The Effects of Nigella Sativa seed and Nigella Sativa Honeybee on Hypercholesterolemic Rats

Introduction

Plants have been an important source of medicine for thousands of years. The World Health Organization (WHO) estimated that up to 80% of people still rely on herbal remedies for their health care (Farnsworth et al., 1985). The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main sources of drugs used for therapeutic purpose (Hernández-Ceruelos et al., 2002). Plants have always been a major source of nutrition and health care for both humans and animals. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Schwartsmann et al., 2002).

The Nigella sativa (black seed) is a type of plant that belongs to the Ranunculaceae family (El-Dakhakhny et al., 2000). It has been used as a herbal medicine for more than 2000 years. It is also used as a food additive and flavouring agent in many countries. The black seed oil is reported to be beneficial due to its content of over a hundred components such as aromatic oils, trace elements, and vitamins (Ali and Blunden, 2003). The seeds of Nigella sativa Linn have been used in Southeast Asia, Middle and Far East as a natural remedy to treat many diseases, including asthma, hypertension, diabetes hypercholesterolemia, inflammation, arthritis, tumors, gastrointestinal disturbances and gynecological disorders for over 2000 years (Ali and Blunden, 2003, Kamal El-Din et al., 2006 and Ramadan, 2007).
Honey is a natural mixture of fructose-glucose along with some oligosaccharides, proteins, vitamins and minerals with PH between 3 and 4. Some studies demonstrated that consumption of honey decreases body weight, total cholesterol, low-density lipoprotein-cholesterol and triglyceride, while high-density lipoprotein-cholesterol increased significantly (Bahrami et al., 2009). Most studies indicated that honey administration include oral, topical and parenteral route Many complications have been attributed to oxidative damage, including atherosclerosis, aging, and cancerous diseases. Antioxidant foods that are rich in flavonoids are protective agents against these ailments (Perez et al., 2006).

Hypercholesterolemia is one of the crucial risk factors for the development of atherosclerosis and subsequent cardiovascular diseases (Steinberg, 2002). Cholesterol-rich diets are associated with free radicals production, followed by oxidative stress and hypercholesterolemia (Stehbens, 1986 and Bulur et al., 1995). Hypercholesterolemia is a causal factor of atherosclerosis, a process histologically characterized by lesions processing from fatty streaks to fibrous plaques, ultimately occludes the lumen of the affected artery (Ross, 1993 and Lee and Libby, 1997).

**Aim of the study**

This study is conducted to investigate the effects of Nigella Sativa seed and Nigella Sativa Honey bee and mixture of them on hypercholesterolemic rats.

**Material and Method**

**Materials:**-
- Cholesterol, white crystalline powder was obtained from Infinity Company for Chemical, Cairo, Egypt.
Nigella sativa seed and nigella sativa honeybee were obtained from ministry of Agriculture, Giza, Egypt.

Casein, vitamins, minerals, cellulose, Choline chloride, Sodium selenite and Zinc carbonate were obtained from El-Gomhoria Company – Cairo – Egypt.

Kits for blood analysis were purshed from Gama Trade Company for Chemical, Cairo, Egypt.

Thirty male albino rats (Sprague–Dawley strain), weighting 120 ± 5 g were used in the present study. The animals were obtained from Helwan experimental animal station. Rats were housed in well aerated cages under hygienic condition, and fed on basal diet for one week for adaptation in biological studies lap of faculty of Home Economics, the experimental diet were prepared according to Reeves et al., (1993).

Method:-

Hypercholesterolemia was induced in rats according to Rodas et al., (1995) by adding (1.5% cholesterol and bile salts 0.25%) to basal diet for two weeks.

Experimental design:-

Rats were divided into two main groups as follows; the first main group (6 rats) fed on basal diet (as a control negative group), the second main group (twenty four rats) fed on hypercholesterolemic diet, then was divided into 4 sub groups, each subgroup consisted of (6 rats each, these rats were divided as follows; Subgroup (1) fed on basal diet (as a control positive group). Subgroup (2) fed on basal diet supplemented with (2% grinded nigella sativa seed /kg diet). Subgroup (3) fed on basal diet supplemented with (5% nigella Sativa Honeybee /kg diet). Subgroup (4) fed on basal
diet supplemented with (2% grinded nigella sativa seed plus 5% nigella sativa Honeybee /kg diet).

**Blood samples:-**

Blood samples were collected from each rat, after the end of the experimental period (6 weeks), and centrifuged at 3000 r.p.m. for 10 minutes, and serum was separated to estimate some biochemical parameters.

**Biological evaluation:-**

Daily feed intakes (FI) per each group were recorded throughout the experimental period. Body weight gain percentage and feed efficiency ratio will be calculated according to the method of Chapman et al., (1959).

\[
\text{BWG}\% = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100
\]

\[
\text{FER} = \frac{\text{Gain weight}}{\text{feed consumed}}.
\]

**Biochemical analysis:-**

Serum total cholesterol (TC) concentration was determined according to the method of Allain et al., (1947). Serum triglyceride (TG) concentration was determined according to the method of Fossati and Precipe (1982). High density lipoprotein-cholesterol (HDL-c) concentration was determined according to the method of Lopes et al., (1977). Concentrations of LDL-c and VLDL-c are calculated by the equation of Friedwald et al., (1972). Aspartate amino transferase (AST) and Alanine amino transferase (ALT) was measured according to the method of Retiman and Frankel (1972).
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Statistical Analysis:

The obtained results were analysed statistically, according to SPSS program, on way ANOVA test SPSS (1986).

Results and Discussion

As shown in Table (1), the comparison between the mean value of feed intake (FI) of control negative group (-Ve), control positive group (+Ve) and the treated groups. The mean value of feed intake in the negative control group recorded 10.42 g/day for each rat, while the mean values of feed intake in the positive control group which suffering from hypercholesterolemia recorded 14.05 g/day for each rat. Also, feed intake increased in the positive and all treated groups, as compared to negative control group.

The mean value of BWG% significantly increased (p < 0.05) of the positive control group, as compared to the negative control group (36.33±8.40 vs. 20.63±4.06). Also, table (1) indicated that all treated groups’ tended to have significantly decreased (p < 0.05) in BWG%, as compared to the positive control group. It show be noted that there is no significant difference between all treated groups, Whereas, the mean values of body weight gain had significantly increased as compared to the negative control group. Abd El-Maksoud et al., (1996) reported that rats fed the hyperlipidemic diet with 5 % raw nigella sativa seed showed decrease in body weight, as compared to the negative control group, On the other hand they found that the weight gain increased in rats fed hyperlipidemic diet with 2.5 % raw nigella sativa for 4 weeks. With respect to feed efficiency ratio, the mean value of positive control group (0.22±0.01) was significantly higher than that of the
negative control group (0.10±0.01). Also, all treated groups decreased significantly as compared to the positive control group.

Table (1): Effect of Nigella Sativa seed powder and Nigella Sativa Honeybee supplementation on feed intake (FI), body weight gain % (BWG) and feed efficiency ratio (FER) of hypercholesteremic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>FI (g/day)*</th>
<th>BWG (%)</th>
<th>FER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control negative (-)</td>
<td>10.42</td>
<td>20.63±4.068c</td>
<td>0.107±0.013c</td>
</tr>
<tr>
<td>Control positive (+)</td>
<td>14.05</td>
<td>36.33±8.401a*</td>
<td>0.222±0.019a</td>
</tr>
<tr>
<td>2% NS seed /kg diet</td>
<td>12.75</td>
<td>32.92±3.168b</td>
<td>0.120±0.015b</td>
</tr>
<tr>
<td>5% NS Honeybee /kg diet</td>
<td>13.39</td>
<td>30.01±3.435b</td>
<td>0.126±0.006b</td>
</tr>
<tr>
<td>2% NS seed plus 5% NS Honeybee /kg diet</td>
<td>14.11</td>
<td>31.92±3.159b</td>
<td>0.114±0.014b</td>
</tr>
</tbody>
</table>

* Mean g/day/6 rats.

Values are expressed as mean ± S.E.

Values at the same column with different letters are significant at p< 0.05.

From data recorded in Table (2), it could be noticed that the mean value of serum cholesterol significantly increased (p <0.05) of the positive control group, as compared to the negative control group (161.75±6.78 vs. 75.77±5.76 mg/dl), respectively. The mean values of total cholesterol in all treated groups are significantly increased, as compared to the negative control group. Also, From table (2) can note that all treated groups decreased significantly as compared to the positive control group. Bhatti et al., (2016) showed that Nigella sativa and honey lowered significantly the levels of total cholesterol (TC), after 30 days of in hyperlipidemic smokers. Buriro and Tayyab (2007) indicated that Nigella sativa seeds in the diet has a favorable
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effect on lipid profile by lowering the total cholesterol in albino rats.

Table (2): Effect of Nigella Sativa seed powder and Nigella Sativa Honeybee supplementation on lipid profiles Total Cholesterol (Initial and Final) of hypercholesteremic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial cholesterol (mg/dl)</th>
<th>Final cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control negative (-)</td>
<td>77.312 ± 3.284b</td>
<td>75.775 ± 5.766a</td>
</tr>
<tr>
<td>Control positive (+)</td>
<td>128.448 ± 6.779a</td>
<td>161.755 ± 6.782a</td>
</tr>
<tr>
<td>2% NS seed /kg diet</td>
<td>130.058 ± 5.354a</td>
<td>128.183 ± 5.689b</td>
</tr>
<tr>
<td>5% NS Honeybee /kg diet</td>
<td>129.412 ± 5.715a</td>
<td>102.755 ± 5.684c</td>
</tr>
<tr>
<td>2% NS seed plus 5% NS Honey bee /kg diet</td>
<td>132.238 ± 4.879a</td>
<td>93.432 ± 3.766d</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.

Values at the same column with different letters are significant at p< 0.05.

Results illustrated in Table (3) showed that the mean value of positive control group (81.926 ± 5.281 mg/dl) was significantly higher than that of the negative control group (49.35 ± 6.31 mg/dl). Whereas, the mean values of serum triglyceride in all treated groups significantly increased, as compared to the negative control group (49.35 ± 315 mg/dl). It can note that all treated groups decreased significantly as compared to positive control group. Burio and Tayyab (2007) indicated that Nigella sativa seeds in the diet has a favorable effect on lipid profile by lowering the triglyceride. Also, Gargari et al., (2009) indicated that the concentration of triglycerides was significantly lowered in black seed group ( p < 0.05), as compared to control group at the end of study. Moreover demonstrated that dietary black seed can
favorably decrease serum lipid profile and lipid peroxidation levels in hyperlipidemic rabbits.

**Table (3):** Effect of Nigella Sativa seed powder and Nigella Sativa Honeybee supplementation on lipid profiles (Triglycerides) of hypercholestermic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control negative (-)</td>
<td>49.35±6.315⁺</td>
</tr>
<tr>
<td>Control positive (+)</td>
<td>81.92±5.281ᵃ</td>
</tr>
<tr>
<td>2% NS seed /kg diet</td>
<td>76.25±5.354ᵇ</td>
</tr>
<tr>
<td>5% NS Honeybee /kg diet</td>
<td>63.41±3.715ᵃ</td>
</tr>
<tr>
<td>2% NS seed plus 5% NS Honey bee /kg diet</td>
<td>55.23±4.123ᶜ</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.
Values at the same column with different letters are significant at p< 0.05.

The data presented in Table (4), showed that, positive control group (+Ve group) was significantly decreased (p <0.05) in high density lipoprotein- cholesterol, while low and very low density lipoprotein- cholesterol increased in the serum, as compared to the negative control group (37.93±2.89, 107.43±2.66 and 16.38±0.07 mg/dl) vs. (50.67±2.97, 15.16±2.87 and 9.87±1.26 mg/dl) respectively. The HDL-c decreased significantly in positive control group (37.93±2.89 mg/dl), as compared to the negative control group (50.67±2.97 mg/dl). The mean values of HDL-c in all treated groups are significantly decreased, as compared to the negative control group. Also, it can noted that all treated groups in HDL-c increased significantly as compared to the positive control group, except group fed on 2 % grinded nigella sativa. These results agree with, **Buriro and Tayyab (2007)**, whom found that Nigella sativa seeds in the diet has a favorable effect on
lipid profile by increasing the HDL cholesterol in albino rats. Also, El-Shafey et al., (2015) found that after one and two months, honeybee treated group showed significant increase in antioxidant enzymes and HDL-c. Abd El-Maksoud et al., (1996) reported that supplementating the control diet with 2.5 or 5 % nigella sativa seeds to hyperlipidemic diets for 4 weeks increased significantly the serum HDL-c.

The LDL-c increased significantly in positive control group (107.43 ± 2.66 mg/dl) as compared to negative control group (15.16 ± 2.87 mg/dl). Moreover the mean values of LDL-c in all treated groups, were significantly increased, as compared to the negative control group. Also, the same table cleared in that all treated groups, LDL-c decreased significantly as compared to the positive control group. This finding agree with, Burio and Tayyab (2007) whom indicated that Nigella sativa seeds in the diet has a favorable effect on lipid profile by lowering total cholesterol and LDL cholesterol in albino rats. Also, El Shafey et al., (2015) found that after one and two months, honeybee treated group showed significant decrease in LDL-c. Bhatti et al., (2016) reported that Nigella sativa (Kalonji) and honey were as effective as Atorvastatin or more effective in some cases in reducing LDL-c of hyperlipidemic smokers significantly. Shahid et al., (2016) showed that dietary supplemention on Nigella sativa (kalonji) has hypolipidemic effect by lowering LDL-c level in rats fed on diet rich in poly-unsaturated fatty acid. Abd El Maksoud et al., (1996) found that supplementing the hyperlipidemic diet with 2.5 % or 5 % nigella sativa seeds decreased the LDL-cholesterol. Also supplementing the control diet with 2.5 or
5% nigella sativa seeds slightly decreased the LDL-cholesterol.

With respect to VLDL-c, Table (4) shows that VLDL-c increased significantly in the positive control group (16.38 ± 0.07 mg/dl), as compared to the negative control group (9.87 ± 1.26 mg/dl). Also, the same table cleared that all treated groups decreased significantly, as compared to the positive control group. These results agree with, El-Shafey et al., (2015), whom reported that after one and two months, honeybee treated group showed significant decrease in VLDL-c.

Table (4): Effect of Nigella Sativa seed powder and Nigella Sativa Honeybee supplementation on lipid profiles (HDL-c, LDL-c and VLDL-c) of hypercholestermic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>HDL-c (gm/dl)</th>
<th>LDL-c (mg/dl)</th>
<th>VLDL-c (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control negative (-)</td>
<td>50.671±2.972a</td>
<td>15.162±2.874e</td>
<td>9.870±1.263e</td>
</tr>
<tr>
<td>Control positive (+)</td>
<td>37.936±2.895d</td>
<td>107.433±2.669a</td>
<td>16.385±0.076a</td>
</tr>
<tr>
<td>2% NS seed /kg diet</td>
<td>39.840±4.123cd</td>
<td>73.365±1.574b</td>
<td>14.123±0.999b</td>
</tr>
<tr>
<td>5% NS Honeybee /kg diet</td>
<td>41.281±3.643cb</td>
<td>45.714±3.998c</td>
<td>12.767±0.513c</td>
</tr>
<tr>
<td>2% NS seed plus 5% NS Honey bee /kg diet</td>
<td>45.128±2.753b</td>
<td>38.618±0.763d</td>
<td>11.515±0.843d</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.
Values at the same column with different letters are significant at p<0.05.

The data presented in Table (5) showed that the effect of different levels from nigella sativa, nigella sativa honeybee and their combination on Aspartate Amine Transferase (AST), Alanine Amine Transferase (ALT) and Alkaline phosphatase (ALP) U/L of rats suffering from...
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hypercholesterolemia. It could be noticed that, control positive group showed a significant increase (p <0.05) in the mean values of AST, ALT and ALP (105.87±5.57, 49.84±4.70 and 150.24±2.08) U/L, as compared with control negative group (76.42±6.04, 24.55±3.06 and 70.12±1.92) U/L, respectively.

The AST enzyme increased significantly in positive control group (105.87± 5.57 U/L), as compared to the negative control group (76.42 ± 6.004 U/L). Also, the data in this table showed that all treated groups were significantly decreased AST enzyme, as compared to the positive control group, except group fed on 2 % grinded nigella sativa seed / kg diet. The mean values of AST enzyme in all treated groups, were significantly decreased, as compared to the negative control group, except group fed on 2 % grinded nigella sativa seed plus 5 % nigella sativa honeybee group. The mean value of AST enzyme in the group fed on 2 % grinded nigella sativa seed plus 5 % nigella sativa honeybee, decreased significantly, as compared to the group fed on 5 % nigella sativa honeybee.

The ALT enzyme increased significantly in positive control group (49.84±4.70 U/L), as compared to the negative control group (24.55 ± 3.06 U/L). The mean value of serum ALT enzyme in all treated groups significantly increased, as compared to the negative control group. Also, the mean values of all treated groups were significantly decreased (p < 0.05), as compared to the positive control group. These finding are in agreement with Hassanin and Hassan (1996), whom recorded that significant decrease in ALT and AST activities due to treatment by nigella sativa seeds for 9 days. Abd El-Megeid et al., (2005) and Gouda
(2007), revealed that honeybee realized the best effect on liver functions.

Treating rats (which suffering from hypercholesterolemia) with different levels from nigella sativa and nigella sativa honeybee and their combination led to significantly increase (p <0.05) in serum ALP enzyme of positive control group (150.24 ± 2.08 U/L), as compared to the negative control group (70.12 ± 1.92 U/L). The mean values of all treated groups were significantly decreased in ALP (p <0.05), as compared to the positive control group. The mean values of ALT enzyme in all treated groups, were significantly decreased, as compared to the negative control group, except group fed on 5 % nigella sativa honeybee group and 2 % grinded nigella sativa seed plus 5 % nigella sativa honeybee. A study by Al-Waili (2003) showed that the activities of several enzymes such as AST, ALT and ALP were markedly or moderately reduce in healthy individuals administered honey. Muneera et al., (2015) indicated that Nigella sativa showed protective role in terms of hepatic dysfunction and can be used as a cholesterol lowering agent. Also, Klip and Vranic (2006) reported that honey supplementation might retard deterioration and improve hepatic function.
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Table (5): Effect of Nigella Sativa seed powder and Nigella Sativa Honeybee supplementation on serum liver biomarkers (AST, ALT and ALP) of hypercholesteremic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control negative (-)</td>
<td>76.427 ± 6.046c</td>
<td>24.558 ± 3.064d</td>
<td>70.122 ± 1.920c</td>
</tr>
<tr>
<td>Control positive (+)</td>
<td>105.876 ± 5.576a</td>
<td>49.841 ± 4.708a</td>
<td>150.24 ± 2.081a</td>
</tr>
<tr>
<td>2% NS seed /kg diet</td>
<td>95.272 ± 5.568ab</td>
<td>43.654 ± 3.465b</td>
<td>130.245 ± 3.237b</td>
</tr>
<tr>
<td>5% NS Honeybee /kg diet</td>
<td>86.995 ± 4.810b</td>
<td>33.221 ± 2.189c</td>
<td>83.455 ± 4.654c</td>
</tr>
<tr>
<td>2% NS seed plus 5% NS Honey bee /kg diet</td>
<td>79.300 ± 1.870c</td>
<td>30.555 ± 2.326c</td>
<td>77.251 ± 1.021c</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.

Values at the same column with different letters are significant at p< 0.05.

Conclusion

The present study concluded that treatment with grinded nigella sativa and nigella sativa honeybee for hypercholesterolemic rats lower the elevated serum levels of cholesterol, lipid profile and liver enzyme. These effects are associated with their contents especially thymoquinone. Therefore, fortification and supplementation of food with nigella sativa and nigella sativa honeybee may beneficial for patients who suffering from elevated level of cholesterol.

References

Nehal Mostafa Ebrahim


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