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The Potential of Black Cumin (*Nigella sativa*, L.) Seeds Extract to Prevent Polyphagia and Weight Loss in *Rattus Norvegicus* of Diabetes Mellitus-Type 2

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Abstract

Patients of Diabetes Mellitus-type 2 (DM-2) is not only characterized by an increase of blood glucose levels, but also it is characterized by polyphagia and weight loss. This research aimed to discover the potential of 80% ethanol extract of Indonesia Nigella sativa L. (black cumin) seeds to the feed consumption level and changes in body weight of DM-2 rats model due to the administration of High Fat Diet (HFD) followed by the induction of streptozotocin (STZ) in a dose of 30 mg/kg Body Weight (BW). The DM-2 rats as the induction results divided into five groups, it was given a treatment using N. sativa extract with different doses, i.e. 0, 24, 48 and 72 mg/kg BW (DM-0, DM-24, DM-48, DM-72), positive control used metformin 45 mg/kg BW (DM-Metf). Non-DM rats (Normal) used as reasonable control. HFD induction carried out for 14 weeks, and N. sativa therapy conducted for four weeks after the oral glucose tolerance test. During the treatment, an observation of the feed consumption level and weekly weight gain were carried out. The data obtained were tested using one-way ANOVA, and it continued by Duncan Multiple Rank Test (DMRT), α= 5%. The research results indicated that the administration of 80% ethanol extract Indonesia N. sativa using the doses of 24 mg/kg BW and 48 mg/kg BW can control the feed consumption levels and prevent significant weight loss (p<0.05).

1. INTRODUCTION

More than 90% of Diabetes Mellitus-type2 (DM-2) patients are suffered by those who are obese [1]. The DM-2 of non-obesity often found in European and Asian countries [2]. Several types of research shows that people

with DM-2 who have average weight have more visceral fat. The abdominal fat release a hormone that affects glucose metabolism and interferes with fat metabolism. The belly fat can make a person's typical metabolic profile looks like someone's profile that is overweight, maybe even they looked slim. However, visceral fats are closely related to the occurrence of insulin resistance, including slim individuals [3].

Both DM-2 obese and non-obese have the same pathogenesis due to a decrease in insulin production; the cells also proficiency insulin resistance [4; 5]. There is a slight difference between the two, in which the DM-2 of non-obesity in a decrease of insulin production and insulin resistance occurs not as massive in the DM-2 obesity [2]. The reduction of insulin level and even insulin resistance causes the cells to experience glucose deficiency as a source of energy through the levels in the blood are very high. Limitations of glucose in cells of DM patients force lipolysis of adipocyte cells, release energy deposits into the circulation and hepatocytes that perform gluconeogenesis [6; 7]. Even though the DM patients are suffering often accompanied by polyphagia, decreased digestive efficiency and increased gluconeogenesis, it causes on the weight loss [8]; hence, the body of DM patients quickly becomes very emaciated, especially in DM-2 of non-obesity.

Weakened insulin production and physiological function in both types of DM-2 have the same general principle of treatment, namely increasing insulin production and decreasing insulin resistance to reduce blood glucose levels. However, there are slightly different parameters between the two. Since in the DM-2 obesity is triggered by being overweight, one of therapies of DM-2 obesity is by carrying out weight loss [9; 10], whereas therapy of DM-2 of non-obesity is expected to be able to decrease the blood glucose levels accompanied by weight loss recovery. Nevertheless, weight normalization is an indicator that accompanies the success of therapy all diabetes.

Consumption of black cumin seeds customarily has been reported to the public and laboratory tests related to N. sativa as an antioxidant, antibacterial, anti-fungal, anti-cancer, anti-inflammatory, antiallergic, hepatoprotector [11], antifertility [12]. The ethanol extract of N. sativa seeds can decrease blood glucose levels and able to improve the lipid profile of serum DM-2 rats [13]. Thymoquinone is the main active compound in N. sativa as an antidiabetic, one of which is through its potential to reduce liver gluconeogenesis [14]. However, the potential of N. sativa in improving weight loss for those who are DM-2 patients still requires empirical data from laboratories. This research aims to discover the effectivity of N. sativa seeds extract as antidiabetic, in which it is indicated through the existence of decreased feed consumption, increased digestive efficiency, to the increased weight loss in DM-2 rats.

2. MATERIALS AND METHODS

Study area

The animals model used are *Rattus norvecicus starin wistar* male, 3-4 months old, 150-200 g body weight obtained from the Integrated Research and Testing Laboratory of Gajah Mada University, Yogyakarta. The *N. sativa* seeds obtained from Balitro, Bogor, Indonesia and the metformin was from Kimia Farma, Indonesia. Rat feed of Broiler feed-1 (BR-1) was from Pokphand, Indonesia, and Streptozotocin (STZ, Merck).

Research design

The DM-2 rats divided into five groups using the treatment extract of *N. sativa* seeds, in which the doses were different, i.e. doses of 0 mg/kg BW, 24 mg/kg BW, 48 mg/kg BW, 72 mg/kg BW (DM-0, DM-24, DM-48, DM-72), metformin 45 mg/kg BW (DM-Metf), and control of non DM (Normal). The rats' group of DM-0 and Non-DM got 2.5 ml Na-CMC 0.5%. The treatment was given orally for four weeks.

Induction of DM-2

After acclimation for two weeks, it continued by inducing the DM-2 with HFD administration (BR-1: cow fats = 2:1, 40 g/head/day) [15] for eleven weeks; furthermore, the intraperitoneal injection of STZ (in the citrate buffer of 0.01, pH 4.5) carried out using the doses of 30 mg/kg BW in twice in the 10^{th} and 11^{th} weeks of the HFD induction period. Glucose tolerance test carried out on day five after the last STZ injection. The rats used as the examination samples had a minimal glucose level of 200 mg/dl [16].

Extract Preparation

The seeds of *N. sativa* were dried up using an oven in 40 ^{o}C for 2x24 hours, and the powder were mashed and sieved using a 60-mesh sieve until subtle powder obtained. The triturate were soaked in 80% alcohol (1:5 = b: v) for 24 hours repeatedly until the filtrate was clear. The filtrate evaporated using a vacuum rotary evaporator at 40 ^{o}C , the concentrated extract obtained.

Treatment and Data Collection

The rats maintained in the individual cage. The daily temperature as room temperature of 22 oC to 25 oC, lighting with twelve hours of dark and twelve hours of light. The feeds were given dayli or 40 grams each, and the drinking water was given in ad libitum. Weighting the remaining feeds was carried out everyday, the body weighting carried out once a week. The data collected during the research were as follows:

Weekly Feed Consumption, it was carried out by adding up the amount of daily feed consumption for seven days during sixteen weeks of the research.

Weekly Weight Gain, it was carried out by subtracting the final body weight with early week weight for sixteen weeks of research.

Data Analysis

The data were analyzed statistically using the SPSS ver. 16 software. The data which had a normal distribution and homogenous variants (Kolmogorov-Smirnov and Levene tests) were analyzed using one-way ANOVA; then, it followed by the DMRT test, α=5%.

3. RESULTS

The research result indicated that the data normally distributed and it had homogenous data variants ($p>0.05$). The results of the ANOVA test produced the value of $p<0.01$. At the same time, the results of the DMRT test showed that the treatments of DM-24 and DM-48 gave the best results to improve the feed consumption levels of DM-2 rats. The effect was as good as the treatment of DM-Metf until the feed consumption rate was the same as the normal rats (Table 1, Figure 1). On the DM-0 rats group, the feed consumption level continued to increase until the end of the study.

Table 1. The results of Duncan Test on the Average of Feed Consumption Level and Body Weight of Rats (*Rattus norvegicus*) of Diabetes Mellitus-type 2 Model.

Treatment	Feed consumption mean ± SD (g)	Body weight mean ± SD (g)
Normal	208.50 ± 1.93^{c}	335.38 ± 30.90^{d}
DM-Metf.	229.06 ± 2.98^{bc}	307.25 ± 21.06^{bcd}
DM-0	237.44 ± 1.27^{a}	256.00 ± 33.25^{a}
DM-24	202.44 ± 3.08^{abc}	296.00 ± 33.83^{bc}
DM-48	203.81 ± 8.27^{c}	323.63 ± 35.49^{cd}
DM-72	232.81 ± 4.13^{ab}	265.06 ± 11.11^{ab}

Description: Different notations showed significant differences, p= <0.05

The research result indicated that the data normally distributed and it had homogenous data variants ($p>0.05$). The results of ANOVA test showed that the 80% ethanol extract of Indonesia *N. sativa* was very significant ($p<0.01$) affected to body weight of *R. norvegicus* with diabetes mellitus-type 2 model. Furthermore, the results of the DMRT test showed that the treatment of DM-48 gave the best results to improve the body weight of DM-2 rats, the effect was as good as the treatment of DM-Metf, only the DM-48 and DM-Metf treatments which had the similar dody weight but not the same as the normal body weight (Table 1, Figure 2).

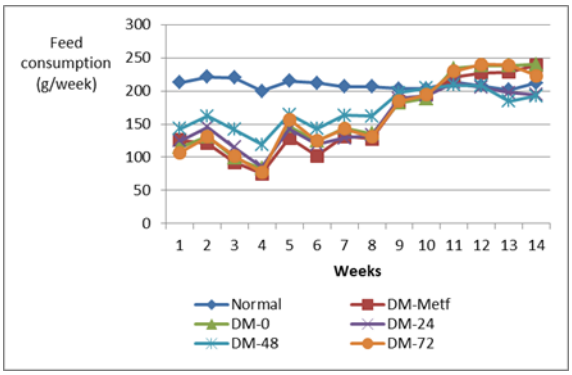

Figure 1. Feed Consumption Levels of Diabetes Mellitus-Type 2 Rats Model

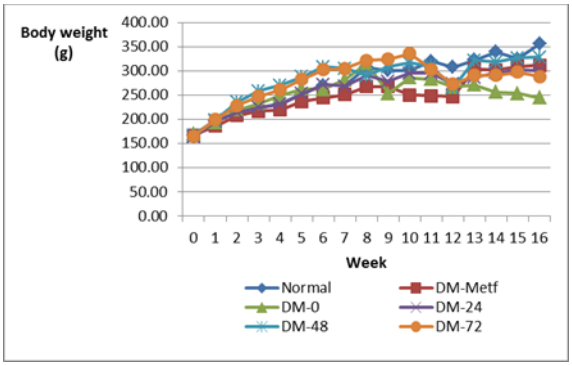

Figure 2. Body Weight of Diabetes Mellitus-Type 2 Rats Model

4. Discussion

The HFD feed initially less favoured by the rats (Figure 1 weeks 1 to 8), yet the preference of HFD-diet continued to increase over time. The STZ injection on all of the DM rats starting from week 10 increased the feed consumption numbers, which exceeded the normal rats (Figure 1). This result in line with the findings reported that there is a powerful correlation between DM and polyphagia [17]. This is due to the pancreas DNA of DM rats that oxidized and apoptotic [15]; thus, the insulin production is reduced [18]. Insulin limitations caused cells unable to take glucose from the blood, lack of energy on cells, cells sent signals to the hypothalamus that the cells required energy intake which manifestated as starvation and showed the level of food consumption exceeded the normal at 11-14 weeks.

The treatment use black cumin extract on this enquiry results could suppress and normalize the feed consumption levels (Table 1, Figure 1). The use of ethanol extract of N. sativa the doses of 24 and 48 mg/kg BW for twenty-eight days in this study was the best way to affect the feed consumption levels so that it was the same as the effects of the standard drug metformin even to the same as normal rats. This finding was accordance with a research report N. sativa treatment in improving the rats body weight through a therapy compared to the DM rats of non-treatment. This condition is due to improvements in the structure of the pancreas [19]. The consumption of N. sativa also rectified the insulin functional; this was coherent to the statement that the consumption of N. sativa oils in 3 g/day for the DM patients during 12 weeks can slightly reduce the insulin resistance and dietary intake [20].

Typically, high feed consumption level enhanced the weight gain. However, in the DM-2 rats model was inversely proportional, the feed consumption of diabetic rats (DM-0) which was high had low body weight (Table 1), the body weight of the DM-0 group rats continued to decline until the end of the research (Figure 1 week 11 to 14). This result was in accordance with the fact of weight loss in individuals who suffered from the DM-2 [21; 8].

Diabetic rats in this research experienced not only polyphagia but also weight loss (Table 1, Figure 2). This result relative to those experienced by diabetics in general [22]. This study also indicated the feed efficiency that was very low because of glucose of absorption results and the results of gluconeogenesis as the body's response to low glucose level. The energy source in cells could not be utilized properly. The DM rats experienced thermodynamic balance disorder and efficiency of energy [23].

The *N. sativa* extract treatment in this examination could prevent weight loss and could normalize it. This results were better than the finding on the therapy of thymoquinone 10 mg/kg BW on male diabetic rats for 32 days that could not prevent the weight loss [24]. The DM-48 dose in this inquiry could increase the body weight so that it was the same as the normal rats (Table 1, Figure 2). This finding powered by a report that thymoquinone, carvacrol, t-anethole and even 4-terpineol in *N. sativa*, also used as an excellent free radical scavenger [25]. Thymoquinone acted as an antioxidant to prevent oxidative stress of pancreatic beta cells and body cells [26]. Another research reported that the *N. sativa* able to improve the proliferation and regeneration of pancreatic beta cells that had damaged and it played by thymoquinone [27; 28].

Furthermore, the enhancement of consumption of DM-48 rats in this study also reinforced by the research results which proved that the *N. sativa* could rectify the structure and physiological function of rats pancreas of DM-24 and DM-48. The ethanol extract of *N. sativa* improved the insulin secretion from pancreas [29], and the insulin sensitivity of Guinea Pigs Non-Insulin-Dependent Diabetic (NIDDM) [30]. Another researcher also reported similar findings that the *N. sativa* oils improved the signalling and could prevent the degradation of the insulin enzyme [31]. The increasing of insulin production and functional could facilitate the transportation of glucose from the circulation so that the glucose requirements in congregation the energy needs and other related to the metabolisms in various cells and tissues were improving. This condition allowed the cells and tissues to grow so that there was an amendment on the body weight. The research findings were coherent with a report on the insulin therapy that could improve the body weight of DM-2 patients [32]. Hence, the normalization of the structure and function of the pancreas by *N. sativa* extract can facilitate the transport of glucose into cells. This amelioration was followed by optimize the glucose in the blood to be regenerated into energy so that there is a decrease in the feed consumption level, and finally increase the body weight of rats that given the treatment of *N. sativa*.

5. CONCLUSION

The extract of black cumin (*Nigella sativa, L.*) has a potential to prevent the occurrence of polyphagia and it can normalize the feed consumption levels to prevent the weight loss of Diabetes Mellitus-Type 2 rats.

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