorium Bio Mice and Rat dan Laboratorium Imunologi Poltekkes Kemenkes Denpasar. Penelitian ini menggunakan metode eksperimen sejati dengan Post-test Only Control Group Design. Sebanyak 24 ekor tikus jantan sebagai sampel dibagi menjadi 4 kelompok. Kelompok I (DS): tikus diberi pakan standar; kelompok 2 (DTK): tikus yang diberi diet tinggi kolesterol; kelompok 3 (Ss): tikus diberi pakan standar, diet tinggi kolesterol dan simvastatin 0,8 mg/kgBB, kelompok 4 (Cc): tikus diberi diet tinggi kolesterol dan ekstrak etanol daun kenikir 200mg/kg BB. Perlakuan diet tinggi kolesterol diberikan kepada kelompok DTK, Ss dan Cc selama 20 minggu. Pada minggu ke 17 hingga minggu ke 20, pada kelompok Ss diberikan tambahan simvastatin, kelompok Cc diberikan ekstrak etanol daun kenikir (C.caudatus), kelompok DS hanya diberikan pakan standar selama 20 minggu. Kadar serum PON3 diperiksa setelah 20 minggu perlakuan. Hasil menunjukkan kadar PON-3 serum tikus kelompok Cc lebih tinggi dibandingkan kelompok DTK. Uji one way anova menunjukkan bahwa ada perbedaan yang signifikan dengan p=0,006 dan kadarnya hampir sama dengan kelompok DS (p=0,891), namun lebih rendah dibandingkan Ss. Dari hasil tersebut disimpulkan bahwa terdapat pengaruh dari pemberian ekstrak etanol daun kenikir terhadap kadar PON-3 serum pada tikus.

***Kata kunci**: Ekstrak Etanol Daun Kenikir (Cosmos Caudatus), Kolesterol, Paroxonase-3*

ABSTRACT

Paraoxonase-3 (PON-3) expression in HDL is related to the antioxidant function of HDL. PON3 is a protein that is important in protecting blood vessels from atherosclerosis. Cosmos caudatus is one of the natural ingredients that increase HDL levels. The study aimed to show the effect of ethanol extract from kenikir leaves (C.caudatus) on serum PON-3 levels of rats fed a high-cholesterol diet. This research was conducted at the Bio Mice and Rat Laboratory and Immunology Laboratory at the Health Polytechnic, Ministry of Health, Denpasar. This research used a true experimental method with Post-test Only Control Group Design. A total of 24 male rats were used as samples in this study and

divided into 4 groups. Group 1(DS): rats fed standard diet; group 2 (DTK): rats fed high cholesterol diet; group 3 (Ss): rats fed standard diet, high cholesterol diet, and simvastatin 0.8 mg/kgBW; group 4 (Cc): rats given a high cholesterol diet and ethanol extract of kenikir leaf 200mg/kg BW. High-cholesterol diet treatment was given to the DTK, Ss, and Cc groups for 20 weeks. From week 17 to week 20, the Ss group was given additional simvastatin, and the Cc group was given the ethanol extract of kenikir leaves (C.caudatus). Meanwhile, the DS group was only given standard feed for 20 weeks. Serum PON3 levels were checked after 20 weeks of treatment. The results showed that serum PON-3 levels of rats in the Cc group were higher than in the DTK group. The one-way Anova test showed a significant difference with p=0.006, and the levels were almost the same as in the DS group (p=0.891), but lower than Ss. The study concluded that administering kenikir leaf extract ethanol affected serum PON-3 levels in rats.

Keywords: Ethanol Extract of Kenikir Leaves (Cosmos Caudatus), Cholesterol, Paroxonase-3

INTRODUCTION

In other to avoid atherosclerosis, the antioxidant enzyme paraoxonase (PON) works in conjunction with High Density Lipoprotein (HDL) to shield both HDL and low-density lipoprotein (LDL) from lipid peroxidation[1]. PON prevent oxidation of lipoproteins and arterial cells from oxidation, particularly phospholipids and cholesterol esters. When compared to healthy individuals, patients with diabetes mellitus, familial hypercholesterolemia, and post-myocardial infarction patient had lower serum PON levels[2]. Studies in rabbits have shown that Paraoxonase-3 (PON 3) protects LDL from oxidation[3],while studies in mice have shown that PON 3 has a protective role against atherosclerosis, gallstone disease, and obesity[4].

The activity of paraoxonase-3 (PON 3) prevents LDL from oxidizing. Foods high in unsaturated fat, when consumed in moderation, can raise blood PON, while those high in saturated fats can lower serum PON[5]. In rats with chronic hyperhomocysteinemia, low-dose red grape polyphenol extract dramatically decrased plasma homocysteine levels and restored liver function and plasma PON[6].The defense system regulates the production and elimination of oxidants and is very important in dealing with damage during the oxidative fight process. Defense against ROS includes enzymatic scavengers and antioxidants obtained from the outside, namely from food.

Kenikir leaves (Cosmos caudatus) contain phenolic compounds which are one of the compounds in kenikir leaves that have antioxidant properties. Antioxidants are compounds that have the potential to fight harmful oxidants that can damage body cells. Extraction of kenikir leaf phenolic compounds with Pulse Electric Field (PEF) using a variation of the ratio of material: solvent 1:8, and the PEF exposure time for 6 seconds was 5.87 mg GAE/g fw and the antioxidant capacity was 70.74% [7].

Research on the benefits of kenikir leaf (Cosmos caudatus) as a treatment for hyperlipidemia has been carried out in rats fed a high cholesterol diet and given ethanol extract of kenikir leaf 200 mg/kg body weight compared to atorvastatin weighing 35 mg/kg for four weeks. Mice given C. caudatus extract showed a significant decrease in plasma triglycerides in total cholesterol, LDL and a significant increase in high-density lipoprotein-cholesterol (HDL)[8]. The aimed of this study was to show the effect of ethanol extract of kenikir leaves (C. caudatus) on serum levels of paraoxonase-3 (PON-3) in rats fed high-cholesterol feed.

METHODS

1. Research design

This study used a true experimental method with a Post-test Only Control Group Design.

2. Research sites

The study was conducted in multiple locations. First it involved treating experimental animals in the laboratory for maintaining and breeding of experimental animals Bio Mice and Rat. Subsequently, the serum PON-3 levels were analyzed in the laboratory of the medical laboratory technology department of the Denpasar Health Polytechnic. The study was carried out from March to October 2021.

The Denpasar Health Polytechnic's Health Research Ethics Commission has granted ethical clearance for this study with the reference number LB.02.03/EA/KEPK/0583/2021.

3. Population and research sample

The research sample was 24 male Wistar rats aged 8-12 weeks with a body weight of 100-150 g.

4. Research materials and tools

Measurement of serum levels Rat Paraoxonase-3 used an ELISA kit from the Bioassay Technology Laboratory, and the examination tool used Elisa Rider Phomo Autobio.

5. Collection/research stages

After being chosen at random, twenty-four male Wistar rats that met the criteria were given standard feed and drinking water for a week to help them get used to their new surroundings. Additionally, they were split up into four groups: the DTK group, the Ss group, the Cc group, and the DS group. While DTK, Ss, and Cc had a high cholesterol diet for 20 weeks, DS group received regular feed for 16 weeks. The high cholesterol diet included 50% standard feed, 31.8% wheat, 1% cholesterol, 0.2% cholic acid, 10% pork oil, 2% pig brain, and 5% egg yolk. From week 17 to week 20, the Ss group received an extra dose of 0.8 mg/kgBW/day of simvastatin, whereas the Cc group received a 200 mg/kgBW/day dose of kenikir leaf extract and high-cholesterol meals. Serum PON-3 levels were measured using blood drawn at the conclusion of the fourth week of treatment.

6. Data analysis

The data were not normally distributed and continued with the Kruskal Wallis test, and one way anova test

RESULT

Qualitative and quantitative phytochemical tests of kenikir leaves were carried out in the Food Technology laboratory of UNUD, qualitatively the content of phenols and flavonoids was obtained. By using the spectrophotometric method, the phenol content was 11.26844 g/100 ml GAE (galic acid equivalent) and flavonoids as much as 11.05352 g/100 ml QE (quercetin equivalent).

The study was conducted using 24 male Wistar rats with the age of 12 weeks. Tabel 1 shows, wight of rats weighing 100-120 grams and an average of 110.21 grams. Weight assessments were conducted prior to administering treatment at the conclusion of the 16th week, followed by post-treatment evaluations at the cumulation of the 20th week.

Table 1. Average Body Weight of Rats Before and After Treatment

Group	Average Rat Body Weight (grams)	
	Before Treatment	After Treatment
DS	187,83	201,67
DTK	235	253,67
Ss	233,33	235,5
Cc	231,17	246,83

Tabel 2 indicates that upon analyzing serum PON3 levels, it was observed that the group administered simvastatin (Ss group) exhibited the highest mean serum PON3 level, whereas the group solely fed a high-cholesterol diet displayed the lowest average level.

Table 2. Average levels of Paraoxonase-3 in Rat Serum

Group	Serum PON3 levels (ng/ml)	
	Average	Standard deviation
DS	17,02	1,74
DTK	14,33	1,41
Ss	18,24	3,57
Cc	17,15	1,30

The homogeneity assessment indicated that the data exhibited homogeneity, although not all data followed a normal distribution, necessitating the utilization of the Kruskal-Wallis test for data analysis. Normality was examined via the Shapiro-Wilk test, while homogeneity was assessed using the Levene Statistic.

Tabel 3 indicated that the Kruskal-Wallis test revealed a significant disparity among the treatment groups with a p-value of 0.019. Subsequent analyses employing the Mann-Whitney test and Independent T-test demonstrated variances between the DTK group and DS, Ss, and Cc groups, whereas no dictinctions were observed between DS, Ss, and Cc.

Table 3. The results of the different test data on levels of Paraoxonase-3

Group	Test results	
	Mann-Whitney	T test
DTK - DS	0,016	
DTK - Ss	0,016	
DTK - Cc	0,006	
DS - Ss		0,469
DS - Cc		0,891
Ss - Cc		0,497

DISCUSSION

The findings presented in Table 3 revealed that the use of kenikir leaf extract impacted the serum levels of PON-3 in rats consuming a high-cholesterol diet. This association is attributed to the kenikir leaf extract's capacity to enhance lipid profiles, specifically by elevating High Density Lipoprotein (HDL) levels and reducing LDL levels. These effects contribute to mitigating the risk of atherosclerosis.

Kenikir leaf extract boasts abundant levels of phenols and flavonoids, with polyphenols known for their capacity to enhance plasma HDL levels [9]. PON3, primarily synthesized in the liver and released into the bloodstream, binds with HDL and is

transported to various organs by HDL carriers[10][11]. It is associated with circulating HDL and is also detected in atherosclerotic plaques[12]. Analyzing Apolipoprotein profiles using HPLC (High Performance Liquid Chromatography) revealed the presence of PON-3 within fractions 28 to 31, alongside PON1, in APOA-I-containing particles, while absent in APOA-II or APOE particles[13]. Studies in apolipoprotein-E deficient mice indicated that PON3 augments HDL function[14]. Notably, decreased PON3 levels in HDL of individuals with subclinical atherosclerosis suggest its pivotal role as an antioxidant protein in atherosclerosis prevention. With PON3 identified in APOA-1 HDL particles, an elevation in HDL prompts a corresponding increase in APOA-1, thereby augmenting PON3 levels[15].

Quercetin and PON3 both play a role in preventing atherosclerosis. Kenikir leaf extract contains quercetin. Quercetin is included in the flavonols which are part of the flavonoids[16]. The intake of flavonols is associated with various health benefits which include antioxidant potential and reduced risk of vascular disease, namely atherosclerosis. Macrophages play a vital role in the accumulation of intracellular lipids within the arterial wall, leading to the formation of foam cells within developing atherosclerosis plaques[17]. Quercetin exhibits anti-atherogenic properties by decreasing cholesterol levels in macrophages. This action is achieved through upregulating the expressions of genes associated with peroxisome proliferator-activated receptor γ (PPARγ), liver X receptor α (LXRα) and ATP binding cassette transporter A1 (ABCA1)[18]. In vitro investigations have demonstrated that cosmos caudate extract/fraction exerts its anti-atherogenic effects by mitigating the migration and invasion of smoth muscle cells[19].

The role of quercetin which is one of the antioxidants in kenikir leaf extract in preventing atherosclerosis is also owned by PON3. Paraoxonase acts as an important endogenous enzyme against oxidative stress involved in the pathogenesis of cardiovascular disease. PON-3 functions in thwarting atherosclerosis by hindering LDL-C peroxidation and neutralizinf oxidized phospholipids originating from LDL[20]. Consequently, this diminishes the extent of oxidation implicated in the onset of atherosclerosis[21]. Through in vitro analysis of recombinant HDL (rHDL), it was observed that PON3 engages with the apoA1-HDL particle in a similar way to PON1[15]. This interaction is accompanied by specific modulations at the enzyme's active site, enhancing the stability of rPON3-apoA1-HDL (half-life) significantly in the presence of a calcium chelating agent (>45-fold increase). Serving as an antiatherogenic agent, rPON3 also promotes the HDL-mediated extraction of cholesterol from macrophages[11][22].

The liver serves as the primary location for lipid metabolism[23]. Oxidized LDL (Ox-LDL) enhances oxidative stress by elevating the generation of intracellular reactive oxygen species (ROS) and lipid peroxidation products[21]. Increased ROS is associated with an increased risk of atherosclerosis. Kenikir leaf extract containing phenol and flavonoids can reduce LDL levels. Quercetin which is a flavonoid found in kenikir leaves can reduce LDL levels[8]. Lowering LDL is associated with a reduced risk for atherosclerosis. PON3 mitigates the risk of atherosclerosis by decreasing the initial oxidative products, thereby impeding the propagation of oxidation through the hydrolysis of biologically active oxidized phospholipids and lipid hydroperoxides in Ox-LDL[24]. The efficacy of purified rabbit PON3 in safeguarding LDL-C from oxidation surpasses that of rabbit PON1 by 100-fold[25]. When LDL-C is exposed to stably transfected cells overexpressing human PON3, it exhibits notably reduced lipid hydroxide levels and diminished monocyte chemotactic activity[26]. Furthermore, for the first time, PON3 has been associated with modulating ROS levels in ex vivo and in vivo experimental models, elucidating its role in oxidative stress regulation[27]. In the preventing LDL oxidation induced by copper,PON-3 demonstrates a 100-fold superiority over PON1; however, its efficacy is about 200 times lower than PON1 in HDL rabbits[20]. Likewise, research conducted by Shih et al. on the development of human PON3 transgenic mice sheds

light on the atheroprotective function of PON3. The study reveals a 4-7 times higher expression of PON-3 in the liver of transgenic mice, which exhibited a reduction in lesions comparable to controls when fed an atherogenic diet[4].

Quercetin and PON3 demonstrate potential as anti-inflammatory agents. Kenikir leaf extract, rich in quercetin, influences the serum levels of IL-6 and serum glutathione (GSH) in rats consuming a high-cholesterol diet[28]. The precise mechanism underlying the anti-inflammatory action of quercetin remain uncertain. It is suggested that its effect may involve inhibition of Cyclooxygenase-2 (COX-2) and Inducible nitric oxide synthase (iNOS), nuclear factor (NF)-κB, Activator protein (AP)-1, or mitogen-activated protein kinase (MAPK)[29]. Inhibition these enzymes yields an anti-inflammatory outcome. Nitric oxide (NO), a proinflammatory mediator synthesized by iNOS in response to proinflammatory compounds such as lipopolysaccharides (LPS).

Quercetin induces a transient reduction followed by full restoration of intracellular GSH concentrations. The initial decline in GSH levels does not stem from oxidation to GSSG but results from the formation of quercetin-glutathione conjugates. Studies have shown that quercetin prompts GSH loss in human aortic endothelial cells (HAEC) not through oxidation but via the generation and cellular export of quercetin-glutathione conjugates. Quercetin induction of GCL subsequently replenishes GSH levels, thereby suppressing oxidant production triggered by lipopolysaccharide (LPS)[30]. A critical role of cellular glutathione is to bind to free radicals and peroxides generated during normal cellular respiration, preventing the oxidation of proteins, lipids, and nucleic acids. One mechanism countering oxidative damage involves the transactivation of genes encoding enzymes involved in glutathione metabolism and synthesis. Quercetin enhances the body's antioxidant capacity by modulating GSH levels. When oxygen free radicals are produced, superoxide dismutase (SOD) rapidly captures O^{2-} and converts it into H_2O_2. This enzyme further decomposes H_2O_2 into non-toxic H_2O, requiring GSH as a hydrogen donor. Animal and cell studies demonstrate that quercetin stimulates GSH synthesis[31], and regulates GSH-related redox homeostasis in enterocytes. At high doses of quercetin, the dynamic balance of GSH is disrupted, leading to the conversion of H_2O_2 [32] to H_2O and the oxidation of GSH to GSSG (oxidized glutathione disulfide) by GSH peroxidase[33]. The anti-inflammatory and antioxidant potential of PON3 is evidenced in a murine model of liver injury induced by carbon-tetrachloride (CCl4) over expressing PON3, resulting in elevated glutathione levels and reduced levels of malonyl-di-aldehyde (MDA), tumor necrosis factor (TNF-α)), and interleukin-(IL-1β)[11][34].

Kenikir leaf extract contains phenol, which exhibit antioxidant properties. Phenolics within polyphenols can receive electrons, forming relatively stable phenoxyl radicals, thus disrupting chain oxidation reactions within cellular components[35]. Consequently, foods and beverages abundant in polyphenols can heighten the antioxidant capacity of plasma. The surge in plasma antioxidant capacity following the intake of polyphenol-rich foods may be attributed to the concentration of other reducing agents (known as the sparing effect of polyphenols on other endogenous antioxidants), or their influence on the absorption of pro-oxidative food components, such as iron[36]. Antioxidants have the potential to increase serum PON3 levels. Treatment with insulin sensitizers, ezetimibe, and valsartan, possessing antioxidant properties, can impact PON3 activity, ultimately normalizing oxidative stress in rat serum and liver. This observed effect might be due to an upsurge in PON3 expression[37].

CONCLUSION

Based on the findings of the study, it can be inferred dthat the ethanol extract derived from kenikir leaves influences the serum levels of PON3 in rats subjected to a high cholesterol diet. However, the mechanism for increasing PON3 by ethanol extract

of kenikir leaves is not clear. It is recommended that further research be carried out regarding its effect on genes involved in cholesterol biosynthesis.

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