Studying the effect of *Anethum Graveolens* extract on parameters of lipid metabolism in white rat males

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Abstract:

This study was taken place in the animal house of Al- Najrain University. It studies the effect of *Anethum Graveolens* plant extract on cholesterol and lipid profile of the animals experiment. The results show that using of alcoholic extract of *Anethum Graveolens* leads to a reduction in triglycerides, cholesterol, LDL and VLDL levels in comparison with control group. The concentration of triglycerides in control group was 39.21 mg/ 100 ml, while the concentrations of triglycerides in the treated group were 31.88 mg/ 100 ml and 32.6 mg/ 100 ml, for 0.5 ml and 1 ml of treated groups respectively. On the other hand, cholesterol concentration was also tested and its concentration was found to be 88 mg/ 100 ml and 92.2 mg/ 100 ml for the 1 ml and 0.5 ml treated groups respectively in comparison with the control group, which recorded 112.40 mg/ 100 ml. The results also indicate a reduction in the total cholesterol level comparing with control group, which shows low level of good cholesterol HDL 48.7 mg/ 100 ml. On contrary, the treated groups exhibit a high level of HDL, 59 mg/ 100 ml and 56.46 mg/ 100 ml in 1 ml and 0.5 ml treated groups respectively,. Further, the concentration of bad cholesterol LDL dropped into 29.37 mg/ 100 ml and 22.48 mg/ 100 ml in 1 ml and 0.5 ml treated groups in comparison to the control group, which was 55.86 mg/ 100 ml,. Regarding VLDL levels, the control group recorded 7.84 ml/ 100 ml, while were 6.37 mg/ 100 ml and 6.52 mg/ 100 ml for both treatments of 0.5 ml and 1 ml respectively.

Keywords: *Anethum Graveolens*, lipid metabolism, white rat males

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Introduction:

Hyperlipidemia is a dangerous cardiac disease that causes increasing rate of death around the world and it is associated with arteriosclerosis disease. These diseases are mainly caused by increasing in cholesterol level in plasma (1). The increasing of a special kind of fatty proteins related to the increasing of having the first arteriosclerosis disease LDL which has a high ratio of hyperlipidemia, so that it is the cause of having...
arteriosclerosis disease in contrary with HDL which has high ratio of protein and an acceptable ratio of cholesterol, but its function is to carry this cholesterol from body cells and tissues to the liver to destroy it there. So it is related to decreasing having arteriosclerosis. (2) Also, increasing the level of cholesterol is directly related with some types of cancer such as colon cancer (3). Indeed, increasing coronary heart disease and heart attacks within a ratio of 35 and 45% respectively (4).

Food with high cholesterol level is the biggest cause of arteriosclerosis, which is a physiological defect affects walls of arteries in general and heart arteries in particular (5). High fat food would lead to accretion of fatty matters, which have 70% cholesterol, on the cells lining the walls of arteries, leading to settle calcium and forming complicated carbohydrates settlements. This would eventually develop to form a plaque and then blood clot (6). Therefore, there is an increasing demand to use herbs and natural plants that play an important role in alternative medicine. (7).

Anethum Graveolens has drawn much attention in alternative medicine specially in Asian such as in Iran, Iraq and also in North Africa, East and West area of the Mediterranean Sea, (8). This aromatic herb has many medical uses as it is used as one of the component of “gripe water”, which is given to treat some intestinal disorders in infants. In addition, it is used as a diuretic and as a galactagogue. Moreover, this herb considers as an anti spasmodic agent in white rat males (9), and anti inflammatory agent (10).

The aim of the study is to explore the effect of Anethum graveolens extract on some parameters associated with hyperlipidemia, which would cause arteriosclerosis and cardiac diseases, such as total cholesterol level, low-density lipoproteins LDL; triglycerides level (TC) and High-density lipoproteins HDL as a result reducing infection in arteriosclerosis and cardiac diseases.

Materials and Methods:

- **Anethum graveolens Extraction:**

Anethum graveolens plants were collected from local markets in Baghdad’s regions then cleaned up with water and dried in shadow at the room temperature. After completing the drying, plants were grind into soft powder by an electric grinder (brand name) then saved in plastic containers in order to extract it.

Alcoholic extract was prepared by taking 60 g of the plant’s powder and added to 500 ml of ethyl alcohol with a concentration 80% by using soxhlet extractor at 70 C° for 24 hours, after that the extract was concentrated by rotary evaporator (brand name) at 40 – 50 C° for then put in glass plates and inserted inside the incubator at 30 C° for in order to finish drying operation then took off the extract and weighted the product and saved until the time of use.

- **Experimental design**

This lab experiment has been studied in the animal house of Al-Nahrain University on adult, white, male rats from albino type taken from National Center For Drug Control And Research/ Ministry of Health/ Baghdad. Adult Rat male aged 6-7 weeks were kindly provided from National Center For Drug Control And Research/ Ministry of Health.
Health/ Baghdad and they were brining up in controlled environment in term of air and temperature, which was kept at 25±2 C°.

Three groups were considered and every group has 7 rats. The first and second groups were fed with 0.5 ml and 1 ml separately from *Anethum graveolens* extract. Whereas the third group was the control and was not fed any extract and used for comparison purposes.

- **Sampling**

At the end of the experiment, rats were fasting for 12 hours then anesthetized by chloroform and blood samples were collected from hearts and left for 30 minutes at room temperature. Then, serum was collected by centrifuging the blood for 15 min and 3000 rpm at kept in freezer at– 18 until usage to keep its composition and to prevent microbial pollution

- **Determination of parameters’ levels**

Enzymatic colorimetric test described by the Rando’s instructional manual, was used to determine levels of cholesterol, triglycerides, high- density lipoproteins and low- density lipoproteins.

I. **Cholesterol levels**

Enzymatic colorimetric test was used. wherein this method, enzyme is oxidized the free cholesterol into cholest-4-en-3-one and oxygen peroxide with the existence of peroxides enzyme and oxygen donor, would change the uncolored solution to pink colour. The intensity of the color is directly proportion with cholesterol level in the serum. 10 µl from serum sample and the standard soluble were taken and added up 1 ml. from cholesterol reagent to all of them. Then added 1 ml. from the cholesterol reagent to third test tube and considered blank. All the tubes were shaken well and incubated for 10 minutes at 37 C°. After that, absorbability of the standard soluble, serum sample and the blank were read on 550 nm. Total cholesterol concentration calculated with milligram in 100 milliliter from serum according to the following equation:-

\[
\text{Cholesterol (mg./100 g.)} = \frac{\text{absorbability of sample reading}}{\text{absorbability of the standard soluble}} \times 200
\]

II. **HDL levels** :-

This method considered from the enzymatic methods in evaluating high- density fatty proteins. In this test added 100 µl. from the precipitate to 500 µl. serum in a test tube and mixed well and left for 10 minutes at room temperature then put in the centrifuge (300 rpm) for 10 minutes in order to have two parts from the soluble in the
tube, the pellet and the supernatant part. The latter part was used to determine the high-density lipoprotein. The supernatants were moved to another tube and then they were shaken well and incubated for 10 minutes at room temperature then absorbability of the standard soluble, the serum sample and the blank were read at 550 nm.

HDL concentration was calculated in 100 milliliter of serum according to the following pattern:

\[
\text{HDL concentration} = \frac{\text{absorbability of sample reading}}{\text{absorbability of the standard soluble}} \times 50 \times \text{standard soluble concentration} \times 2
\]

III. Triglycerides levels:

Enzymatic colorimetric test was used to measure triglycerides levels. 3.Glycerol phosphate oxidase enzyme oxidizes Glycerol 3-p to Dihydroxyacetone-p and hydrogen peroxide with the existence of peroxidase enzyme and hydrogen donor, would change the uncolored solution to pink colour. The intensity of the color is direct proportion with triglycerides concentration in the serum. 10 µl. from every serum sample and the standard soluble were taken and added up to 1 ml. from triglycerides reagent to all of them. Then added 1 ml. from triglycerides reagent to third test tube and considered blank. All the tubes were shaken well and incubated for 10 minutes at room temperature. After that, absorbability of the standard soluble, serum sample and the blank were read on 550 nm.

Triglycerides concentration calculated with milligram in 100 milliliter from serum according to the following equation:

\[
\text{Triglycerides (mg./100 g.)} = \frac{\text{absorbability of sample reading}}{\text{absorbability of the standard soluble}} \times 200
\]

IV. VLDL levels

VLDL level has been calculated according to Freid mathematical equation (11).

\[
\text{VLDL- Cholesterol} = \frac{\text{Triglyceride}}{5}
\]
VI. LDL Evaluation:-

LDL level has been calculated according to Freid mathematical equation (11).

\[ \text{LDL-Cholestrol} = \{\text{Total-Cholestrol}\} - \{\text{HDL-cholestrol}\} + \{\text{Triglyceride}\} \]

Results and Discussion:-

Table (1) shows *Anethum graveolens* extract effect on triglycerides, cholesterol, LDL, HDL and VLDL levels in blood. Results shows decreasing in triglycerides, cholesterol, LDL and VLDL in comparing with control treatment. Triglyceride concentration in control treatment was 39.21 mg. /100 ml, and this concentration was decreased in treated groups with 0.5 ml and 1 ml, to 31.88 mg./100 ml and 32.6 mg./ 100 ml. respectively. The treated group with 1 ml extract was the best in comparison with other group, 0.5 ml treated group. Cholesterol ratio was reduced from 112.40 mg/100 ml into 88 mg. / 100 ml in 1 ml treated group and into 92.2 mg/100 ml in 0.5 ml treated group this results support the decreasing in total cholesterol level comparing with control treatment. These results agree with the results obtained by (12) and (13)

Also, high-density lipoproteins levels were increased as a result of feeding with *Anethum graveolens* extract and it hits its highest in 1 ml treated group, 59 mg / 100 ml, with less in 0.5 ml treated group, 55. 86 mg. / 100 ml, in comparison with 48.7 mg / 100 ml in control group. On contrary, low-density lipoprotein levels show decrease as a result the extract treatment and its concentration decreased to 29.37 mg. /100ml and 22.48 mg. /100ml for 0.5 and 1 ml treated group respectively in comparison with the control group, 55.86 mg. / 100 ml. Similarly, VLDL levels were decreased from 7.84 mg / 100 ml in control group in comparison with 0.5 and 1 ml treated group, which recorded 6.37 mg. /100ml and 6.52 mg. /100 ml respectively.

*Anethum graveolens* extract has many active compounds with anti-oxidation activities such as Gingerols , shogaols and some related Phenolic Ketone derivatives, which may be the reason behind reducing the concentration of cholesterol in the blood of the rate males (14,15).

Studies have shown the ability of *Anethum graveolens* to reduce total cholesterol and triglycerides levels in addition to increase high- density lipoproteins HDL abstractly comparing with diabetes mice(16). Also, studies mentioned that mixing Anethum Graveolens with garlic has reducing facts to sugar and fat of Albino rats’ blood. (17,18)
Table 1: The effect of *Anethum Graveolens* on lipids profile

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol</th>
<th>Triglyceride</th>
<th>LDL</th>
<th>HDL</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control treatment</td>
<td>112.21</td>
<td>39.21</td>
<td>55.86</td>
<td>48.7</td>
<td>7.84</td>
</tr>
<tr>
<td>0.5 ml. concentration</td>
<td>92.2</td>
<td>31.88</td>
<td>29.37</td>
<td>56.46</td>
<td>6.37</td>
</tr>
<tr>
<td>1 ml. concentration</td>
<td>88</td>
<td>32.6</td>
<td>22.48</td>
<td>59</td>
<td>6.52</td>
</tr>
</tbody>
</table>

It also mentioned that consumption ethanolic *Anethum graveolens* extract reducing cholesterol plasma and oxidizing low-density lipoproteins protein low-density LDL in mice affected with arteriosclerosis. To our knowledge, no clear mechanism of action was documented so far; a pathway was suggested by Haghighi et al. (19), who proposed hepatic phosphatidate phosphhydrolase enzyme as a key player in this mechanism; however, negative results were obtained and therefore, the hypothesis was dismissed. Another proposed pathway was suggested by [19], which involves active compounds such as flavonoids such as carvon, limonene, or αphellandrene and their direct biological effect on key enzymes such as HMG-CoA reductase or acyl CoA carboxylase [19]. It has been suggested that anethum components may also increase LDL receptors in the liver, stimulating cholesterol clearance from the blood; while many other suggestions including a decrease in cholesterol absorption from the intestine as well as hypotriglyceridemic effect due to a decrease in fatty acid synthesis [20]. Furthermore, *Anethum graveolens* may improve hypercholesterolemia by modification of lipoprotein metabolism mainly through enhancement of lipoprotein metabolism [21]. On the other hand, it has been proposed that *Anethum graveolens* exerts its hypolipidemic effect via antioxidant effect [22, 20]; where it has been reported that Anethum Graveolens might have hepatoprotective effect against harmful effects of oxidized fats due to polyphenol contents of *Anethum graveolens* [19]. Determination of the exact mechanism by which *Anethum graveolens* exerts its hypolipidemic effect, would need further investigation on molecular level, while large scale clinical trials with large sample size and multicenter studies are also crucial to clarify the role of *Anethum graveolens* as hypolipidemic agent.

**Conclusion**

Hyperlipidemia is increasing death threat in Iraq and worldwide. Thus, there is increasing demand for synthetic and natural reagents to treat hyperlipidemia patients. This study comes in this line to seek new and affordable natural herbs that can be used to treat this dangerous cardiac disease. Results obtained in this study suggesting that administration of *Anethum graveolens* extract is effective to treat hyperlipidemia cases in white rat males, and its effect seem to cause decreasing cholesterol, triglycerides and LDL levels and increasing HDL level. Consequently, it could be considered as an option to treat hypercholesterolemic cases in man after testing its toxic effects and potential interactions with other medications.

Reference


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