



Effects of patin fish oil and turmeric extract on levels of malondialdehyde and superoxide dismutase in wistar rat models of metabolic syndrome

Efek minyak ikan patin dan ekstrak kunyit terhadap kadar malondialdehida dan superoksida dismutase pada model tikus wistar sindrom metabolik

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Abstract

Increased oxidative stress in metabolic syndrome conditions leads to increased malondialdehyde and decreased superoxide dismutase. Patin fish oil (*Pangasius hypophthalmus*) and turmeric extract (*Curcuma longa* Linn.) contain bioactive compounds that play a role in improving malondialdehyde and superoxide dismutase levels. The research obtained to measure the effectiveness of patin fish oil, turmeric extract, and their combination against MDA and SOD levels. The research applied an experimental design of randomized pre-post test control groups. Research location at PSPG Animal Laboratory, UGM Yogyakarta in August to October 2023. Wistar male white rats (thirty rats) were randomly divided into five groups, namely K- (Normals), K+ (HFFD), P1(HFFD+catfish oil dose of 0,08 ml/200grBB), P2(HFFD+turmeric extract dose of 5,04 mg/kgBB), and P3(HFFD+catfish oil dose of 0,08 ml/200grBB+turmeric extract dose of 5,04 mg/kgBB). Intervention for 28 days. Information analysis utilizing combined t-test and one-way ANOVA test taken after by post-hoc test. Outcome, catfish oil, turmeric extract, and their combination of them all had an significant effects on MDA levels ($p=0,000$) and SODs ($p=0,000$). The mean reduce in MDA levels and increment in SOD were more prominent in P3, which amounted to $5,29\pm 0,28$ nmol/ml and $22,62\pm 7,03$ units/ml. In conclusion, catfish oil and turmeric extract successfully decrease MDA and increment SOD. The combination of both have the potential to improve metabolic syndrome.

Keywords: Fish oil, malondialdehyde, metabolic syndrome, superoxide dismutase, turmeric extract

Abstrak

Peningkatan stres oksidatif pada kondisi sindrom metabolik menyebabkan peningkatan malondialdehida dan penurunan superoksida dismutase. Minyak ikan patin (*Pangasius hypophthalmus*) dan ekstrak kunyit (*Curcuma longa* Linn.) mengandung senyawa bioaktif berperan dalam memperbaiki kadar malondialdehida dan superoksida dismutase. Penelitian bertujuan untuk mengukur efektivitas minyak ikan patin, ekstrak kunyit, dan kombinasinya terhadap kadar MDA dan SOD. Penelitian menggunakan desain eksperimen randomized pre-post test control group. Lokasi penelitian di Laboratorium Hewan Coba PSPG, UGM Yogyakarta pada bulan Agustus hingga Oktober 2023. Tikus putih jantan wistar (30 ekor) dikelompokkan secara acak menjadi lima, yaitu K- (Normal), K+ (HFFD), P1 (HFFD+minyak ikan patin dosis 0,08 ml/200 grBB), P2 (HFFD+ekstrak kunyit dosis 5,04 mg/kgBB), dan P3 (HFFD+minyak ikan patin dosis 0,08 ml/200 grBB+ekstrak

kunyit dosis 5,04 mg/kgBB). Intervensi selama 28 hari. Uji-t berpasangan dan analisis varians satu arah digunakan untuk analisis data, diikuti dengan uji post hoc. Hasil, minyak ikan patin, ekstrak kunyit, dan kombinasinya semua berpengaruh signifikan terhadap kadar MDA ($p=0,000$) dan SOD ($p=0,000$). Rata-rata penurunan kadar MDA serta peningkatan SOD lebih besar pada P3, yaitu sebesar $5,29\pm 0,28$ nmol/ml dan $22,62\pm 7,03$ unit/ml. Kesimpulan, minyak ikan patin dan ekstrak kunyit efektif menurunkan MDA dan meningkatkan SOD. Kombinasi keduanya berpotensi untuk perbaikan sindrom metabolik.

Kata Kunci: Ekstrak kunyit, minyak ikan, malondialdehida, superoksida dismutase, sindrom metabolik

Introduction

Metabolic syndrome is one of the most common public health problems worldwide (Monserrat-Mesquida et al., 2020). As the prevalence of obesity has increased worldwide, metabolic syndrome has developed into a global epidemic. The World Obesity Atlas 2023 estimates that 38% of people are overweight and obese in 2020, and this will increase to 51% by 2035 (Lobstein et al., 2023). As the prevalence of obesity has increased worldwide, metabolic syndrome has developed into a global epidemic. The World Obesity Atlas 2023 estimates that 38% in 2020 were overweight and obese and this will increase to 51% by 2035.

(Justin et al., 2017), 12-37% of Asians and 12-26% of Europeans experience metabolic syndrome (Ranasinghe et al., 2017). This condition also occurs in Indonesia (28% of men and 46% of women) and is still a very worrying problem with a much higher prevalence rate compared to other countries (39%, 29,2% and 30% respectively in adults in Indonesia, the Netherlands and India) (Sigit et al., 2020)(Krishnamoorthy et al., 2020).

Various metabolic disorders and chronic diseases, such as hypertension, type 2 diabetes, hyperlipidemia, obesity, osteoporosis, and cancer, are influenced by lifestyle changes, such as consumption of foods high in carbohydrates, cholesterol, trans fats, and low physical activity (Zerga & Bezabih, 2020). In Indonesia, the proportion of metabolic syndrome components includes central obesity (41,5%), high triglyceride (TG) levels (27,1%), hypertension (HT) (61,3%), high density lipoprotein (HDL) levels. low (38,7%), and high fasting blood glucose (GDP) levels (51,0%) (Sigit et al., 2020).

Under conditions of metabolic syndrome, there is an increase in oxidative stress and inflammation caused by the disruption of

homeostasis in the production of reactive oxygen species (ROS) and a decrease in the antioxidant defense system (Cheng et al., 2017). Previous research has shown that lower levels of antioxidant enzyme activity in the plasma and higher levels of markers of oxidative damage (especially lipid peroxidation) lead to increased oxidative stress in metabolic syndrome conditions (Vona et al., 2019). Malondialdehyde (MDA) is the end product of lipid peroxidation resulting from the peroxidation of polyunsaturated fatty acids and functions as a marker for lipid peroxidation as well as a hallmark of oxidative damage associated with obesity and metabolic syndrome. Increased systemic oxidative stress leads to increased MDA and decreased superoxide dismutase (SOD) (Francisqueti et al., 2017) (Yadav et al., 2015).

Omega 3 (EPA and DHA) and active secondary metabolites, such as flavonoids, tannins, saponins, and curcumin, are bioactive compounds that can synergistically fight lipid peroxidation and improve endothelial function (Francisqueti et al., 2017). Omega 3 fatty acids or polyunsaturated fatty acids are essential fatty acids that cannot be synthesized by the body and are only obtained from outside the body, most of which are found in food, especially fatty fish, such as catfish (Ateya et al., 2017). Catfish (*Pangasius hypophthalmus*) is a type of Indonesian freshwater fish that has a higher protein and fat content (16,1% and 5,7 %, respectively) than other types of freshwater fish (Hashim et al., 2015). Previous studies have shown that the administration of fish oil to mice with dyslipidemia can reduce serum and liver triglyceride concentrations and increase SOD activity in erythrocytes and liver (Lima Rocha et al., 2022). Other studies have also report that

omega 3 significantly increases serum total antioxidant capacity (TAC) and glutathione peroxidase (GPx) activity, and reduces malondialdehyde (MDA) levels, thereby helping in antioxidant protection against reactive oxygen species (ROS) (Heshmati et al., 2019). Catfish oil can be obtained from an extraction (rendering) process, one of which is the wet rendering method (Nurfadilah, 2020).

In conditions of metabolic syndrome, antioxidants in the body (endogenous) decrease, so antioxidants from outside (exogenous) are needed to increase the cellular antioxidant defense system to prevent free radicals from damaging cells (Francini-Pesenti et al., 2019) one of which is made from natural ingredients, namely turmeric (*Curcuma longa* Linn.). The secondary metabolites contained in turmeric are flavonoids, alkaloids, terpenoids, saponins, tannins, and polyphenols, which function as antioxidants, anti-inflammatory agents, blood anticoagulants, anticancer agents, and antibiotics, and are able to inhibit carcinogenic effects (Vaou et al., 2021). Previous studies have shown that turmeric contains flavonoids, phenolics, and curcumin, which is one of the species with the highest antioxidant potential compared to other species. These compounds protect cells and cell parts from damage caused by reactive free radicals (Muflihah et al. 2021). The content of biologically active compounds in turmeric can be obtained through an extraction process by soaking in 96% ethanol to produce a thick turmeric extract (Ningsih et al., 2020). Administration of turmeric ethanol extract at a dose of 200 mg/kg BW prevents oxidative stress by reducing plasma and brain MDA levels and increasing the activity of SOD, CAT, GPx, and GSH enzymes in the brain, thus providing a neuroprotective effect in the rat brain (Yuliani et al., 2019).

Catfish oil and turmeric extract can provide alternative therapies to improve the condition of metabolic syndrome, and their combination is expected to reduce cell and tissue damage due to oxidative stress and improve the cellular antioxidant defense system. Metabolic syndrome therapy with functional foods has been performed, but the use of a combination of catfish oil (*Pangasius hypophthalmus*) and turmeric extract (*Curcuma longa* Linn.) as support for improving the condition of metabolic syndrome is not widely

known. Therefore, this study aimed to measure the effectiveness of catfish oil (*Pangasius hypophthalmus*) and turmeric extract (*Curcuma longa* Linn.) and their combination on MDA and SOD levels.

Methods

This study was a real experiment using a randomized pre-post-test design with a control group. Research and data collection were carried out from August to October 2023. The research began with the process of making the intervention material, namely catfish oil, using the wet rendering method and purifying by adding 4% bentonite adsorbent. Turmeric extract was processed using the immersion method with 96% ethanol solvent, followed by preservation of the turmeric extract using freeze-drying techniques. Preclinical tests on mice were performed at Gadjah Mada University (UGM), Yogyakarta, at the Experimental Animal Laboratory at the Center for Food and Nutrition Studies (PSPG).

This study used samples of male Wistar white rats (*Rattus norvegicus*), obtained from the Experimental Animal Laboratory, Center for Food and Nutrition Studies (PSPG), Gadjah Mada University (UGM), Yogyakarta with inclusion criteria, namely rats aged 6-8 weeks (adult rats), the body weight of the mice ranged from 150-200 grams, and the mice met three of the four criteria for metabolic syndrome, namely Lee index >300, total cholesterol >129,52 mg/dL, LDL cholesterol >81,55 mg/dL, and fasting blood sugar (GDP) >111,7 mg/dL. The sample size of this study was 30 mice (Percie du Sert et al., 2020). The selection of experimental animals was carried out randomly (Purposive Random Sampling) into five groups consisting of: negative control (K-), namely healthy mice without intervention; positive control (KP), namely the metabolic syndrome group given HFFD feed; treatment group 1 (P1) was given HFFD feed + catfish oil intervention at a dose of 0,08 ml/200 grBW per day; treatment group 2 (P2) was given HFFD feed + turmeric extract at a dose of 5,04 mg/kgBW per day, and treatment group 3 (P3) was given HFFD feed + a combination of catfish oil at a dose of 0,08 ml/200 grBW per day and turmeric extract dose of 5,04 mg/kgBW per day, each group consisting of six mice. To reduce the possibility of bias,

random allocation was used and tightened with negative and positive control groups.

The independent variables were catfish oil (*Pangasius hypophthalmus*) and turmeric extract (*Curcuma longa* Linn.) The dependent variables were malondialdehyde (MDA) and superoxide dismutase (SOD). The raw materials used in this study were Siamese catfish, fresh turmeric rhizome, 4% bentonite, 96% ethanol, distilled water, 0,5% CMC powder, Comfeed AD II standard feed containing 12% water, and 15% crude protein., 3-7% crude fat, and 6% crude fiber. High fat fructose diet (HFFD) feed contains a composition consisting of 3 grams of pork fat, 2 grams of duck egg yolk, and 1% fructose equivalent to 2 ml, as well as MDA and SOD reagents. The HFFD dose administered to mice was based on calculating the dose of pork fat to humans, which was converted to experimental animals by multiplying the human dose of 150 mg/day by the human dose to mice with a conversion factor of 0,018, to obtain a dose of 2,7 gr/200 grBW/ day or 3 gr/200 grBB/day. The dose for duck egg yolk was 2 gr/200 grBB/day. The HFFD feed is made from a mixture of 3 g of pork fat, 2 g of duck egg yolk, and 2 ml of fructose (Harsa, 2014).

The dose of catfish oil in mice was determined based on previous research in humans which used fish oil at a dose of 4 g/day (Albracht-Schulte et al., 2018) and converted to experimental animals, the human dose obtained for white mice was 0,072 gr/200 grBW/day. It is known that the density (density) of catfish oil is 0,8924 gr/ml, so the dosage formula calculation is obtained as follows:

$$v = \frac{0,072 \text{ gr/hari}}{0,8924 \text{ gr/ml}} = 0,08 \text{ ml/200 grBW}$$

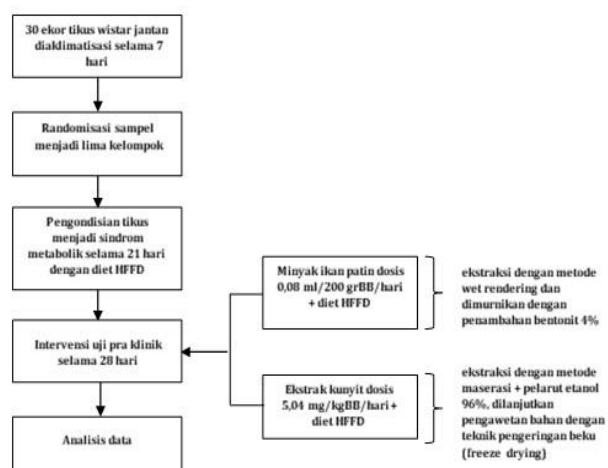
The dose of turmeric extract determined based on PMK of the Republic of Indonesia Number 6 of 2017 concerning the Formulation of Indonesian Herbal Medicine for turmeric in humans is 200 mg/kg BW (Kementerian Kesehatan Republik Indonesia, 2016); therefore, the dose calculation formula used is: Human dose 200 mg/kgBW \times 70 kg = 1400 mg/200 gBW/day, then multiplied by the mouse conversion factor to calculate the formula to 1400 mg/200 g BW \times 0,018 = 25,2 mg, multiplied by 0,2 kg so a total dose of 5,04 mg/kgBW/day.

Thirty male Wistar rats were acclimatized for seven days by providing standard Comfeed AD II feed (20 g/day) and water ad libitum. After the adaptation period was complete, the body weight of the mice was measured, and the mice were randomly divided into five groups using simple random sampling. The five groups were the negative control (K-), positive control (K+), treatment 1 (P1), treatment 2 (P2), and treatment 3 (P3). The mice were then fed a high-fat fructose diet (HFFD) of 20 g per day along with water ad libitum for 21 days, while the mice experienced metabolic syndrome, especially in the positive control group (K+), treatment 1 (P1), and treatment 2 (P2), and Treatment 3 (P3). The negative control group (K-) was given standard food and water ad libitum. Before intervention, the mice's body weight was measured first, and then blood was collected from the eyes (plexus retro orbitalis) to examine lipid profiles and fasting blood glucose (criteria for metabolic syndrome), MDA levels, and superoxide dismutase (SOD) levels as pretest data.

During the intervention period, treatment groups P1, P2, and P3 continued to receive HFFD feed until the end of the study for 28 days, along with the intervention materials and water ad libitum. Group P1 received HFFD feed with catfish oil at a dose of 0.08 ml/200 grBW/day, group P2 received HFFD feed and turmeric extract at a dose of 5.04 mg/kgBW/day, group P3 received HFFD feed and a combination of catfish oil at a dose of 0 .08 ml/200 grBB/day and turmeric extract dose 5,04 mg/kgBB/day, The two intervention materials were suspended in 0,5% carboxymethyl cellulose (CMC) solvent, prepared by weighing CMC powder (0,5 g), dissolved in 100 ml of distilled water, homogenized by heating using a hot plate stirrer, and then cooled. The intervention was performed via a nasogastric tube. The negative control group (K-) received standard feed and water ad libitum until the end of the study. At the end of the study, the body weight of the mice was measured again, and blood was collected via the retroorbital plexus to analyze MDA and SOD levels as post-test data. Rats that have been used for observation in research will be terminated and then destroyed by burning in the available place (burning furnace).

The data collected included the body weight of the mice from the acclimatization

period until the end of the study. MDA levels were measured using the Thiobarbituric Acid Reactive Substance (TBARS) method. The principle of the TBARS method is that MDA levels react with thiobarbituric acid (TBA) and produce a pink color with a wavelength of 532 nm (Tsikas, 2017), while SOD levels were measured by enzyme-linked immunosorbent assay (ELISA) using the water-soluble Tetrazolium Salt-1 (WST-1) method. The principle of the WST-1 method is that the reaction between xanthine and xanthine oxidase produces superoxide radicals that reduce nitroblue tetrazolium (NBT) to purple formazan (Novarini et al., 2022). All protocols in this study were approved by the Health Research Ethics Committee of the Faculty of Medicine, Diponegoro University, Semarang No. 57/EC-H/KEPK/FK-UNDIP/VI/2023.



Picture 1. Research flow diagram

The collected data were analyzed using statistical applications. The normality of the MDA and SOD levels was tested using the Shapiro-Wilk test, which showed that all variables, both MDA and SOD levels in the intervention group and control group, were normally distributed, and the p-value was greater than 0,05 {MDA level: K- (0,212); K+ (0,785); P1 (0,338); P2 (0,189); P3 (0,778) and SOD levels: K- (0,167); K+ (0,091); P1 (0,097), P2 (0,341); P3 (0,161)}. The paired t-test was used to determine differences in MDA and SOD levels before and after intervention, while the ANOVA test and the post hoc test, namely the Bonferroni post hoc test for the same data variance and Tamhane test, were used to determine variations between the five groups.

Post-hoc test for data with different variants. Kruskal Wallis test was followed by the Mann-Whitney test for data that were not normally distributed (Dahlan, 2015).

Result and Discussion

Patin Fish Oil (*Pangasius hypophthalmus*)

From the extraction of Siamese catfish (*Pangasius hypophthalmus*) using the wet rendering method, catfish oil was obtained at a yield of 7,28%. Catfish oil was purified by adding an adsorbent in the form of 4% bentonite and then centrifuged for 20 min at 3000 rpm to obtain 180 ml of pure catfish oil. This study tested and analyzed the quality parameters of catfish oil based on the International Fish Oil Standard (IFOS) reference and analyzed the omega 3 fatty acid content, including EPA and DHA, carried out at the PT Laboratory. Saraswanti Indo Genetech (SIG) Semarang. The test parameters for the catfish oil are presented in Table 1.

Table 1. Characteristics of catfish (*Pangasius hypophthalmus*) oil based on IFOS standards.

Test Parameters	Result
DHA (mg/100gr)	6093,2
EPA (mg/100gr)	4770,7
Peroxide Number (mEqO2/kg)	0,978
Free Fatty Acid (%)	0,46

Table 1 presents the quality parameters of catfish oil (*Pangasius hypophthalmus*), including peroxide and free fatty acid numbers, meeting the standards set by IFOS, namely peroxide numbers ≤ 5 mEqO2/kg and free fatty acid numbers $\leq 1,50\%$. The lower the peroxide number, the better is the oil quality (Syarifuddin et al., 2022). The higher the amount of free fatty acids, the lower the quality of fish oil (Putri et al., 2020).

Turmeric Extract (*Curcuma longa* Linn.)

The turmeric extract in this study was obtained from the extraction of 350 g of fine turmeric simplicia using the maceration method and a 96% ethanol solvent. From the evaporation of the turmeric filtrate, 100,87 gr of the thick turmeric extract was obtained. The thick turmeric extract was preserved in a

preservation process using freeze-drying techniques, resulting in a total weight of turmeric extract of 44,58 gr. The yield of turmeric extract obtained in this study was 28,82%. Table 2 shows the results of the quantitative analysis of the content of secondary metabolite compounds in turmeric extract, which was carried out at the Agricultural Technology Laboratory, Soegijapranata Christian University (UNIKA), Semarang. The freeze-drying method was used to preserve the extract because it maintains the quality of the drying results, inhibits microbial activity, and maintains the stability of the extract. Thus, the compounds contained in the extract are safe against degradation (Suhesti, 2019). Pada Tabel 2 diketahui bahwa kandungan senyawa tertinggi pada ekstrak kunyit yaitu total flavonoid, hal ini dikarenakan kunyit memiliki polaritas yang lebih tinggi, sehingga ekstraksi maksimum dapat dicapai pada pelarut yang sama (Khanifah et al., 2021).

Tabel 2. Kandungan metabolit sekunder pada ekstrak kunyit (*Curcuma longa* Linn.) dengan metode pengeringan beku (*freeze drying*)

Parameter	Hasil (gr/100 gr)
Total flavonoid	18,728
Tanin	1,752
Saponin	4,457

Body Weight of Test Animals During Metabolic Syndrome Conditioning

There was a significant difference in the mean body weight of mice before and after HFFD feeding (Table 4) in K- (P =0,000), K+ (P =0,000), P1 (P =0,000), P2 (P =0,000), and P3 (P =0,000). Meanwhile, the results of the one-way ANOVA statistical test showed that there was a significant difference in the mean body weight after feeding HFFD in the five groups (p=0,000), and there was a significant difference in the mean change in body weight after feeding HFFD between the five groups (p=0,000).

Rats in the K+, P1, P2, and P3 groups all experienced an increase in body weight after being fed HFFD, although the difference in the increase was not much different. Consuming a diet that is high in carbohydrates and fat, such as HFFD feed containing pork fat, duck egg yolk, and fructose, can cause changes in the body weight of experimental animals. This causes an increase in the amount of fat, especially that

stored in adipose tissue under the skin and abdominal cavity. Previous studies have shown that administering a high-fat, high-fructose diet (HFHF) and a high-fat diet (HFD) for two months could increase rat body weight and levels of oxidative stress in plasma and tissue (Lozano et al., 2016). There was no difference in the mean body weight before HFFD feeding in the five groups (p=0,754) because all groups of mice were still in normal condition (had not received treatment), so there was no difference in the statistical tests.

Metabolic Syndrome Mice

The results presented in Table 3 indicate that the K+, P1, P2, and P3 groups showed an increase in metabolic syndrome indicators such as obesity, dyslipidemia, and hyperglycemia. In addition, compared with the other groups of mice, the K+ group showed the highest indicators of metabolic syndrome. Previous studies have shown that the combination of dietary fat and sugar in feed and drink is associated with the development of type 2 diabetes mellitus and long-term effects such as hyperglycemia, insulin resistance, and dyslipidemia (hypertriglyceridemia and hypercholesterolemia) (Taskinen et al., 2019). Other studies have also shown that administering a high-fat and fructose diet (HFFD) to mice for eight days can increase body fat, blood sugar, triglycerides, and reduce HDL cholesterol, but there is no increase in LDL cholesterol and total cholesterol levels (Vidal et al., 2019).

The Effectiveness of Catfish (*Pangasius hypophthalmus*) Fish Oil, Turmeric Extract (*Curcuma longa* Linn.) and a Combination of Both on Rat Body Weight

Based on the research results (Table 4), a significant difference was observed in the mean body weight of mice before and after the intervention in groups K- (P =0,000), K+ (P =0,000), P1 (P =0,000), P2 (P =0,000), and P3 (P =0,000). There was a significant difference in the mean body weight of the mice between the five groups (p=0,000). Group P3 experienced lower weight loss after the intervention than groups P1 and P2. In Table 4, the body weights of groups P1, P2, and P3 still increased after the intervention, but compared with the K+ group, the body weights of groups P1, P2, and P3 experienced a significant decrease.

Table 3. Mean metabolic syndrome indicators after HFFD feeding

indicators of metabolic syndrome	Mean Metabolic Syndrome Indicator (Mean ± SD)				
	K- (n=6)	K+ (n=6)	P1 (n=6)	P2 (n=6)	P3 (n=6)
Lee Index	281,72 ± 5,44	357,90 ± 3,28	320,83 ± 2,20	323,96 ± 2,49	316,68 ± 2,93
Total cholesterol (mg/dL)	95,07 ± 3,19	249,82 ± 5,08	183,75 ± 4,18	181,11 ± 3,46	171,84 ± 2,92
Fasting Blood Sugar (mg/dL)	73,19 ± 2,32	159,27 ± 3,12	122,96 ± 1,96	118,82 ± 2,32	114,16 ± 3,54
Fasting Blood Sugar (mg/dL)	27,13 ± 2,22	90,15 ± 3,35	59,79 ± 2,14	57,90 ± 1,52	54,12 ± 3,01

Table 4. Effectiveness of HFFD feeding and intervention of patin fish oil and turmeric extract in metabolic syndrome rats

Category	Group	n	Median (Mean±SD)			p-value
			Before	After	Δ	
Body weight of mice fed HFFD (gr)	K-	6	188,33±4,84	210,00±4,47 ^a	21,67±1,37 ^a	0,000*
	K+	6	187,50±5,93	254,83±6,15 ^b	67,33±0,82 ^b	0,000*
	P1	6	189,17±3,66	255,33±3,93 ^b	66,17±0,75 ^b	0,000*
	P2	6	185,50±4,04	252,17±3,49 ^b	66,67±1,03 ^b	0,000*
	P3	6	187,83±5,42	254,33±3,83 ^b	66,50±1,76 ^b	0,000*
	p'			0,754**	0,000**	0,000**
Body Weight of Rats Given Intervention (gr)	K-	6	210,00±4,47 ^a	239,17±4,02 ^a	29,17±1,72 ^a	0,000*
	K+	6	254,83±6,15 ^b	354,67±5,35 ^b	99,83±1,17 ^b	0,000*
	P1	6	255,33±3,93 ^b	330,17±3,87 ^c	74,83±1,47 ^c	0,000*
	P2	6	252,17±3,49 ^b	335,67±3,93 ^c	83,50±0,55 ^d	0,000*
	P3	6	254,33±3,83 ^b	329,50±4,85 ^c	75,17±1,17 ^c	0,000*
	p'			0,000**	0,000**	0,000***
Malondialdehyde (MDA) levels (nmol/ml)	K-	6	1,93±0,15 ^a	2,13±0,14 ^a	0,198±0,05 ^a	0,000*
	K+	6	10,14±0,40 ^b	11,76±0,36 ^b	1,62±0,15 ^b	0,000*
	P1	6	10,295±0,34 ^b	6,17±0,21 ^c	4,13±0,23 ^c	0,000*
	P2	6	10,40±0,45 ^b	5,94±0,14 ^c	4,47±0,50 ^c	0,000*
	P3	6	10,28±0,40 ^b	4,99±0,19 ^d	5,29±0,28 ^{c,d}	0,000*
	p'			0,000**	0,000**	0,000**
Superoxide Dismutase (SOD) levels (units/ml)	K-	6	83,04±3,34 ^a	79,46±4,34 ^a	3,57±1,59 ^a	0,000*
	K+	6	25,89±3,34 ^b	22,92±4,14 ^b	2,98±1,46 ^a	0,000*
Body weight of mice fed HFFD (gr)	P1	6	25,595±2,92 ^b	34,82±3,34 ^c	9,23±4,29 ^b	0,000*
	P2	6	25,89±3,34 ^b	38,39±3,34 ^c	12,50±4,52 ^b	0,000*
	P3	6	24,70±4,14 ^b	47,32±3,34 ^d	22,62±7,03 ^{b,c}	0,000*
	p'			0,000**	0,000**	0,000**

Information. *Uji Paired t-test; **Uji One-Way ANOVA; ***Uji Kruskal-Wallis

This increase was caused by an HFFD diet containing fat and fructose, which continued until the end of the intervention (28 days). Therefore, in obese mice, there is an increase in the amount of fatty acids in the body that are stored in the tissues, especially under the skin. Based on the Kruskal-Wallis test, there was a difference in the average body weight of mice before and after intervention between the five groups (p=0,000). Meanwhile, based on the Mann-Whitney test, there was a difference in the average body weight of mice before and after

intervention in all groups. Previous research on mice with a metabolic syndrome model induced by a high-fat diet showed that mice experienced an increase in body weight of more than 20% during the study period (Auberval et al., 2017). Another study analyzing the administration of catfish oil to hypercholesterolemia model mice showed that catfish oil was able to prevent weight gain and liver fat accumulation in the intervention group compared with the control group (Firmansyah et al., 2017). Another study on animal models of mice induced with a high-

fat diet (HFD) analyzed the effects of the bioactive compound quercetin as a natural antioxidant and reported that the addition of 0,1% quercetin to the HFD could reduce weight gain in mice for 17 weeks (Dong et al., 2014).

Effectiveness of Giving Catfish (*Pangasius hypophthalmus*) Fish Oil, Turmeric Extract (*Curcuma longa* Linn.), and a Combination of Both on Malondialdehyde (MDA) Levels in Rats

The results in Table 4 show that the average MDA levels of mice decreased after intervention in the P1, P2, and P3 groups compared to the K- and K+ groups. There was a significant difference in MDA levels before and after the intervention in the K- (P =0,000), K+ (P =0,000), P1 (P =0,000), P2 (P =0,000), and P3 (P =0,000) groups. There was a significant difference in the MDA levels before and after the intervention between the groups (p=0,000). There were differences in changes in MDA levels before and after the intervention between the K + and K+ groups and the P1, P2, and P3 groups. There was no difference in MDA levels before and after the intervention between groups P1 and P2 (p=0,866) and between groups P2 and P3 (p=0,083). This is because there was a decrease in MDA levels in the three groups which were not much different, so they were not statistically different.

The decrease in MDA was greater in the P3 group than in the P1 and P2 groups, because omega 3 fatty acids bind to COX1 and COX2 to prevent the formation of PGE2, which acts as an anti-inflammatory agent to reduce inflammatory cell activity and reduce inflammation (Wahjuni, 2015). The antioxidant activity of omega 3 fatty acids and flavonoid compounds in turmeric extract neutralizes free radicals by donating electrons to free radical compounds, making them more stable and not causing lipid peroxidation (Yassir et al., 2023).

The Effectiveness of Pin-fish Oil (*Pangasius hypophthalmus*), Turmeric Extract (*Curcuma longa* Linn.) and a Combination of Both (SOD) Levels

The results presented in Table 4 show an increase in SOD levels after intervention in the P1, P2, and P3 groups compared to the K+ group. The greatest increase in SOD occurred in the P3 group, because omega 3 fatty acids (EPA and

DHA) in fish oil activate the nuclear factor erythroid 2 related factor 2 (NRF2) gene, which plays a role in increasing the synthesis of endogenous antioxidants by increasing antioxidant enzymes such as SOD and glutathione peroxidase. and catalase, as well as reducing ROS production through the endogenous antioxidant activity of the SOD enzyme (Hendrawati, 2017). Previous studies have reported that administration of omega 3 fatty acids at a dose of 36 mg/kg BW/day had a significant effect on increasing SOD levels in a mouse model of dyslipidemia (Yassir et al., 2023). There was a significant difference in SOD levels before and after intervention in K- (p=0,000), K+ (p=0,000), P1 (p=0,000), P2 (p=0,000), and P3 (P=0,000). There was a significant difference in the SOD levels before and after the intervention between the groups (p=0,000). There were differences in changes in SOD levels before and after intervention between the K- group and the P1 group (p=0,004), P2 (p=0,001), P3 (p=0,002) and between the K+ group and the P1 group (p=0,005), P2 (p=0,002), P3 (p=0,002), and between groups P1 and P3 (0,037).

Flavonoid compounds act as antioxidants that can reduce and capture free radicals due to fat peroxidation by supporting the activity of SOD in neutralizing superoxide ions, thus preventing cell damage due to free radicals (Sunaryo et al., 2015). Administration of 200 mg/kg BB ethanolic turmeric extract prevented increased oxidative stress and reduced MDA levels in the plasma and brain while increasing the activity of SOD, CAT, GPx, and GSH enzymes in the rat brain (Yuliani et al., 2019).

This study did not fully control for other components in the diet of mice that could influence the results of the study because the observation duration was short (28 days) and the focus of the study was only on MDA and SOD levels, which may limit our understanding of the effects of catfish oil and turmeric extract.

Conclusion

The intervention of catfish oil, turmeric extract, and a combination of both was effective in reducing MDA levels and increasing SOD levels in Wistar rats with metabolic syndrome. The greatest decrease in

MDA levels and increase in SOD levels occurred in P3 group mice that were given a combination intervention of catfish oil at a dose of 0,08 ml/200 grBW/day and turmeric extract at a dose of 5.04 mg/kgBW/day.

Recommendations for further research include determining the appropriate dosage for catfish oil and turmeric extract, especially so that MDA and SOD levels reach normal values, encouraging the development of packaged products, and collaborating with the pharmaceutical industry to support government efforts in creating environmental health to prevent metabolic diseases or non-communicable diseases (NCDs).

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