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Effect of Giving Black Seed (Nigella Sativa L) On Il-10 Levels, And CRP Levels (Experimental Study on Wistar Strain Male White Rats Induced By A High Fat Diet)

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ABSTRACT: Dyslipidemia is a disease that often occurs in Indonesia. The impacts of this disease include inflammation and various types of cytokines. This study aimed to determine the effect of Black Seed on Interleukin 10 levels and CRP levels in rats induced by a high-fat diet. This research is a posttest-only control group type of research. Twenty-five male white Wistar rats were taken as samples and divided into 5 groups: K0 (pellets and water), K – (high-fat diet), K + (high-fat diet + simvastatin 1.8 mg/day), P1 (high-fat diet + Black Seed 3 ml/KgBB), P2 (high-fat diet + black seed 4 ml/KgBB). The data required in this study was obtained through the ELISA test. The collected data was analyzed using one-way ANOVA and post hoc LSD test. From the results of the analysis carried out, it was found that (1) the difference in mean IL-10 levels between groups was significant with a p-value <0.05; (2) The difference in average CRP levels between groups where K0, K-, and P1 is significant, while between K (+) and P2 is not significant with a $p>0.05$. Administration of Black Seed affected IL-10 levels and CRP levels in white mice induced by a high-fat diet.

KEYWORDS: High fat diet, IL 10, CRP, Black Seed

I. INTRODUCTION

Dyslipidemia is a condition characterized by increased levels of total cholesterol, blood fat, LDL cholesterol, and decreased levels of HDL cholesterol, which in the long term can cause chronic inflammation and the formation of atherosclerotic plaque, which is the main cause of coronary heart disease. Traditional statin treatment can lower cholesterol levels, but long-term use often causes side effects such as myalgia, rhabdomyolysis, and myositis. The development of alternative therapies with natural ingredients such as black seed oil (Nigella sativa L) in reducing blood lipid levels has been widely carried out, but further research on the role of black seed in treating chronic inflammation in dyslipidemia conditions is based on levels of Interleukin-10 (IL 10) and C-Reactive Protein (CRP) as a marker of inflammation still needs to be done. In Indonesia, the high prevalence of dyslipidemia is a major risk factor for cardiovascular disease, especially Coronary Heart Disease (CHD). Habbatussauda has potential as an antioxidant and anti-inflammatory thanks to the content of active substances such as flavonoids and thymoquinone (TQ). Previous research shows that black seed can increase levels of IL-10, which acts as an inflammation regulator, and reduce levels of MDA and NO. Apart from that, Black Seed can also regulate pro-inflammatory cytokines such as IL-1α, IL-2β, and IL-6 and reduce inflammation in various conditions. However, research on the effect of Black Seed in inhibiting chronic inflammation in dyslipidemia by measuring IL-10 and CRP levels in male Wistar rats induced by a high-fat diet still needs to be deepened [1-15].

This study aimed to evaluate the effect of Black Seed on IL-10 (Interleukin-10) levels and CRP (C-Reactive Protein) levels in male Wistar rats that had been induced with a high-fat diet. This study aimed to understand how Black Seed affects IL-10 and CRP levels in these conditions. Additionally, specific objectives involve measuring mean levels of IL-10 and CRP and analyzing mean differences between different groups to understand the potential impact of Black Seed on the inflammatory response in the body of mice exposed to a high-fat diet.

II. MATERIAL AND METHOD

Study Design and Experimental Animals

This type of research is a laboratory experiment using a post-test-only control group design with male Wistar white rats given an induction diet high in fat and black seed simultaneously. The subjects were male Wistar rats with a body weight of 180-220 grams, intensively developed and maintained at the UGM Yogyakarta Center for Food and Nutrition Studies according to inclusion and inclusion criteria. Samples were obtained through Randomized Sampling Allocation, which will be adapted for 7 days. A total of 25 male Wistar rats that met the research inclusion criteria were randomly divided into 5 groups : healthy group without treatment and given standard feed (K0), negative control group, namely given a high-fat diet induction for 4 weeks without giving Black Seed (K-), positive control group, namely given a high-fat diet induction with simvastatin 1.8 mg/day for 2 weeks (K+), the treatment group was given a high-fat diet induction by administering black seed oil 3 ml/kgBB for 2 weeks (P1), and the treatment group was given a high-fat diet induction by administering black seed oil 4 ml/kgBB for 2 weeks (P2). In the fifth week after treatment, IL-10 and CRP levels were measured using the ELISA method from blood samples taken via the orbital vein.

Research Materials

The research material consists of male Wistar rats with body weight characteristics ranging from 180 to 220 grams and 8 weeks of age, standard feed (COMFEED AD II from PT. Japfa Comfeed Indonesia), additional feed consisted of 2% cholesterol and 9 grams of saturated fat, aquades, and black seed oil.

Research Equipment

This study used several equipment, including a rat cage with appropriate places to eat and drink, special water bottles for rats, hematocrit tubes, injection syringes, sondes, digital scales, masks, sterile gloves, and laboratory equipment such as a microplate reader was used along with a CRP-level examination kit and an IL-10-level examination kit.

How to Prepare Before Treatment

The research material involved 25 male Wistar rats with body weight characteristics ranging from 180 to 220 grams and 8 weeks of age as research subjects. These rats were given standard feed COMFEED AD II from PT. Japfa Comfeed Indonesia, Tbk as their main food. In addition, to induce a high-fat diet, additional feed consisted of 2% cholesterol and 9 grams of saturated fat, which was mixed into the rat's feed and converted into pellet form and given for 4 weeks. Drinking water is provided freely using Aquades.

How to Induce a High Fat Diet Condition

A high-fat diet was prepared by mixing 2% cholesterol and 9 grams of saturated fat into rat feed, which was then converted into pellets and given for 4 weeks. In the third and fifth weeks, lipid profiles such as total cholesterol, HDL, and LDL levels were assessed.

How to Give Black Seed Oil Dosage

Black Seed (Black Seed oil) is given in capsule form from PT. Habbasyi Niaga Utama Depok, Indonesia, and the feeding was carried out for 14 days after being fed a high-fat diet. Black Seed oil is given orally in various doses, 3 ml/KgBB and 4 ml/KgBB, measured in millilitres (ml).

IL-10 and CRP levels Measurement Procedure using ELISA kit

The blood sampling technique was carried out carefully, involving holding the rat and collecting blood from the orbital vein using a microhematocrit. IL-10 levels were measured using the ELISA method after blood plasma was homogenized. CRP levels were also measured using the ELISA method after separating the serum from the blood sample via centrifugation. IL-10 and CRP (C-Reactive Protein) levels in the blood of experimental animals are also expressed in ng/L units and measured using the ELISA kit rat CRP method at PAU UGM by a laboratory analyst assistant.

III. RESULT

Research on the effect of Black Seed on IL-10 levels and CRP levels was carried out at PAU UGM Yogyakarta and the Faculty of Medicine Unissula Semarang from 10 July to 15 August 2023. The research subjects consisted of 25 male white rats of the Wistar strain, which were divided into five groups randomly, namely one the healthy control group (K(0)) and four other groups (K(-), K(+), P1, and P2) were induced on a high-fat diet for four weeks by providing feed containing 2% cholesterol and 9 grams of saturated fat in pellet form. During the last 14 days of induction, group K(+) received simvastatin of 1.8 mg/day, while P1 and P2 were given Black Seed oil at doses of 3 ml/kgbb and 4 ml/kgbb, respectively. In the fifth week after treatment, IL-10 and CRP levels were measured using the ELISA method from blood samples taken via the orbital vein. The high-fat diet induction aimed to create a model of dyslipidemia, which was confirmed by examination of the lipid profile in the second post-induction week and at the end of the study. During the study, monitoring the rats' body weight was also carried out, the results of which were recorded for further analysis.

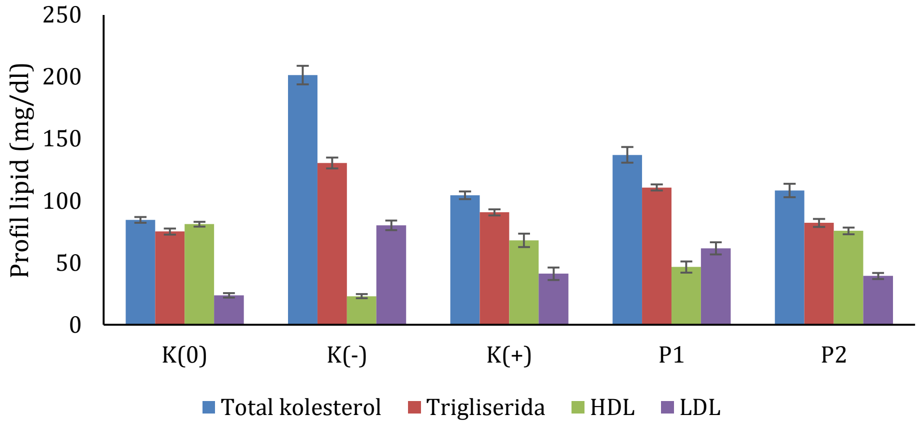


Figure 3. Line chart of the average lipid profile of rats between groups at the end of the study period (fifth week)

Figure 3 shows that at the end of the study, mice in the K(0) group had the highest levels of total cholesterol, triglycerides and LDL and the lowest HDL compared to the other four groups.

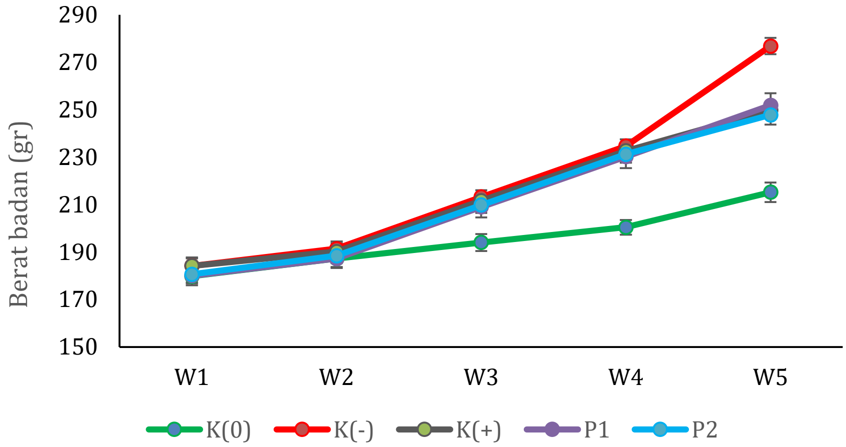


Figure 4. Line chart of average body weight of mice between groups for 5 (five) weeks

Information: W1 = BW after 7 days of adaptation, W2 = BW 1 week after high fat diet induction, W3 = BW 2 weeks after high fat diet induction, W4 = BW 3 weeks after high fat diet induction + treatment, W5 = BW 4 weeks post induction high fat diet + treatment

Figure 4 shows that the mice's average weight increased from the second week (W2) to the fifth week (W5) for each group. The increase in weight in K(0) was the lowest every week. In the group (K-), there was an increase in weight, which was relatively similar to K(+), P1 and P2 until the fourth week (W4). In the fifth week, the average weight in K(-) increased the most, while in K(+), P1 and P2, the increase was relatively similar and lower than in K(-). Rats induced by a high-fat diet appear to be obese because the average weight reaches > 220 grams.

As for the results of measuring IL-10 levels and CRP levels in male white Wistar rats induced by a high-fat dissemination diet, namely:

IL-10 levels

An overview of the mean IL-10 levels in each group along with the results of the analysis of normality of data distribution, homogeneity of variance, and mean difference tests between groups is shown:

Table 1. Results of mean analysis, normality test, homogeneity test and test for differences in IL-10 levels between groups

IL-10 Levels (pg/mL)	Groups					*p-value*
	K(0)	K(-)	K(+)	P1	P2	
Mean±SD	111,41±4,40	31,52±2,45	82,45±3,53	60,31±2,44	89,61±4,07	
*Shapiro Wilk**	0,118	0,740	0,995	0,823	0,900	
Levene test						0,791
One way anova						<0,001

Information: * = *p-value*

The study found that the highest IL-10 levels were in the K(0) group, namely 111.41 ± 4.40 pg/mL and the lowest in K(-), 31.52 ± 2.45 pg/mL. In the Shapiro-Wilk test on IL-10 levels in the five groups, the p-value was > 005, which means the IL-10 level data was normal. IL-10 levels in the five measurements were also homogeneous, as indicated by the p-value from the Levene test, namely 0.791 or p>0.05. The requirements for data normality and homogeneity of changes were met so that the average correlation of IL-10 levels between the five groups was broken down using a one-way ANOVA test, and a p-value <0.001 was obtained so that it was stated that there was a very large influence. contrast of mean IL-10 levels between groups K(0), K(-), K(+), P1 and P2.

Using a post hoc LSD test, further differences in mean IL-10 levels between the five groups were significant, as shown in Table 2.

Table 2. The results of the comparative analysis of the mean IL-10 levels between the two groups

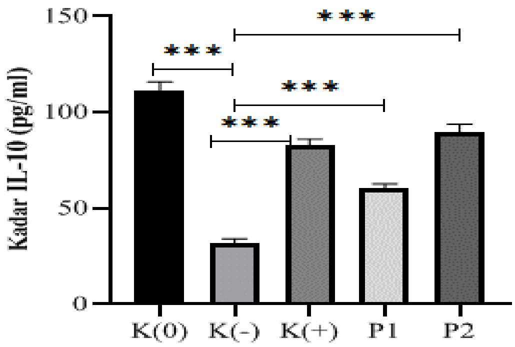
Group comparison	**p**
K(0) vs K(-)	<0,001
K(0) vs K(+)	<0,001
K(0) vs P1	<0,001
K(0) vs P2	<0,001
K(-) vs K(+)	<0,001
K(-) vs P1	<0,001
K(-) vs P2	<0,001
K(+) vs P1	<0,001
K(+) vs P2	<0,001
P1 vs P2	0,004

* = significantly different (p<0,05)

Comparison of the mean IL-10 levels between the two groups was significant, as indicated by the p-value of the LSD post hoc test, each of which was below 0.05 (p<0.05). The results of the LSD post hoc test showed that the treatment given had a significant effect on IL-10 levels. IL-10 levels at K(-) were significantly lower than K(0), indicating that induction of a high-fat diet reduced IL-10 levels. IL-10 levels in K(+), P1 and P2 were significantly higher than in K(-), indicating that simvastatin and Black Seed oil doses of 3 ml/kgbb and 4 ml/kgbb affected increasing IL-10 levels in mice treated with induced by a high-fat diet.

IL-10 levels in P1 and P2, which were significantly lower than K(+), showed that simvastatin administration was more effective in increasing IL-10 levels in rats induced by a high-fat diet than Black Seed oil doses of 3 ml/kgbb and 4 ml/kgbb. IL-10 levels in P1, significantly lower than P2, showed that administering Black Seed oil at 4 ml/kgbb was more effective in increasing IL-10 levels in rats induced by a high-fat diet than a dose of 3 ml/kgbb. IL-10 levels in K(+), P1 and P2 were significantly lower than in K(0), showing the effect of simvastatin or Black Seed oil 3 ml/kg and 4 ml/kg on IL-10 levels in rats induced by a high-fat diet not optimal.

Comparison of IL-10 levels between the two groups can also be seen in Figure 5.



Keterangan: *** = p<0,001

Figure 5. Graph of average IL-10 levels between groups

CRP levels

An overview of the average CRP levels in each group along with the results of the analysis of normality of data distribution, homogeneity of variance, and the mean difference test between groups is shown in Table 3.

Table 3. Results of the mean analysis, normality test, homogeneity test and test for differences in CRP levels between groups

CRP Levels (pg/mL)	Groups					*p-value*
	K(0)	K(-)	K(+)	P1	P2	
Mean±SD	0,74±0,02	2,28±0,01	0,83±0,01	1,08±0,01	0,85±0,03	
*Shapiro Wilk**	0,611	0,493	0,634	0,313	0,958	
Levene test						0,052
One way anova						<0,001

Information: * = *p-value*

The study results found that the highest CRP level was in the K(-) group, 2.28 ± 0.01 ng/mL and the lowest was in K(0), 0.74 ± 0.02 ng/mL. In the Shapiro-Wilk test for CRP levels in the five groups, the p-value was > 005, meaning the CRP level data is normal. CRP levels in the five measurements were also homogeneous, indicated by the p-value of the Levene test, which was 0.052 or p>0.05. The one-way ANOVA test shows that there is a significant difference in average CRP levels between groups K(0), K(-), K(+), P1 and P2 with a p-value of less than 0.001 which indicates normal data distribution requirements and homogeneity of variance. Fulfilled. Large differences in mean CRP levels between the five groups were also examined using the LSD post hoc test, and the results obtained in Table 4 or Figure 5.

Table 4. The results of the comparative analysis of the mean CRP levels between the two groups

Group Comparison	**p**
K(0) vs K(-)	<0,001
K(0) vs K(+)	<0,001
K(0) vs P1	<0,001
K(0) vs P2	<0,001
K(-) vs K(+)	<0,001
K(-) vs P1	<0,001
K(-) vs P2	<0,001
K(+) vs P1	<0,001
K(+) vs P2	0,071
P1 vs P2	<0,001

* = significantly different (p<0,05)

The results of the analysis of the comparison of mean CRP levels between the two groups showed that most of the comparisons were significant, with the p values from the LSD post hoc test all being below 0.05 ($p < 0.05$), except for the comparison between K(+) and P2 with values p was 0.071 ($p > 0.05$). The results of this test confirm that the treatment given has a significant influence on CRP levels. CRP levels in the K(-) group were significantly higher than those in the K(0) group, indicating that high-fat diet induction impacted increasing CRP levels. On the other hand, CRP levels in the K(+), P1, and P2 groups were significantly lower than those in the K(-) group, indicating that administering simvastatin and black seed oil in doses of 3 ml/kgbb and 4 ml/kgbb were able to reduce CRP levels in mice induced by a high-fat diet. There were differences between the K(+), P1, and P2 groups in CRP levels, where the CRP levels in the K(+) group were significantly lower than those in the P1 group, indicating that simvastatin was more effective in reducing CRP levels compared to 3 doses of black seed oil. ml/kgbb. In addition, CRP levels in group P1 were significantly higher than in group P2, indicating that 4 ml/kgbb of black seed oil was more effective in reducing CRP levels than 3 ml/kgbb. However, it should be noted that the CRP levels in the K(+), P1, and P2 groups were still significantly higher than in the K(0) group, indicating that the effect of administering simvastatin or black seed oil in doses of 3 ml/kgbb and 4 ml/kgbb The reduction in CRP levels in mice induced by a high-fat diet did not reach optimal levels.

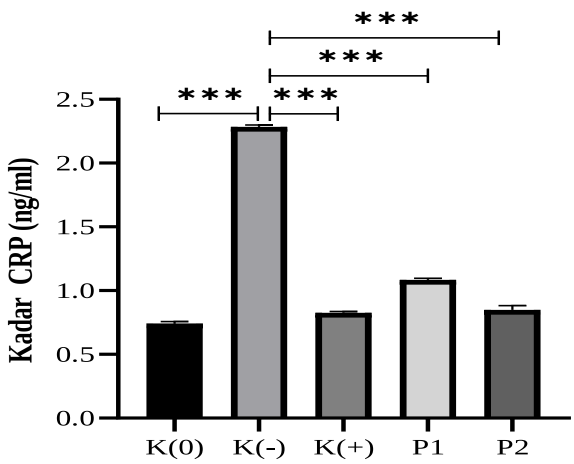


Figure 6. Bar graph of average CRP levels between groups

Next, to provide support for whether body weight and lipid profile affect IL-10 levels and CRP levels, a correlation analysis was carried out, and the results were obtained, namely:

Table 5. Correlation of body weight and lipid profile with IL-10 levels and CRP levels

	p-value (r correlation)	
	IL-10 Levels	CRP Levels
Body weight^	<0,001 (-0,831)	<0,001 (0,813)
Total cholesterol level^	<0,001 (-0,908)	<0,001 (0,916)
Triglyceride levels^	<0,001 (-0,926)	<0,001 (0,870)
LDL levels*	<0,001 (0,957)	<0,001 (-0,935)
HDL levels^	<0,001 (-0,972)	<0,001 (-0,875)

* = Pearson correlation test, ^ = Spearman rank correlation test

Table 5 shows that body weight, total cholesterol levels, triglyceride levels and LDL levels have a very strong negative correlation with IL-10 levels. In contrast, HDL levels positively correlate ($p<0.001$). On the other hand, body weight, total cholesterol levels, triglyceride levels and LDL levels had a very strong positive correlation with CRP levels. In contrast, HDL levels had a negative correlation ($p<0.001$). The r correlation values obtained each ranged from 0.800-1.000, indicating that the relationship between body weight and lipid profile with IL-10 levels and CRP levels was classified as very strong. These results can show the effect of Black Seed on IL-10 levels and CRP levels, which are also related to body weight and lipid profile.

IV. DISCUSSION

IL-10 levels in rats induced by a high-fat diet for 4 (four) weeks decreased significantly compared to healthy mice's IL-10 levels. The decrease in IL-10 levels occurred because the induction of a high-fat diet increased the body weight of the mice or had an impact on obesity, which was characterized by an increase in body weight up to > 220 gr. Similar results were also shown in the study by Kondo et al., who stated that obesity associated with the induction of a high-fat diet significantly resulted in a significant decrease in serum IL-10 levels in wild-type mice. [15] Obesity is characterized by low-grade inflammation in adipose tissue due to changes in adipokines and the release of various cytokines. Obesity associated with a high-fat diet causes a decrease in the differentiation of naive T cells into Th2 anti-inflammatory cells that secrete anti-inflammatory cytokines such as IL-10, IL-13 and IL-4.[17], high TNF-α secretion from the accumulation of visceral fat,[18] and an increase in the mass or size of adipose tissue.[19]

Apart from being obese, decreased IL-10 levels also occur due to the induction of a high-fat diet that contributes to dyslipidemia, which in this study was indicated by total cholesterol levels, namely 201.5 mg/dl; triglyceride levels, namely 130.56 mg/dl; LDL levels were 80.28 mg/dl, and HDL levels were 23.12 mg/dl. The lipid profile is different from that shown by normal/healthy mice, namely 84.6 mg/dl; 75.32 mg/dl; 23.81 mg/dl each for total cholesterol levels, triglyceride levels, and LDL and HDL levels, namely 81.21 mg/dl. Evidence of mice experiencing dyslipidemia was also shown in previous studies, which showed that the cholesterol levels of the Wistar rats significantly reached 95.7 mg/dl (from the normal 36.2 mg/dl); HDL level is 6.6 mg/dl (from normal 5.2 mg/dl), LDL level is 83.2 mg/dl (from normal 24.2 mg/dl). Triglyceride level is 10.5 mg/dl (from normal 6.7 mg/dl). [20] According to a study by Kim et al., IL-10 levels were negatively correlated with changes in total cholesterol and LDL levels. IL-10 levels decrease when total cholesterol levels and LDL levels increase

A high-fat diet increases levels of free fatty acids, which can directly affect intestinal cells and, cause an increase in proinflammatory cytokines and decrease anti-inflammatory cytokines. The high free fatty acids can upregulate the toll-like receptor (TLR) expression in circulating macrophages so that macrophages (M1) are activated. Then, M1 secretes TNF-α, IL-1, and mass cell protease-1 (MCP-1) to recruit monocytes to adipose tissue and increase the ratio of M1 to M2, causing a decrease in IL-10. On the other hand, increased levels of triglycerides and total cholesterol can increase the role of free fatty acids in regulating TLR2 and TLR4 to trigger NFkB activation, which results in the release of proinflammatory cytokines and decreased levels of IL-10.[22]

Black seed administration can increase IL-10 levels in rats induced by a high-fat diet at 3 ml/kg and 4 ml/kg. This result is because Black Seed is rich in bioactive compounds that can improve lipid profiles and reduce rat weight. Several previous studies in mice, rats, or clinical trials have shown that Black Seed can improve lipid profiles by reducing cholesterol levels, LDL levels, triglyceride levels, and VLDL, and increasing HDL levels; it can also reduce body weight, as well as body mass index (BMI).[23]

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that plays an important role in shaping the immune response by inhibiting the expression of major histocompatibility complex (MHC) class II and reducing the expression of pro-inflammatory cytokines (IL-1 and IL-6) from macrophages. [24] Increased IL levels -10 in mice with high-fat diet induction occurred because black seed oil contains the active compound thymoquinone. [25] Administration of black seed oil in previous studies showed a reduction in serum cholesterol and triglyceride levels as well as levels of pro-inflammatory cytokines consisting of TNF-α, IL-1, IL-6, and lactate dehydrogenase,[26] as well as increasing IL-10 levels. [27] Research on lipopolysaccharide (LPS)-induced bovine adipocytes also shows that Black Seed water extract has an immunomodulatory effect by reducing IL-6 and TNF-α levels and increasing the expression of IL-10. [28]

Thymoquinone regulates inflammatory processes in various ways, including inhibiting cyclooxygenase-2 (COX-2), NFκB and nitric oxide (NO) to reduce inflammatory cytokines. Thymoquinone inhibits NO production by reducing the expression of inducible nitric oxide synthase (iNOS),[29] and from the NFκB pathway, thymoquinone inhibits the nuclear expression of the P65 NFκB subunit and the binding of the P50 subunit to the TNF-α promoter. [30] This study's results are similar to previous studies that thymoquinone improves oxidative stress. Moreover, reduces the inflammatory response by reducing TNF-α and increasing levels.

IL-10 in the non-alcoholic fatty liver disease (NAFLD) rat model. Clinical trials in NAFLD patients can also prove that giving Black Seed seeds 2 g/day can reduce TNF-α and NFκB. [31]

This study found that administering black seed oil in a higher dose, namely 4 ml/kgbb, had a better effect in increasing IL-10 levels compared to a dose of 3 ml/kgbb. However, the effect of increasing IL-10 levels produced by black seed oil was still lower than that of simvastatin, and IL-10 levels in the group receiving black seed oil still did not reach normal levels. This is likely caused by the continued administration of a high-fat diet, which is a contributing factor. High CRP levels in this study's K(-) group were also associated with obesity and an unbalanced lipid profile. Obesity can lead to increased inflammatory cytokines and decreased adiponectin, while increased adipose size contributes to increased free fatty acids and increased CRP levels. CRP has a significant relationship with lipid profiles, such as total cholesterol, triglyceride, LDL, VLDL and HDL levels, which was also observed in this study. These results show that lipid metabolism disorders occur simultaneously with the inflammatory process. [32] Increased CRP levels are one of the main causes of atherosclerosis and heart disease, along with VLDL levels, total cholesterol, LDL, triglycerides and decreased HDL levels.[33]

By administering Black Seed, the increase in CRP levels due to induction of a high-fat diet can be suppressed through weight loss and improvement in lipid metabolism disorders as indicated by a decrease in total cholesterol levels, triglyceride levels, LDL levels and an increase in HDL levels. A literature review conducted by Al Asoom states that long-term administration of Black Seed (6-12 weeks) can significantly reduce body weight and anthropometric indices and improve lipid profiles, glucose levels, and insulin resistance. This effect is demonstrated because the fatty acid and thymoquinone content in black seed can suppress appetite, reduce calorie intake and inhibit intestinal glucose absorption. [34] Reducing body weight will reduce low-grade systemic inflammation, characterized by decreased IL-6 levels released by adipose tissue. Subcutaneous or abdominal visceral, so that stimulation of acute phase protein (CRP) production by the liver also decreases.[35]

Improving the lipid profile of Black Seed comes from its essential oils, namely nigellone, thymoquinone, phytosterols, flavonoids, and other compounds that act synergistically to inhibit de novo cholesterol synthesis, reduce intestinal cholesterol absorption, increase biliary or fecal cholesterol excretion and increase regulation of hepatic LDL receptors. Clinical trials on NAFLD patients can also prove that administering 2 g/day of Black Seed seeds can reduce CRP levels. [36] Systematic reviews and meta-analyses of randomized controlled trials also state that Black Seed seeds can significantly reduce serum CRP levels. [37] Thymoquinone suppresses IL pro-inflammatory factors. -6 through inhibition of the AP-1/NFκB pathway associated with IRAK. [38] Suppression of IL-6 causes CRP production to decrease because IL-6 is involved in CRP regulation.

Administration of simvastatin also significantly reduces CRP levels, which occurs by directly reducing CRP production in hepatocytes through inhibiting protein geranylgeranylation and inhibiting STAT3[39] phosphorylation.

In this study, administering a higher dose of black seed oil of 4 ml/kgbb had a better effect on CRP levels than a dose of 3 ml/kgbb. The effect was similar to simvastatin, but the effect obtained was not equivalent to normal conditions. This result was also caused by the treatment of dyslipidemia not being accompanied by cessation of the high-fat diet, as well as the duration of administration being less long.

V. CONCLUSION

1. Black Seed significantly affected IL-10 levels and CRP levels in male Wistar rats that had been induced on a high-fat diet.
2. The results of the analysis showed that the mean IL-10 level in the control group (K(0)) was 111.41 ± 4.40 pg/ml; negative group (K(-)) was 31.52±2.45 pg/ml; positive group (K(+)) was 82.45±3.53 pg/ml; treatment group 1 (P1) was 60.31 ± 2.44 pg/ml; and treatment group 2 (P2) was 89.61 ± 4.07 pg/ml. Meanwhile the mean CRP level in the control group (K(0)) was 0.74 ± 0.02 ng/ml; negative group (K(-)) was 2.28±0.01 ng/ml; positive group (K(+)) was 0.83±0.01 ng/ml; treatment group 1 (P1) was 1.08±0.01 ng/ml; and treatment group 2 (P2) was 0.85 ± 0.03 ng/ml..
3. The difference in mean IL-10 levels between groups proved significant. In contrast, the difference in mean CRP levels between the two groups was insignificant, especially in comparing the K(+) and P2 groups.

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