Journal of Research in Medical and Dental Science
2018, Volume 6, Issue 4, Page No: 81-87

Available Online at: www.jrmds.in
eISSN No.2347-2367: pISSN No.2347-2545

The Effects of *Nigella sativa* Hydro-alcoholic Extract and Honey on Lipid Profile and Indices of Insulin Resistance in Polycystic Ovarian Syndrome Wistar Rat Model

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common complex endocrine and metabolic disorder. The cause of PCOS is still unknown and may be due to several factors. The aim of the present study was to evaluate the combined effect of Nigella sativa hydro-alcoholic extract and honey on triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C), glucose and insulin in PCOS Wistar rat model.

Materials and Methods: 72 adult Wistar rats, weighting 200-220 g were divided randomly into 9 groups (n=8): including control (intact), Sham (letrozole solvent), control PCOS and 6 experimental PCOS groups. Rats were treated with letrozole for 21 days to induce PCOS. In experimental groups, PCOS rats were treated with 2 doses of honey (1200 and 2400 mg/k), 2 doses of Nigella sativa extract (300 mg/k and 600 mg/k) and 2 doses of combination of Nigella sativa extract and honey by the gavage for 28 days. After harvesting blood serum, lipid profile, glucose and insulin were measured by ELISA method. SPSS software version 16 was used for data analysis.

Results: Serological analysis showed lower TG, TC, LDL, Glucose and insulin in all experimental groups. TG, TC, LDL, Glucose and insulin, particularly at dose 2 of combination of Nigella sativa extract and Honey was significantly decreased ($p \leq 0.05$) as compared with control PCOS group. HDL level was significantly increase in dose2 of combination of N. sativa extract and Honey groups.

Conclusion: Combination of Nigella sativa extract and Honey improves lipid profile and the sensitivity to insulin in patients with PCOS.

Key words: *Nigella sativa*, Honey, Polycystic ovarian syndrome, Insulin resistance, Lipid profile, Wistar rat

HOW TO CITE THIS ARTICLE: Seiyedeh Narjes Naseran, Mokhtar Mokhtari*, Mahmood Abedinzade, Mehrdad Shariati, The effects of *Nigella sativa* hydro-alcoholic extract and honey on lipid profile and indices of insulin resistance in polycystic ovarian syndrome Wistar rat model, J Res Med Dent Sci, 2018, 6(4): 81-87

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Received: 01/07/2018
Accepted: 31/07/2018

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting approximately 5 to 10 percent of women with reproductive age [1]. This syndrome is characterized by high androgen (hyperandrogenism) and disorder of gonadotropin secretion. There are symptoms such as hirsutism and acne, menstrual disorders, anovulation and finally infertility [2,3]. The cause of PCOS is still unknown and may be due to several factors such as genetic causes, neuroendocrine, and metabolism. Some researchers believe that PCOS is not an ovarian disease, but a metabolic disorder [4]. Women with PCOS are more likely to have hyperinsulinemia and IR with higher frequency and severity than the control group. The results of recent studies indicate that the prevalence of type 2 diabetes in patients with PCOS is 7 times higher than the control group [5]. Diabetes can increase blood glucose and lipid disorders such as increased free fatty acid, triglycerides, total cholesterol, LDL, and decreased HDL-cholesterol [6]. Several therapeutic methods have been proposed to control or treat the symptom of PCOS, such as lifestyle changes, surgery, and drug use. Currently, the most well-known therapeutic approach is the use of drugs such as clomifen citrate, metformin, letrozole and tamoxifen [7]. Given that these conventional synthetic drugs can cause many unwanted serious side effects, identifying and providing alternative drugs are important. *Nigella sativa* has a wide range of medical properties including anti-microbial, anti-tumor, analgesic, anti-inflammatory, anti-oxidant, salivation, regulating menstruation, laxative and increasing milk secretion [8,9].

N. sativa contains fiber, salts (ions) and elements such as zinc, copper, sodium, iron, calcium and various vitamins including ascorbic, thiamine, niacin and folic acid. The seeds of this plant are the rich source of fatty acid esters such as lauric acid, myristic acid, stearic acid, palmitic acid, lolic acid and linolenic acid [10]. Prophet Muhammad (peace be upon him) said that the black seed is used to heal every disease except death [11]. In various studies, antioxidant effects of *N. sativa* and its main component, thymoquinone (TQ), have been proven and it has been shown that plant extract and thymocinone have inhibitory effect on lipid peroxidation and free radical scavengers [12,13]. Oral administration of *N. sativa* in diabetic rats caused to decrease in blood glucose, glutathione and glutathione peroxidase levels in the liver and kidneys [14]. Honey is present in nature as an effective antioxidant. It is known as a biological antioxidant, with other important properties such as antimicrobial and anti-inflammatory. Honey's phenols increase its antioxidant capacity. In addition, other compounds such as catalase, fructose and glucose, minerals such as magnesium, potassium and calcium, vitamin C, and all kinds of B vitamins confirm the antioxidant properties of honey [15]. Honey has anti-diabetes property [16]. It has been proven to be effective for controlling lipid metabolism due to antioxidant properties [17]. In Iran, the combination of *N. sativa* with Honey (Dosin) is used for traditional and Islamic medicine [18]. Given that little studies have been done on the combined effects of *Nigella sativa* and Honey, this study focused on the possible therapeutic efficacy of natural supplement of combination of *N. sativa* extract and Honey to improve complications of PCOS and the effect of this supplement on insulin, glucose, triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C).

MATERIALS AND METHODS

Animals

All experiments were conducted in accordance with the national laws for the use of animals in research approved by the local ethical committee at Islamic Azad University of Kazeroon. 72 adult Wistar rats, weighting 200 g-220 g were purchased from the animal house of the Islamic Azad University of Kazeroon. All rats were housed at a temperature of 22°C ± 2°C and exposed to 12 hours of light/darkness, Food and water were provided to the rats without restrictions.

Experiment design

Estrous cyclicity was monitored by vaginal smears. Rats had 2-3 regular estrous cycles during the twelve to fourteen days of vaginal smear, and they were in the estrous phase of their reproductive cycle [19].

In this study hormonal induction of PCOS by letrozole (Aburaihan Co., Iran) was used. To induce PCOS, phenotype letrozole (concentration of 1 mg/kg) was dissolved in 0.5% carboxymethyl-cellulose (CMC) and daily administered to the rats by gavage for 21 days. During this period, the estrus cycle was microscopically evaluated by the analyses of proportion of leukocytes, epithelial and cornified cells [20]. Natural Honey was purchased from the Bozkouye village, Guilan province, Iran.

The *N. sativa* seeds were purchased from a local herbal shop Shiraz, Fars province, Iran. The *N. sativa* hydro alcoholic extract was prepared by "percolation" method [21]. Doses were prepared (300 mg/kg and 600 mg/kg) [22] and mixed with doses of 1200 mg/kg and 2400 mg/kg [23] of Honey solution respectively.

72 Female rats were divided into 9 groups (n=8 each): control had no treatments, sham received letrozole solvent (CMC) for 21 days, control PCOS and 6 experimental PCOS groups (Rats were administered with letrozole at the concentration of 1.0 mg/kg body weight (bw), dissolved in 2.0 ml/kg bw, of 0.5% CMC for 21 days). After ensuring that the syndrome was induced, PCOS rats were divided into 7 groups: Control PCOS group (Group 1), and 6 experimental groups (n=8 each). The experimental groups included Group 2: rats received oral doses of 1200 mg/kg Honey, Group 3: rats received oral doses of 2400 mg/kg Honey, Group 4: rats received oral doses of 300 mg/kg *N. sativa* extract, Group 5: rats received oral doses of 600 mg/kg *N. sativa* extract, Group 6: rats received oral doses of combination of 300 mg/kg *N. sativa* extract and 1200 mg/kg Honey and Group 7: rats received oral doses of combination of 600 mg/kg *N. sativa* extract and 2400 mg/kg Honey for 28 consecutive days.

Termination of the procedure

At the end of the experimental period, the rats were anesthetized by ether and fasting blood samples were drawn directly from cardiac. Then blood samples were centrifuged at 3000 rpm for 15 minutes and serum was separated and used for the biochemical analysis. The serum was utilized to determine the levels of several biochemical factors, including blood insulin (Mouse Insulin, INS ELISA Kit, CSB-E05071m), blood glucose and lipid profile, using commercial kits (Pars azmoon, Iran).

Statistical analysis

The results are presented as mean ± SD. The data was analyzed *via* the SPSS program using Student's t-test to compare two means, or analysis of variance (ANOVA followed by Tukey's test) for multiple comparisons. $p \leq 0.05$ was considered the criterion for significance.

RESULTS

The results of the present study indicate changes in lipid profile, insulin and FBS (Fasting Blood Sugar) in the PCOS group compared to control groups.

The levels of cholesterol, triglyceride, LDL-cholesterol, insulin and FBS in PCOS group showed the significant increase ($p \leq 0.05$) as compared with control and sham

groups (Figure 1), whereas HDL-cholesterol level was significantly reduced ($p \leq 0.05$) (Figure 1).

Serological analysis showed lower TG, TC, LDL, FBS and insulin in all experimental groups. TG, TC, LDL, Glucose and insulin levels in experimental groups, particularly at dose 2 of combination of *N. sativa* extract and Honey (600 mg/k *N. sativa* extract and 2400 mg/k Honey) were significantly decreased ($p \leq 0.05$) as compared with control PCOS group (Table 1).

A significant decrease in TC, TG and LDL was found in the group of PCOS rats treated with dose 2 of combination of *N. sativa* extract and honey, Honey 2 and *N. sativa* 2 as compared with control PCOS group (Table 1). The levels of TG in the all experimental groups showed significant decrease as compared with control PCOS group (Table 1), While PCOS group treated with dose 1 of combination of *N. sativa* extract and Honey, dose 2 of combination of *N. sativa* extract and Honey and *N. sativa* 2 showed a significant increase in serum HDL as compared with control PCOS group ($p \leq 0.05$) (Table 1).

Insulin level was significantly decreased ($p \leq 0.05$) in PCOS rats treated with dose 2 of combination of *N. sativa* extract and Honey as compared with control PCOS group. Also PCOS group treated with dose 2 of combination of *N. sativa* extract and Honey and *N. sativa* 2 showed a significant decrease ($p \leq 0.05$) in serum glucose compared with control PCOS group (Table 1).

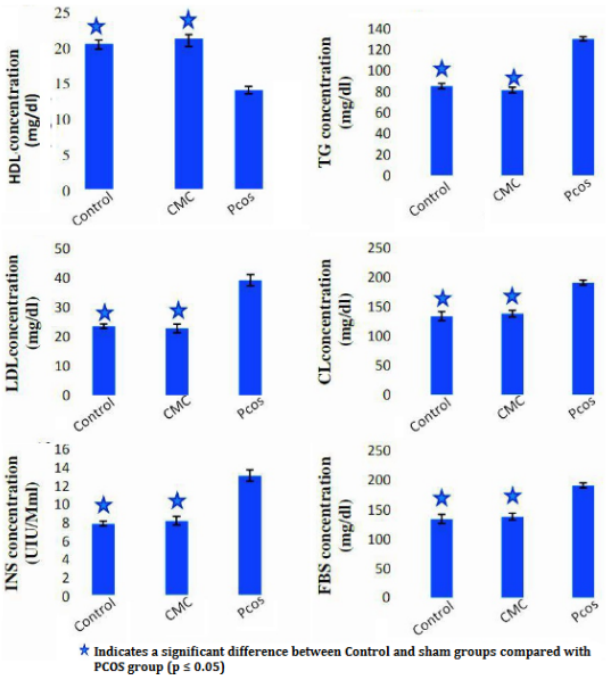

Figure 1: The average levels of triglyceride, cholesterol, LDL, HDL, insulin and FBS in control, sham and PCOS groups

Table 1: The average levels of triglyceride, cholesterol, LDL, HDL, insulin and FBS in different experimental groups

Groups| Variables	TC	TG	LDL	HDL	INS	FBS
Group 1 (PCOS)	130.62 ± 5.55	190.5 ± 11.17	39.37 ± 5.4	14 ± 1.51	13.03 ± 1.76	191.62 ± 12.14
Group 2 (Honey 1)	123 ± 5.63	146.5 ± 22.07*	36.75 ± 3.15	15.87 ± 1.24	11.78 ± 1.53	184.12 ± 16.42
Group 3 (Honey 2)	104 ± 8.34*	138.87 ± 23.33*	28.75 ± 3.91*	17 ± 1.06	10.73 ± 1.07	181.75 ± 25.46
Group 4 (*N. sativa* 1)	121.12 ± 5.56	145.25 ± 27.42*	35.5 ± 3.81	16.87 ± 1.12	11.92 ± 1.91	181.87 ± 19.14
Group 5 (*N. sativa* 2)	93.75 ± 10.2*	133.61 ± 24.41*	27 ± 1.06*	20.12 ± 2.35*	11.05 ± 1.24	145.37 ± 18.3*
Group 6 (*N. sativa* 1+Honey 1)	120.37 ± 8.19	143.37 ± 14.82*	35 ± 3.7	19.75 ± 2.05*	10.75 ± 0.93	160.62 ± 27.54
Group 7 (*N. sativa* 2+Honey 2)	92.37 ± 8.86*	129 ± 18.69*	24.5 ± 3.66*	22 ± 2.8*	8.76 ± 0.9*	140.5 ± 20.69*

*Indicates a significant difference among the experimental groups compared with PCOS group ($p \leq 0.05$)

DISCUSSION

In this study, the effect of *Nigella sativa* hydro alcoholic extract and Honey on lipid profile and indices of IR (insulin and glucose levels) in polycystic ovarian syndrome Wistar rat model were investigated. Our analysis of serum lipids showed significant increase ($p \leq 0.05$) in the TC, TG, LDL-C, insulin and glucose levels after the induction of PCOS by letrozole. Also after the induction of PCOS, HDL-C levels were significantly reduced ($p \leq 0.05$). Serological analysis showed lower TG, TC, LDL, FBS and insulin in all experimental groups. TG, TC, LDL, Glucose and insulin levels in experimental groups, particularly at dose 2 of combination of *N. sativa* extract and Honey (600 mg/k *N. sativa* extract and 2400 mg/k Honey) were significantly decreased ($p \leq 0.05$) as compared with control PCOS group. Also concentration of TG showed significant decrease ($p \leq 0.05$) in the all experimental groups as compared with control PCOS group. Serum HDL levels showed significant increase ($p \leq 0.05$) in the PCOS rats treated with dose 1,2 of combination of *N. sativa* extract and Honey as compared with control PCOS group. IR occurs for 50%-80% of women with PCOS [24] and it is a common symptom among women with PCOS. In IR, cells are not able to respond to insulin. High level of insulin causes the ovaries to not function properly. It is one of the mechanisms behind the development of fatty liver. High level of total cholesterol, LDL, triglyceride and low level of HDL are the most important factors in metabolic syndrome and PCOS. IR increases HDL catabolism and LDL particle formation [19].

In this study, the induction of polycystic ovary syndrome with letrozole had similar results in changing the lipid profiles, insulin and glucose. In addition to IR, hyperandrogenism as a marker of PCOS, contributes to changing lipid profiles. Hyperandrogenism is associated with an increased liver lipase activity affecting catabolism of HDL particles. Therefore, PCOS patients have more atherogenic lipids than the control group [25]. The results indicated that PCOS group treated with maximum dose of alcoholic extract of *N. sativa* seed and honey showed a significant decrease in serum TC, TG, LDL, insulin and glucose levels. In addition TG levels were significantly reduced in PCOS rats treated with minimum dose of alcoholic extract of *N. sativa* seed and Honey While PCOS group treated with dose 1,2 of combination of *N. sativa* extract and Honey showed a significant increase ($p \leq 0.05$) in serum HDL versus PCOS group. *N. sativa* is widely used to treat the various disorders and considered as a miracle herb with a rich historical and religious background [18]. *N. sativa* has substances such as thymol, thymoquinone and dithymoquinone to influence on blood lipid profiles. The study of Pourghassem et al. [26] indicated that administration of 7.5 grams of *N. sativa* daily with 0.5% cholesterol diet for 2 months in hyperlipidemic rabbits leads to decrease total cholesterol, triglyceride and LDL significantly [26]. *N. sativa* extract improves blood lipid profile in the rat, strengthens the antioxidant defense system, decreases blood glucose level in diabetic rabbits and prevents lipid oxidation in carbon tetrachloride-induced hepatotoxity mice [27]. Fararh et al. [28] reported a significant decrease in blood cholesterol and triglycerides levels in diabetic rats treated with thymoquinone [28]. TQ increases LDL-cholesterol uptake by increasing the expression of the liver LDL receptor gene [29]. *N. sativa* regulates the expression of *Apo A1* and *Apo B-100* genes and also cholesterol synthesis [30]. It can also reduce the production of liver fatty acids and, consequently improve insulin resistance by reducing the activity of Acetyl-CoA carboxylase (ACC) enzyme [31]. Soluble fiber (Mucilage) content of *N. sativa* causes to reduce cholesterol absorption, diet and stimulate bile acid synthesis, which leads to more cholesterol excretion [32]. Honey is also very important in traditional medicine [33]. It has high antioxidant effects [34] and able to prevent the various diseases such as cancer, coronary diseases, inflammation, neurodegenerative disorders and aging [35]. The antioxidant activity of Honey results from the activity of its compounds including phenol, peptide, organic acids, enzymes and other compounds [15]. Prophet Muhammad (peace be upon him) said “Honey is very useful to treat physical diseases and Qur’an is an effective book for mindful disorders, therefore it is recommended to use them [36]. 75 gram of Honey for 15 days significantly lowered lipid profile [37]. In a study by Yaghoobi et al. [38] the effects of Honey on fasting blood glucose, total cholesterol, body weight, LDL, HDL, C-reactive protein (CRP) were evaluated in 55 patients and showed that 70 g of Honey for 30 days would reduce LDL, triglycerol, and cholesterol in overweight patients. HDL level increased after long periods of time [38]. In another study by Adnan et al. [39], the effects of Honey on hyperlipidemic dietary rats were evaluated and their anti-hyperlipidemic properties confirmed [39]. There is Niacin in honey. It prevents adipose tissue lipolysis and decreases the synthesis of triglycerides in the liver and plasma triglycerides. Triglyceride synthesis is essential for VLDL synthesis, and LDL is formed from VLDL in the blood plasma. Therefore niacin reduces triglyceride, total cholesterol and LDL, and honey might mediate these effects through niacin [40]. The results of the present study showed significant decrease ($p \leq 0.05$) in insulin and glucose levels for PCOS rats treated with dose 2 of combination of *N. sativa* extract and Honey. *N. sativa* regulates the activity of the liver enzymes associated with the metabolism of glucose and decreases gluconeogenesis. The liver increases the activity of the hexokinase enzyme. It also inhibits the activity of the glucose 6-phosphatase and fructose-1,6-bisphosphatase enzymes, which have an important role in gluconeogenesis. In addition, the *N. sativa* increases the activity of the "glucose-6-phosphate dehydrogenase" enzyme that plays a role in the pathway of pentose phosphate in the cell [41,42]. In consistent with our results, the study of Fararh et al. [41] indicated that *N. sativa* oil (400 mg/kg bw) significantly decreased liver glucose and blood glucose in rats [41]. Oral treatment with 300 mg/kg *N. sativa* extract for 30 days decreased blood glucose, lipid profile, glutathione and glutathione peroxidase levels in the liver and kidneys in diabetic rats [14]. In another study, *N. sativa* extract increased insulin secretion in langerhans islet cells of rat [43]. The effects of *N. sativa* on decreased blood glucose are due to the synergistic effects of its various components, including thymocinone, soluble fibers (mucilages), sterols, flavonoids, and high levels of polyunsaturated fatty acids (PUFA) [30]. The antihyperglycaemia effects of Honey have been conformed in diabetic rabbits by chemotherapy. One study has indicated that different doses of Honey significantly reduce blood glucose level and other relevant parameters [44]. In another study, the effect of different types of honey on fasting insulin level indicated that it increases insulin level in healthy group compared to control group. This difference was not observed in the diabetic group. While Abdulrhman et al. reported that taking Honey for 12 weeks does not affect blood glucose in 20 patients with type 1 diabetes [45]. Münstedt et al. confirmed the ineffectiveness of Honey on short-term blood glucose (OGTT) testing [46]. In fact, Honey stimulates insulin secretion only in healthy mice [47]. Flavonoids have certain antioxidant effects on the pancreas. They have antidiabetic effect on alloxan-induced diabetic rats [48] and protect INS-1 β-cells against oxidative damage [49]. Honey contains nitric oxide metabolites. It probably stimulates nitric oxide synthesis and increases it. Nitric oxide is a stimulant for insulin release [50]. In a study by Mabrouk et al. the combination of honey and black seed increased NO and had a protective effect against oxidative stress [51]. Also, glucose and fructose contents in Honey have hypoglycemic effect. Glucose and fructose exert a synergistic effect on the gastrointestinal tract and

pancreas. These effects increase insulin level [52]. Since the letrozole induced model does not represent all morphological, hormonal and metabolic aspects of polycystic ovary syndrome, it is a limitation of this research.

CONCLUSION

According to the results of this study, combination of *Nigella sativa* extract and Honey can regulate lipid profile and increase the sensitivity to insulin in patients with PCOS and thus reduce the risk of some complication such as metabolic syndrome. The authors suggest that future research be done on the effects of *Nigella sativa* extract and honey on hormonal parameters, morphological and morphometric changes in the ovaries and uterus in the PCOS.

ACKNOWLEDGMENT

Authors thank to Kazerun Islamic Azad University for their technical assistant.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

ETHICAL APPROVAL

All institutional and national guidelines for the care and use of laboratory animals were followed.

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