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Effects of catfish oil and *Curcuma longa* L. extract on ApoB levels and atherosclerosis risk indicators in metabolic syndrome rats

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ABSTRACT

The increase in ApoB levels, atherogenic index of plasma (AIP), and Castelli risk index I-II (CRI) involves cardiovascular disease in metabolic syndrome. Lipid peroxidation could initiate the process of atherosclerosis and involve in the etiology of cardiovascular disease. Dietary fish oil and extract of *Curcuma longa* Linn. have been shown to reduce lipid peroxidation and atherosclerosis. The aim of this study was to determine the effect of catfish oil and *Curcuma* extract on ApoB levels and lipid ratios as protective nutraceutical for metabolic syndrome. Thirty male Wistar rats were randomly divided into 5 groups namely healthy control (HC), high-fat high-fructose diet (HFFD), and intervention group P1 (HFFD + catfish oil 0.08 ml/g/day), P2 (HFFD + *Curcuma* extract 5.04 mg/g/day), and P3 with HFFD + combination of both. HFFD induced metabolic syndrome in rats. ApoB levels were measured by the immunoturbidimetric method and lipid levels with ELISA. Lipid ratios were calculated by AIP (log TG/HDL), and the CRI I-II (TC/HDL, LDL/HDL). The study was showed significantly reduced in ApoB levels, AIP, and CRI in intervention groups. The correlation test results showed that a significant correlation among ApoB levels, AIP, and CRI. In conclusion, combination of both may decrease levels of ApoB and atherosclerosis risk indicators in metabolic syndrome rats.

INTRODUCTION

Non-communicable diseases (NCD) remain to be the primary cause of morbidity and mortality globally [1]. Based on the World Obesity Atlas 2023, the present obesity rate stands at 38% of the population, with projections indicating a potential doubling of prevalence between 2020-2035 to reach 10-20%. Obesity is related to cardiovascular diseases [2]. From 2000 to 2019, the prevalence of metabolic diseases has been increasing over the last two decades with the greatest increase in the high socio-demographic index [3]. Cardiovascular and metabolic diseases are among the most common non-communicable diseases.

Apolipoprotein B (ApoB) is a constituent of chylomicrons, very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), and LDL particles that contribute to the development of atherosclerosis [4]. Dysfunction of apolipoprotein may also result in cardiovascular disease [5]. Nevertheless, it is crucial to assess ApoB levels as a predictor of atherosclerosis, even for those with normal lipid profile levels, to ensure their safety from the risk of cardiovascular disease [6]. Assessing the lipid profile levels is also essential in reducing the risk of cardiovascular disease in metabolic syndrome [7]. The atherogenic index of plasma (AIP) and Castelli risk index I-II (CRI) are simple fractions

that can be calculated in clinical practice. These indices are particularly useful when lipid profile values appear normal or high and can serve as predictors of cardiovascular disease [8].

Metabolic syndrome is a cluster of metabolic disorders defined by three of the five diagnostic criteria including impaired lipid metabolism, glucose abnormalities, hypertension, and central obesity [9]. Research is required to provide therapies using suitable and efficient multitargeted techniques [10]. A meta-analysis study has provided evidence that Omega 3 can decrease the likelihood of developing metabolic syndrome and the risk of cardiovascular disease [11]. Omega 3 also has been shown to reduce macrophages in the inflammatory process and the risk of obesity [12]. A 4 g/day omega 3 dose for 3 months modulates significant changes in plasma fatty acid profile, adipose tissue, and systemic inflammation in obese subjects [13].

Curcuma longa Linn., a genus of *Zingiberaceae* is a traditional medicinal plant used in India for obese humans. Curcumin is considered the most active constituent of *Curcuma longa* including demethoxycurcumin, and bisdemethoxycurcumin [14]. Multiple studies have demonstrated that Curcumin possesses antioxidant and anti-inflammatory properties and effectively decreases cardiovascular disease. These compounds can prevent endothelial dysfunction, and regulate the polarization of macrophages and a dose of 200g/day could improve glycemic factors in the obesity [15]. Secondary metabolites of *Curcuma* extract are essential to determine as antioxidants and anti-inflammatories [16].

To date, no studies have investigated the potential of combining Catfish oil and *Curcuma* extract as a preventive or therapeutic option against cardiovascular disease in individuals with metabolic syndrome [17]. Thus, research is necessary to comprehend the interplay between polyphenols in *Curcuma* extract and fish oil, as these two nutraceuticals have advantageous effects on metabolic health due to their bioactive properties [18].

MATERIALS AND METHODS

Experimental animals

A randomized controlled group design was used in this study. Thirty male Wistar rats 6-8 weeks old and weighing 185-210 g from the Experimental Animal Laboratory of the Centre for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, Indonesia were used. The study was approved by the Research Ethics Committee of the Faculty of Medicine, Diponegoro University (NO.56/EC-H/KEPK/FK-UNDIP/V/2023). Rats were divided into 5 groups (n=6), namely healthy control (HC) which received a standard diet; high-fat high-fructose diet (HFFD), intervention group P1 (HFFD + Catfish oil 0.08 ml/g/day), P2 (HFFD + *Curcuma* extract 5.04 mg/g/day), and P3 with HFFD + combination of both. Carboxyl Methyl Cellulose 0.5% was used as a solvent. HFFD was used to induce metabolic syndrome in rats (Lee Index ≥300, total cholesterol >129.52 mg/dL, triglyceride >108.11 mg/dl, LDL >81.55 mg/dL, HDL <35 mg/dl, and fasting glucose >111.7 mg/dl [19]. The interventions were commenced on the 21st day following the acquisition of metabolic syndrome criteria in rats until the last day of research.

Induction of metabolic syndrome

The HFFD group and intervention groups including P1, P2, and P3 were subjected to a 21-day feeding regimen of HFFD at a dosage of 20 g per day and was maintained until the last day of the intervention to induce metabolic syndrome. The rats get metabolic syndrome if classified into criteria NCEP ATP-III with the increase in body weight, total cholesterol, triglyceride, LDL, blood sugar, and decrease in HDL levels. On day 21 (pre-test) and 28 days (post-test), blood samples were obtained from the retro-orbital vein. Mice were previously anesthetized and fasted for 6-8 hours. The blood samples were analyzed to measure fasting blood glucose, total cholesterol, triglycerides, LDL, and HDL levels using ELISA-KIT from Diasys and manufactured by Holzheim, Germany in the Enzymatic colorimetric method (Cholesterol Oxidase Para Aminophenazone-CHODPAP) and (Glycerol Phosphate Oxidase-Para Aminophenazone-GPO-PAP).

Administration of fish oil extract

Catfish (*Pangasianodon hypophthalmus*) was derived from Waduk Kedung Ombo, Central Java, Indonesia. The process of generating fish oil with high levels of EPA and DHA has been achieved by the wet rendering extraction method and 4% bentonite purification at Laboratory Fishery Products Technology, Diponegoro University, Indonesia. The peroxide number (PV) content was measured by AOAC 965.33 and Free fatty acids (FFA) with titrimetry method. Detection of EPA-DHA content used the GC-FID method [20].

Administration of *Curcuma* extract

Curcuma rhizome was obtained from Kranggan Temanggung, Central Java, Indonesia. The procedure of obtaining *Curcuma* extract involved a straightforward maceration method with 96% ethanol [21-22] and a preservation technique with freeze drying at the Laboratory Food Technology and Innovation, Soegijapranata Catholic University, Semarang, Indonesia. Secondary metabolites in *Curcuma* extract were quantitatively analyzed consisting of flavonoid and tannins by spectrophotometry method, and saponin was analyzed using the gravimetric method [43].

ApoB levels analysis

ApoB levels were measured by an immunoturbidimetric method using an ELISA kit (Biotinylated and Avidin-Horseradish Peroxidase-HRP) 96T/48T from Elabscience, United States. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

Lipid profile analysis

The levels of total cholesterol, LDL, and HDL were measured by Enzymatic colorimetric method (Cholesterol Oxidase Para Aminophenazone-CHODPAP) and Triglyceride levels by Glycerol Phosphate Oxidase-Para Aminophenazone-GPO-PAP. Lipid profiles were analyzed using an ELISA kit (Precipitant - HDL precipitant 1 3540 99 83 885, LDL precipitant 1 4330 99 83 885, cholesterol 1 1300 99 10 023, triglycerides 1 5710 99 83 021) From Diasys manufactured by Holzheim, Germany. Absorbance results were calculated using a wavelength of 500nm.

Lipid ratios analysis

AIP obtained from triglyceride (TG) and HDL levels were calculated using the formula (Log TG/HDL) [44] as well as CRI-I (TC/HDL), and CRI-II (LDL/HDL) as described [45].

Statistical analysis

The statistical analysis used SPSS 24. The Shapiro-Wilk test was employed to assess the normality of the data. The initial investigation compared the data before and after therapy for various parameters, including lipid profile, AIP, and CRI values. The paired t-test was employed for data that followed a normal distribution. The analysis examined the variations in the effect of intervention across five groups. This was conducted using the one-way ANOVA test, followed by the Bonferroni Post Hoc test if the data exhibited equal variances ($p>0.05$) or the Tamhane Post Hoc test if different variances ($p<0.05$). In cases where the data did not follow a normal distribution, the Kruskal Wallis test was utilized and distributed the Spearman correlation to multivariate data analysis test results used to multiple linear regression.

RESULTS

Effects of catfish oil, *Curcuma* extract, and a combination of both on ApoB levels

The range of ApoB levels in P1, P2, and P3 groups before intervention ranged from 354 to 356. After the intervention, the range was 141 to 129 mg/dl. The P1, P2, and P3 groups that were given HFFD (20 g/day) with an intervention for 28 days had a decrease in ApoB levels, while the HFFD group experienced a significant increase in ApoB levels from 358.90 ± 7.36 to 373.76 ± 5.38 mg/dl when compared to the HC group from 35.11 ± 0.72 to 36.81 ± 0.74 mg/dl (Figure 1). The levels of ApoB demonstrated significance in all groups indicating that the data was of normal distribution and showed significance ($p=<0.05$). The data variants were homogenous, and the data of ApoB levels were significantly different. In all groups except the P1 and P2 groups ($p=0.745$), there was a significant difference between the HC groups and HFFD; and HFFD vs PI, P2, and P3 (Figure 1).

Effects of catfish oil, *Curcuma* extract, and a combination of both on AIP

The range of AIP values (TG/HDL ratios) in P1, P2, and P3 groups before intervention was 0.72 to 0.74 mmol/L. After intervention, it was with a range of 0.29 to 0.47 mmol/L. The data also showed that in groups P1, P2, and P3, there was a decrease in AIP compared to HFFD (Figure 2). While the HFFD group experienced a significant increase from 0.71 ± 0.01 to 0.77 ± 0.03 mmol/L compared to the NC group from 0.06 ± 0.016 to -0.03 ± 0.12 mmol/L (Figure 2). Group P3 showed significance at $p<0.05$, indicating an abnormal distribution of data, whereas P1 and P2 group showed $p=0.332$. This demonstrates that the data was significantly different between the HC group and HFFD; and HFFD vs PI, P2, and P3 (Figure 2).

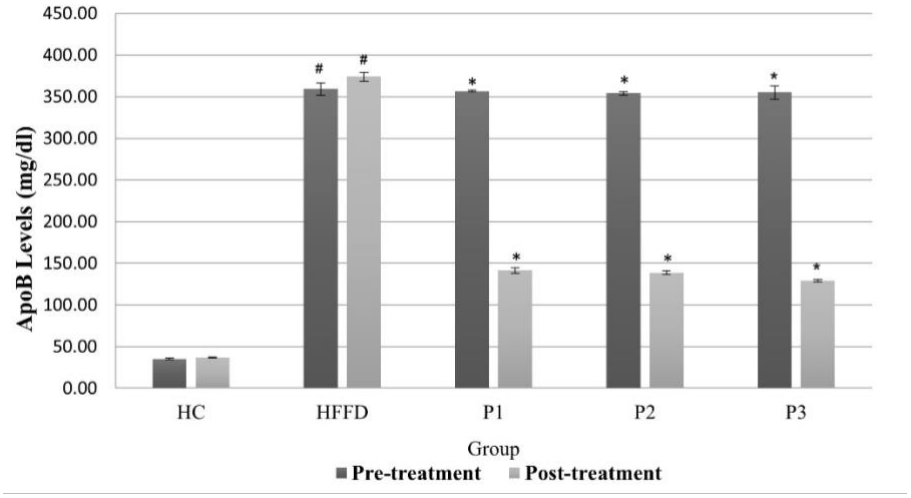

Figure 1. Effects of intervention (pre-and post-treatments) on ApoB levels. According to the results of the one-way ANOVA test, the data variants were homogenous, and the data of ApoB levels were significantly different indicated by # compared to the HC group. Significant differences are indicated by * when compared with the HFFD group. HC (healthy control); HFFD (negative control); and intervention groups (P1, P2, and P3); n=6; *= p<0.05.

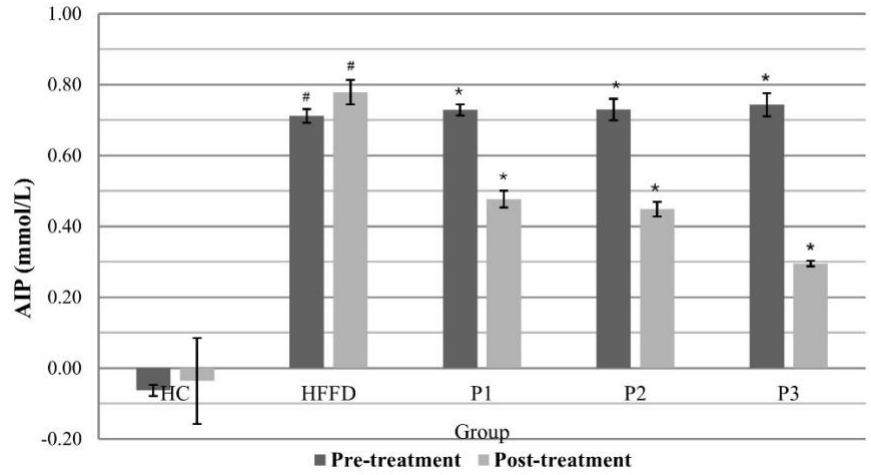

Figure 2. Effects of intervention (pre-and post-treatments) on AIP. The result of the Kruskall-Wallis test resulted in p=0.000 (<0.05). This demonstrates that the data was significantly different and indicated by # compared to the HC group. Significant differences are indicated by * when compared with the HFFD group. HC (healthy control), HFFD (negative control), and intervention groups (P1, P2, and P3), n=6; *= p<0.05.

Effects of catfish oil, *Curcuma* extract, and a combination of both on CRI I-II

The range of CRI-I values (TC/HDL ratio) in the P1, P2, and P3 groups before the intervention ranged from 8.4 to 8.6 mmol/L. After the intervention, the range was 1.5 to 4.6 mmol/L. This showed that the P1, P2, and P3 groups given HFFD (20 g/day) with the intervention for 28 days had a decrease in CRI-I. While the HFFD group experienced a significant increase in CRI-I from 8.44 ± 0.65 to 10.45 ± 0.82 mmol/L when compared to the HC group from 1.11 ± 0.06 to 1.16 ± 0.05 mmol/L (Figure 3).

The range of CRI-II values (LDL/HDL ratio) in the P1, P2, and P3 groups before the intervention ranged from 3.1 to 3.2 mmol/L, and after the intervention, the range was 1.0 to 1.5 mmol/L. This showed that groups P1, P2, and P3 had a decreased CRI-II. The HFFD group had significant difference in CRI-II from 3.18 ±0.29 to 3.77 ± 0.38 mmol/L than the HC group from 0.29 ± 0.22 to 0.33 ± 0.02 mmol/L (Figure 4).

The data findings in all groups for CRI I-II revealed significance (p>0.05) demonstrating that the data had a normal distribution, but the other test result was p<0.05 indicating that the intervention of the differed significantly. In all groups except for PI and P2 (p=0.791; p=0.540), there was a significant difference between the HC group and HFFD; and HFFD vs PI, P2, and P3.

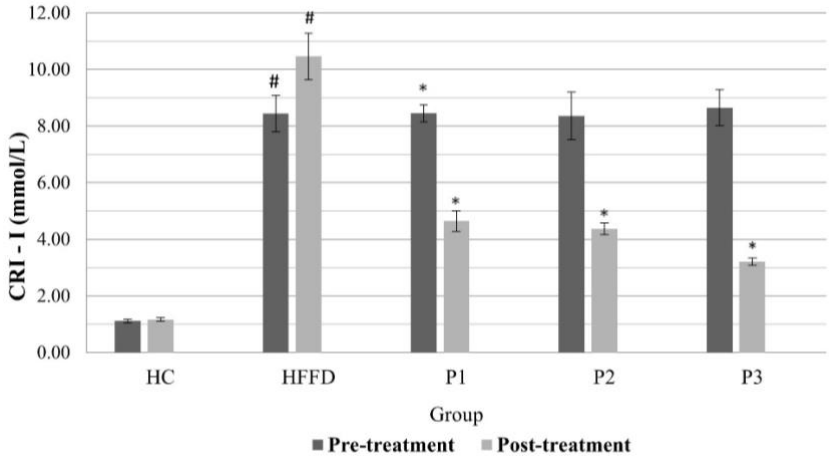

Figure 3. Effects of intervention (pre-and post-treatments) on CRI-I. According to the results of one-way ANOVA test, the data variants were homogenous, and the data of CRI-I had significantly different are indicated by # compared to the HC group. Significant differences are indicated by * when compared with the HFFD group. HC (healthy control), HFFD (negative control), and intervention groups (P1, P2, and P3), n=6; *= p<0.05.

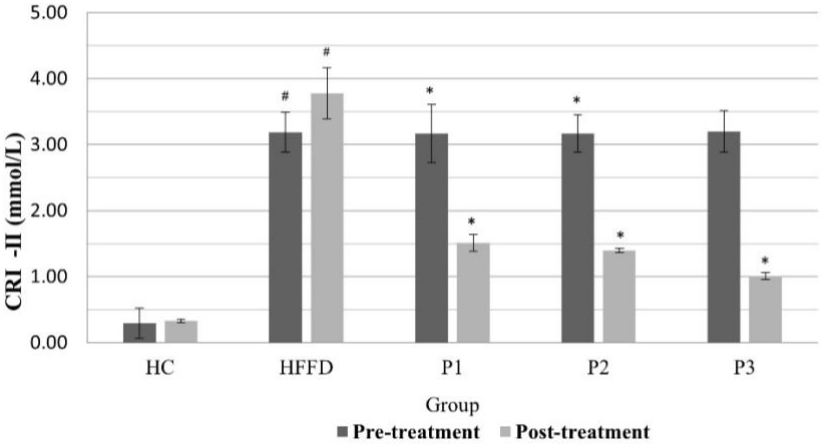

Figure 4. Effects of intervention (pre-and post-treatments) on CRI-II. According to the results of one-way ANOVA test, the data variants were homogenous, and the data of CRI-II had significantly different are indicated by # compared to the HC group. Significant differences are indicated by * when compared with the HFFD group. HC (healthy control), HFFD (negative control), and intervention groups (P1, P2, and P3), n=6; *= p<0.05.

Correlation among ApoB levels, AIP, and CRI I-II

The data for AIP were not normally distributed, so the Spearman correlation test was performed to determine the relationship among ApoB levels, AIP, and CRI I-II. The correlation test results showed that there was a significant correlation among ApoB levels, AIP, and CRI (p=0.000). The Spearman's correlation value and the strength of the relationship are shown in Figures 5-7. The multivariate data analysis test results using multiple linear regression showed an adjusted R square (97.0%). Unstandard

Coefficients showed that with every increase in ApoB levels against AIP and CRI, there was an increase of 35.309mg/dl with the equation Y=-4.320+35.309.

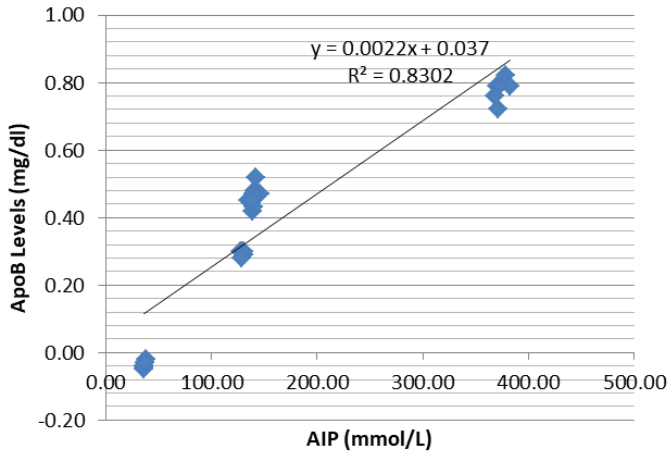

Figure 5. Linear regression of ApoB levels with AIP.

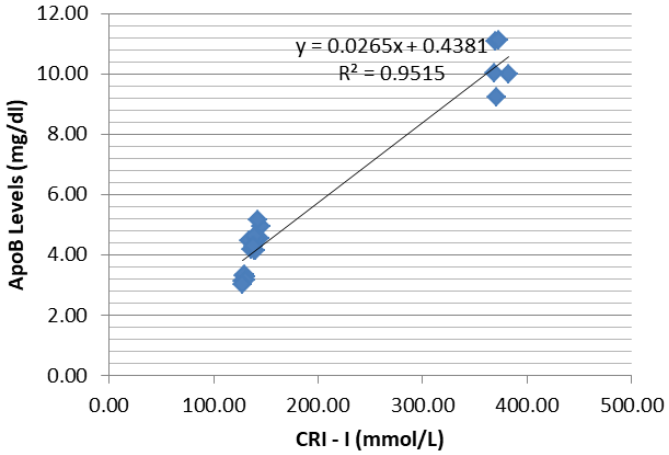

Figure 6. Linear regression of ApoB levels with CRI-I.

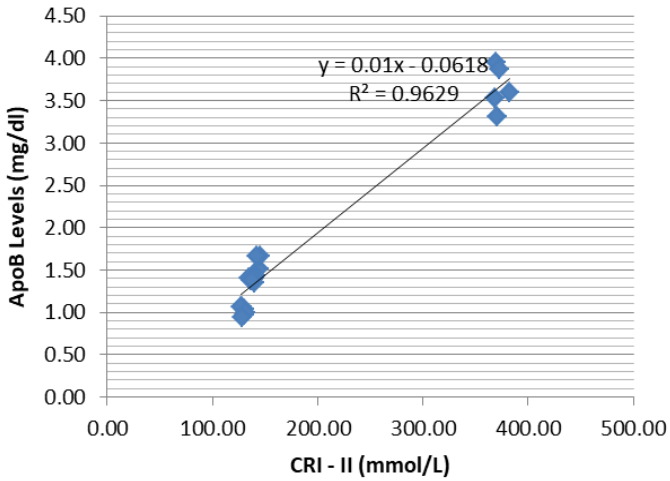

Figure 7. Linear regression of ApoB levels with CRI-II.

DISCUSSION

Our study showed that the fish oil content of EPA 4770.7 mg/100g and DHA 6093.2 mg/100g. In comparison, research with *Atlantic Salmon* showed a 2.2-fold increase in the percentage of EPA, a 1.7 of DHA [23]. This shows that the results of freshwater fish oil in this study are superior to marine fish oil. The PV value was obtained as 0.978mEq O2/kg (<5mEq/kg) and FFA as 0.46% (<1,50%) which shows that the results are in accordance with the quality standards of fish oil eligibility based on the International Fish Oil Standard. *Curcuma rhizomes* extracted with maceration method using 96% ethanol solvent and preservation by freeze-drying produced flavonoid content of 18.728mg/100g, tannin 1.752 mg/100g, and saponin 4.457mg/100g. In the prevention of metabolic syndrome, secondary metabolites of flavonoid, tannin, and saponin compounds have anti-inflammatory and anti-oxidative effects that protect cells from endothelial dysfunction [24-25] and as potential antidiabetic agents [26].

Metabolic disorders including adipose dysfunction, hyperlipidemia, and type 2 diabetes mellitus, can be induced by long-term HFFD [27]. The results of induction using HFFD in this study have successfully induced metabolic syndrome conditions in experimental animals with NCEP ATP-III criteria, the mean of body weight of 245.32 g, total cholesterol 189.5 mg/dl, triglyceride 122.29 mg/dl, HDL 37.07 mg/dl, and LDL 69.10 mg/dl. The result of this study shows that administration of HFFD can elevate the levels of ApoB and all interventions effectively reduce it. The P3 group had a more pronounced reduction in ApoB levels than the P1 and P2 groups. The P3 group demonstrated a more substantial decrease in Δ% (63.55%) compared to a study of the curcumin markedly increased the amount of ApoB mRNA in mouse hepatocytes (27.63%–35.61%) and reduced the amount of ApoB levels [28]. The level of ApoB in plasma relates directly and powerfully to the potential risk of cardiovascular diseases [29]. A decrease in triglycerides and ApoB levels by 1 mmol/L or more than 20% is linked to effectively lowering the risk of cardiovascular disease [30]. The study found that the P3 group demonstrated a significant reduction in ApoB levels and reached normal values (<133mg/dl) [31]. The P1 and P2 groups did not reach normal values after the intervention, but all groups experienced a decrease of 60-63% (>20%).

In this study, the P1 group was given catfish oil of 4g/day which was converted to a rat dose of 0.08ml/g/day. Clinical trial results released in the past year have demonstrated that higher doses of omega 3 (4g/day) led to a significant reduction in ApoB levels and triglyceride by 20-30% in individuals with metabolic syndrome and also an effective adjunct for cardiovascular treatment [32]. The function of Omega 3 as ligands for PPARs involved in lipid metabolism by inhibiting the SREBP-1c receptor [33][34]. The P2 group was given *Curcuma* extract of 200g/day which was converted to a rat dose of 5.04ml/g/day. The bioactive compounds and secondary metabolites found in *Curcuma* can influence cholesterol transportation in maintaining lipid balance and preventing the formation of foam cells and also inhibit the regulation of the LDL receptor (TLR4) which is associated with ApoB levels. Macrophages are the primary focus of the mechanism by bioactive chemicals in *Curcuma.* Metabolic syndrome is a condition that arises from obesity and is characterized by the presence of metabolically active visceral adipose tissue, which contains more macrophages. In addition to the advantages of fish oil, studies have discovered that curcumin decreases the expression of SREBP[35] and ABCA1, which is involved in maintaining lipid balance [36].

Assessing ApoB levels provides a more accurate prediction of cardiovascular disease risk compared to solely testing LDL or triglyceride levels [37]. Nevertheless, individuals who have normal lipid profile levels cannot be exempted from the risk of cardiovascular disease [6]. This pertains to a study on individuals with metabolic

syndrome, which discovered a situation where 22% of the participants exhibited increased LDL but normal levels of ApoB (≤90mg/dL). According to this study, these individuals were less likely to develop arteriosclerosis for 13 years [38]. Hence, assessing ApoB levels as a robust indicator of atherosclerosis is crucial [6].

The findings of this study align with previous research demonstrating that ingesting excessive amounts of HFFD will elevate levels of total cholesterol, triglycerides, and LDL, increasing the ratio of TC/HDL and LDL/HDL [39]. The AIP and CRI are indicators to predict the risk of cardiovascular disease [8]. The changes in the mean of TG and HDL levels before and after the intervention in groups P1, P2, and P3 were in line with the changes in AIP values. Likewise, LDL levels decreased after the intervention have been associated with decreased levels of CRI I-II. The AIP value demonstrated a considerable drop, despite its initial categorization as moderate and remaining in the moderate category (0.1-0.24) after the intervention of the P1, P2, and P3 than NC group [40]. The CRI-I value has decreased significantly, namely before the intervention in the high category (>5), and after the intervention in the low category (<4.5). The CRI- II value decreased significantly, namely before the intervention in the moderate category (3.1-3.4), and after the intervention in the low category (<3.0) [41]. The human study on women with Polycystic Ovary Syndrome often accompanied by metabolic syndrome, has demonstrated that 65%-80% of them experience a deterioration in their lipid profiles, which can lead to the development of atherogenic dyslipidemia. This deterioration is characterized by increased levels of ApoB and triglycerides and decreased levels of HDL [29]. In this study, the P3 group experienced a significant reduction in triglyceride levels following the intervention of 21.68% (>20%). The AIP can serve as an alternative diagnostic tool for predicting cardiovascular disease [8]. Nevertheless, the assessment of ApoB levels is more reliable in characterizing lipid particles undergoing oxidation [37]. Thus, it is recommended that ApoB levels be assessed as the most potent predictor of atherosclerosis. Replacement of LDL levels with ApoB levels is a step that should be taken now in managing lipid-lowering therapy for screening [42].

The research has proposed that fish oil and *Curcuma* are nutraceutical components that can lower the levels of sdLDL, atherogenic apolipoproteins, and LDL particles prone to oxidation [16]. A recent study conducted an experiment where fish oil and *Curcuma longa* were mixed and administered to individuals with type 2 diabetes mellitus. The findings indicated that a blend comprising 2x1000mg of fish oil and 2x500mg of *Curcuma* can potentially diminish insulin resistance. Nevertheless, no substantial alterations were observed in fasting blood glucose levels. Significant alterations were observed solely in the group that received *Curcuma* and the singular fish oil group decreased the AIP and dyslipidemia [17]. The findings of these studies contrast with the findings of this study which demonstrated a significant impact of the combination of fish oil and *Curcum*a extract in reducing fasting blood glucose levels and lipid profiles when compared to receiving a single intervention group. The complementary and synergistic effects between polyphenols of *Curcuma* extract and fish oil have been demonstrated in research studies, showing their beneficial effects on metabolic health as anti-oxidant and anti-inflammatory agents [43].

CONCLUSION

These findings suggest that all groups of rats that were induced with HFFD could exhibit characteristics of metabolic syndrome. The study demonstrated that a combination of catfish oil 0.08 ml/g/day and *Curcuma* extract 5.04 mg/g/day,

significantly reduced the levels of ApoB and improved lipid profile abnormalities by reducing the AIP and CRI I-II. The synergistic effects of a combination of both have demonstrated significant benefits, making them valuable as a nutraceutical with potent antioxidant and anti-inflammatory properties, thereby reducing the risk of cardiovascular disease in individuals with metabolic syndrome.

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AUTHOR CONTRIBUTIONS

VP conceived the manuscript and performed data collection. EM, MS, and DNA contributed suggestions, checked on its interpretation data analysis, and reviewed the manuscript. All authors approved the final version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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