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Antihyperlipidemic Activity and Hematological Effects of Nigella Sativa Seeds Extract in Egg yolk-fed Rabbits: A Comparative Study with Atorvastatin.

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Abstract

The aim of this research work is to evaluate the antihyperlipemic activity of *Nigella sativa* L. seeds in the rabbit model by inducing hyperlipidemia by egg yolk. The study was carried out on 30 female rabbits divided into 5 groups of 6 each: The first received no treatment and served as a control (CRL), the second group was gavaged with the aqueous extract of the of nigella seeds (NS), the 3rd group was gavaged by the egg yolk only (EY), the 4th was gavaged by the egg yolk and treated by atorvastatin drug (EY+ATV) and the last group was gavaged with egg yolk and treated by black seed as NS (EY+NS). The different animal groups were treated for a period of 4 weeks. The haematological results showed a significant decrease at least at $P \leq 0.05$ in hemoglobin, red blood cells and platelets number in EY and EY+ATV groups compared to CRL or to EY+NS groups. The biochemical results showed that administration of egg yolk in the EY group caused an increase in total cholesterol (TC), LDL (Low Density Lipoproteins) cholesterol, as well as triglycerides (TG). The 2 groups (EY+ATV) and (EY+NS) registered a marked decrease in TC, LDL and TG and an increase in HDL cholesterol (High Density Lipoproteins). From the obtained results in this work, it can be concluded that the seeds of *Nigella sativa* may exert antihyperlipemic activity.

Keywors: Nigella sativa; cholesterol; triglycerides; hemoglobin; erythrocytes; platelets.

1. Introduction

Lipids play an essential role in the body, such as the synthesis of steroid hormones and the energy production. Disorders of metabolism that lead to deregulation of lipid levels in the body are called "dyslipidemias"[1].

Dyslipidemia is a primary or secondary pathological change in serum lipids; it is a metabolic abnormality which can be hyperlipidemia or hypolipidemia.

Hyperlipidemia is a biochemical disturbance that is part of asymptomatic disorders. It is characterized by an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids and or plasma lipoproteins along with reduced high-density lipoprotein levels. These disturbances in the blood lead to certain problems, including atherosclerosis, hypertension and other coronary heart disease [2,3].

Recent studies have shown that many plants are able to modify plasma levels of TG, LDL and HDL. Notably, *Nigella sativa* is a medicinal plant

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that has been used for the treatment of various diseases, in particular hyperlipidemia [4-7].

It has been reported that seeds of *N. sativa* contains several active compounds in the classes of essential oil, fixed oil, saponins, and alkaloids [8]. The seeds of this medicinal plant were largely used in different cultures to treat bronchitis, asthma, diarrhea, liver, skin disorders and parasitic infections. It is also used as immunomodulatory natural agent [9-11].

In this context, the present study aimed the evaluation of antihyperlipidemic activity and hematological effects of *N. sativa* L. seeds extract in *Oryctolagus cuniculus* rabbits under hyperlipidemic diet.

2. Material and methods

2.1. Animals and breeding conditions

The *in vivo* study was carried out on 30 *Oryctolagus cuniculus* synthetic female strain rabbits weighing between 1.3 kg and 1.8 kg at the beginning of the experiment. The rabbits were purchased from a private farm in the El Hamma Bouziane region of Constantine.

The rabbits were kept in metallic cages. These animals were fed with a standard food (100g / animal). The food is in the form of granules, it is composed of: wheat bran, barley, corn, total soybean (vegetable proteins), and edible oil. The water was supplied *ad libitum*. The study was approved by the scientific committee of Institute of Veterinary Sciences, University of Mentouri Constantine 1, Algeria.

2.2. Plant material

The plant material used in this study is *Nigella sativa* seeds which were purchased from a grocery store in Ain kachra.

2.3. Chemicals and used products

Atorvastatin, a statin drug, as white tablets (Arovan® 20 mg, lot n°: 181601) was supplied by a private pharmacy in Tamalous region.

The eggs used to obtain egg yolk were purchased from a single supplier in the El Harrouch region.

2.4. Methods

2.4.1. Preparation of the N. sativa aqueous extract

The seeds of black seed were crushed using an electric grinder (Pitra brand: Mr. Mixer) and then sieved in order to be able to recover the finest powder. The obtained powder was then stored in hermetically sealed glass containers.

A quantity of 10 g of *N. sativa* powder was dissolved in 40 ml of distilled water. The aqueous extract obtained after stirring was stored in a glass vial.

2.4.2. Preparation of the egg yolk

To obtain the egg yolk, the eggshell was broken and the egg yolk was then carefully separated from the white. The egg yolk was well mixed before administration to each rabbit.

2.4.3. Experimental protocol

2.4.3.1. Distribution and treatment of rabbits

After an adaptation period of 7 days, the rabbits were randomly divided into five groups each containing six rabbits, the rabbits were treated for 28 days as follows:

Group 01 (CRL): This is the negative control; the rabbits in this group did not receive any treatment.

Group 02 (NS): The rabbits in this group were treated with the aqueous extract of *N. sativa* seeds at a dose of 1g / kg orally.

Group 03 (EY): The rabbits in this group received the egg yolk at a dose of 10 ml for each rabbit, administered orally.

Group 04 (EY+ATV): The rabbits in this group received the egg yolk at a dose of 10 ml for each rabbit, after 15 min the rabbits were treated with atorvastatin at a dose of 2.5 mg / kg orally [12].

Group 05 (EY+NS): The rabbits of this group received the egg yolk at a dose of 10 ml for each rabbit, after 15 min the rabbits were treated with the *N. sativa* aqueous extract at a dose of 1g / kg, administered orally.

The aqueous extract of *N. sativa* seeds, egg yolk and atorvastatin were applied through a gastric tube daily for 4 weeks.

2.4.3.2. Clinical state of rabbits

The general condition of the animals (behavior, appetite, condition of the mucous membranes, condition of excreta, etc.) was checked every day throughout the experimental period. The animals were subjected to a clinical examination once

a week: heart rate, respiratory rate, rectal temperature (Thermovale Basic model thermometer), as well as body weights were monitored.

2.4.3.3. Blood samples and analysis

At the end of the experiment, rabbits from all five groups were fasted overnight. The next day, then the blood was collected from the ear marginal vein of each rabbit, the blood sampled is collected in two different types of tubes containing the anticoagulant: EDTA for haematological parameters, and heparins for lipidemic profile.

The heparin tubes are centrifuged using a centrifuge to recover the serum in dry tubes and then they were stored in a freezer at -22 °C until biochemical analysis.

Haematological and biochemical analyzes were performed in "El-yassamine" medical analysis laboratory in Collo region. The blood count numbering was performed using Sysmex XN-350 electronic analyzer model.

The biochemical studied parameters are: Triglycerides, total cholesterol (TC), HDL-cholesterol, LDL- cholesterol, these parameters were assayed on a Respons® 910 model machine, brand: DiaSys.

2.4.3.4. Statistical analysis

The results are expressed as means with standard deviation (SD). The means of the data of different groups were compared by application of the *ANOVA* variance test using the Origin® software (version 6). The differences are considered significant for $P \leq 0.05$.

2. Results

3.1. Clinical condition and body weights of rabbits

During the experimental period, no case of mortality was recorded in the five groups. The animals did not show significant signs of toxicity. The food and water consumption and appetite of the rabbits were normal. Except, for the group fed with egg yolk and treated with atorvastatin where was noticed a decrease of food and water consumption during the first week of the experiment, noting that the appetite was restored by the following.

Heart rate, respiratory movements and rectal temperature were within the normal values in all the five groups.

The body weight was progressively evolved in all the groups during the experimental period. Except, in the rabbits of the EY+ATV group, a decrease in body weight was observed during the first week (Data not shown).

3.2. Haematological results

The results represented in figure 1 show that the values of the hematological parameters in all the groups were within the standards.

A significant decrease for $P \leq 0.01$ of red blood cells was observed in the rabbits of the group fed with egg yolk and treated with atorvastatin (EY+ATV) compared to the rabbits of the control CRL group. This group showed also a significant decrease for $P \leq 0.05$ compared to the group treated with black seed (NS). On the other hand, in the other comparisons between the different groups, no significant difference is observed ($P > 0.05$).

For hematocrit rates, no significant difference ($P \geq 0.05$) in all comparisons between the different groups was found (Figure 1, B).

For hemoglobin level, the results showed a significant decrease ($P \leq 0.05$) in the two groups EY, EY+ATV compared to the control CRL group. Comparison between the other groups showed no significant difference (Figure 1, C). However, no significant difference ($P \geq 0.05$) in all comparisons between different groups was observed for mean corpuscular hemoglobin concentration (MCHC) (figure 1-D).

As presented in figure 1-E, no significant differences ($P \geq 0.05$) in all comparisons between the different groups were observed in white blood cells. For the blood platelets number, the results represented in figure 1-F, show a significant decrease at least for $P \leq 0.001$ of the platelets in the EY and EY+ATV groups compared to the CRL control group. Likewise, these two groups showed a significant decrease for $P \leq 0.001$ compared to the group treated with black seed "NS". A significant decrease ($P \leq 0.01$) in blood platelets was observed in rabbits in the egg yolk-fed group and treated with black seed compared to rabbits in the CRL control group. While in the EY+NS group a significant decrease for $P \leq 0.05$ compared to the NS group was observed. For the other comparisons, no significant difference is observed.

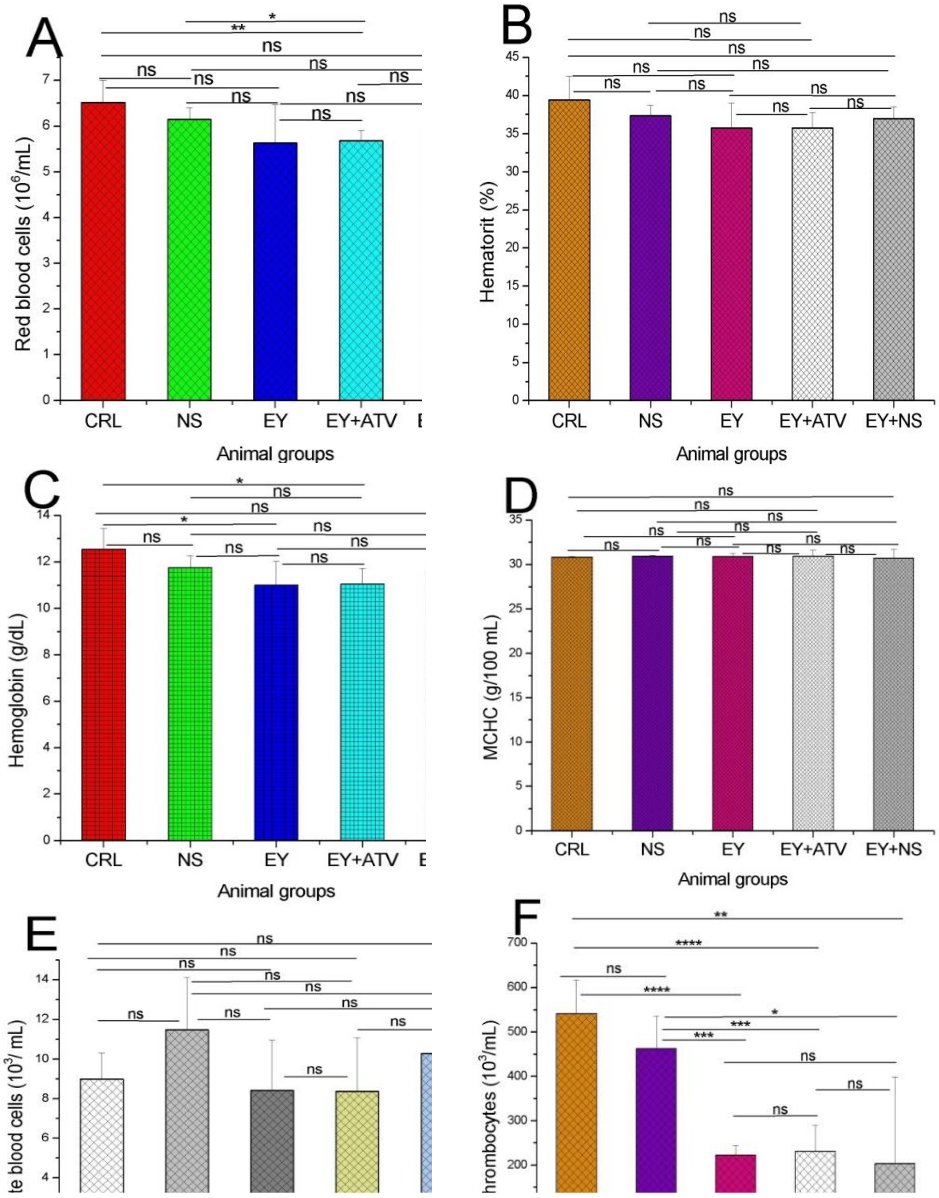

Figure 1 Hematological parameters of the different animal groups: A, Variation in the number of red blood cells in the different groups of rabbits; B, Variation of the hematocrit in the different groups of rabbits; C, Hemoglobin variation in different groups of rabbits; D, Variation of the mean corpuscular hemoglobin concentration (MCHC) in the different groups of rabbits. E, Variation in the number of white blood cells in the different groups of rabbits; F, Variation in the number of blood platelets in the different groups of rabbits. CRL, Negative control group; NS, Group treated with *Nigella sativa* seeds extract; EY, Egg Yolk-fed Group; EY+NS, Group fed with egg yolk and treated with *N. sativa*; EY+ATV, Group fed with egg yolk and treated with Atorvastatin; Results are expressed as means ± SD and compared with *ANOVA*, n = 6, the differences are considered significant at P≤0.05.

3.3. Lipidemic profile

3. 1. Change in total cholesterol level

The results presented in figure 2-

A showed normal values of total cholesterol level in the rabbits of the CRL control group (0.401 ± 0.023g / L). Rabbits in the egg yolk-fed group (1.414 ± 0.374g / L) show a significant increase (P≤0.001) compared to the CRL group. A significant decrease (P≤0.001) was observed in TC level in the EY+NS group compared to the EY group with a non-significant increase (P≥0.05) of this parameter compared to the CRL group. The NS group (0.338 ± 0.094g / L) show a non-significant decrease (P≥0.05) compared to the CRL group. The group fed with egg yolk and treated with atorvastatin (EY+ATV) presented a significant decrease (P≤0.001) compared to the EY group, and also a non-significant increase (P≥0.05) compared to the CRL group and the NS group. It should be noted that no significant difference was observed between the two groups EY+NS and EY+ATV.

3.2. Change in triglycerides levels

The results of triglycerides (TG) represented in figure 2-B, showed values within the physiological range in the CRL control group with an average of (0.601 ± 0.075 g / L). The EY group recorded an average of 1.556 ± 0.114g / L. The comparison between the EY group versus the CRL control group and the NS group showed a significant increase for P ≤0.001. A significant increase (P≤0.01) in the TG level was observed in the rabbits of the egg yolk-fed group and treated with black seed (EY+NS) compared to the rabbits of the CRL and NS control group, and a significant decrease (P≤0.001) in comparison with the group with EY group. A significant decrease (P≤0.01) of TG was observed in the rabbits of the EY+ATV group (0.796 ± 0.465g / L) in comparison with the rabbits of the EY group. No significant difference (P≥0.05) was observed between the two groups EY+ATV and EY+NS.

3.3. Change in HDL cholesterol levels

The CRL control group recorded an average of 0.102 ± 0.017 g / L of HDL cholesterol. A non-significant increase (P≥0.05) of this parameter in the EY group and the NS group compared to the CRL control group was noted. The group fed with egg yolk and treated with black seed (EY+NS) showed a significant increase (P≤0.05) compared to the CRL group and a non-significant increase (P≥0.05) compared to the EY and NS groups. The results obtained also revealed a significant increase (P≤0.001) in HDL cholesterol in the EY+ATV group (0.156 ± 0.02g / L) compared to the CRL group, and also an increase but not significant (P≥0.05) compared to the EY group and the NS group, and a non-significant decrease (P≥0.05) compared to the EY+NS group (figure 2-C).

3.4. Change in LDL cholesterol levels

The results obtained (figure 2-D) showed normal values of the LDL cholesterol level in the rabbits of the CRL control group (0.23 ± 0.069g / L). A significant increase (P≤0.001) in LDL cholesterol was observed in rabbits in the egg yolk-fed group (EY) compared to the rabbits in the CRL control group and in the NS group. A significant decrease (P≤0.01) of this parameter in the rabbits of the two groups NS and EY+NS compared to the CRL group was recorded. The group fed with egg yolk and treated with black seed (EY+NS) presented a non-significant decrease (P> 0.05) compared to the NS group, and a significant decrease (P<0.001) compared to the EY group. A significant decrease for P≤0.001 was observed in the rabbits of EY+ATV group treated with atorvastatin compared to the rabbits of the EY group. The EY+ATV group showed a non-significant decrease (P> 0.05) compared to the CRL control group.

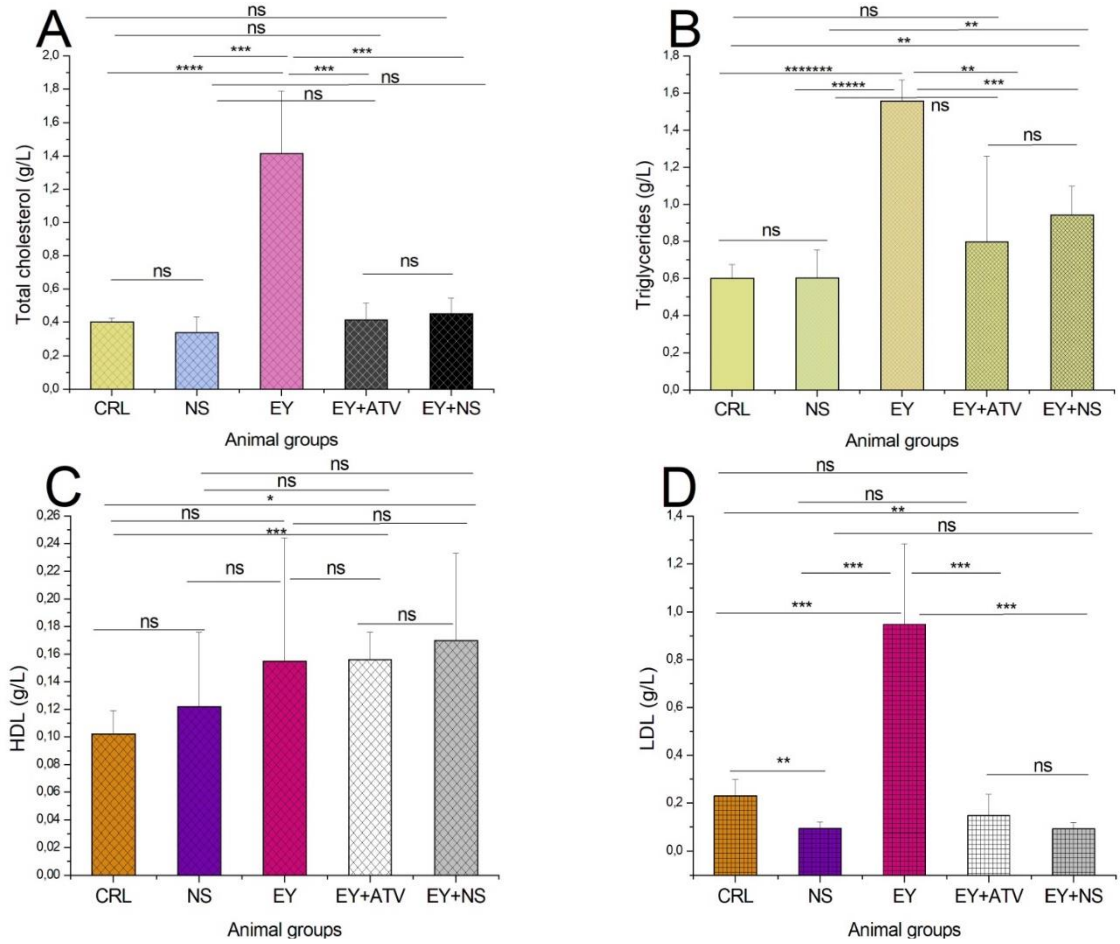

Figure 2 Lipidemic profile in the different animal groups: A, Variation of the total cholesterol level in the different groups of rabbits; B, Variation of the triglycerides level in the different groups of rabbits; C, Variation in HDL cholesterol levels in the different groups of rabbits; D, Variation in LDL cholesterol levels in the different groups of rabbits. CRL, Negative control group; NS, Group treated with *Nigella sativa* seeds extract; EY, Egg Yolk-fed Group; EY+NS, Group fed with egg yolk and treated with *N. sativa*; EY+ATV, Group fed with egg yolk and treated with Atorvastatin; Results are expressed as means ± SD, n = 6, the differences are considered significant at P≤0.05.

4. Discussion

The objective of the present research work was to evaluate the anti-hyperlipemic activity of *Nigella sativa* L. seeds in rabbits. The study used egg yolk as a natural product to disturb lipidemic balance; this product has been used as a food source to induce hypercholesterolemia in several scientific studies [13,14]. Administration of this product to rabbits for a period of 4 weeks resulted in a significant increase in body weight. Egg yolk also affected lipidemic balance; this is due to its high cholesterol content (210 mg per egg).

The hyperlipidemia observed in the egg yolk-fed group (EY) was characterized by an increase in total cholesterol (TC), LDL cholesterol as well as triglycerides. These results are in agreement with those published in several studies [13,14].

According to a study of Radu-Rusu et *al.* [15], the means chemical composition of yolks from eggs produced in standard batteries per 100g are (in %): 55.7±0.74 water, 44.3±0.74 dry matter, 1.29±0.28 ash, 13.7±0.69 proteins, 28.4±1.61 lipids, 0.9±0.14 nitrogen free extract, 1.33±0.05 (MJ/100g) gross energy, and 957±28.7 (µg/100g) cholesterol. It

has been reported that applied housing system altered yolk and egg cholesterol concentration [16].

Our results showed an increase in the level of lipidemic parameters whose cholesterol increased in the groups fed with egg yolk (EY, EY+ATV and EY+NS), and thus for the triglycerides and the LDL cholesterol level increased compared to the control group.

The results also showed that the administration of the aqueous extract of black seed as well as atorvastatin in rabbits fed with egg yolk (groups EY+NS and EY+ATV respectively) resulted in prevention of the hyperlipidemia observed in the EY group.

A study by Aminian et *al.* [13], carried out on rabbits-fed with egg yolk at the same dose (10 ml per rabbit) for a period of 6 weeks, showed a profound effect of egg yolk on total cholesterol (TC) as well as LDL cholesterol (LDL-C). Both parameters increased to 10 times baseline by 6th week, but the changes observed in HDL cholesterol (HDL-C) and triglycerides (TG) levels were minimal and statistically insignificant.

In a study by Buriro and Tayyab, the application of *Nigella sativa* extract incorporated in the diet showed that these seeds have a favorable effect on the lipid profile by lowering triglycerides, total cholesterol and LDL cholesterol and by lowering HDL cholesterol in albino rats [17].

According to studies on the chemical composition of the *Nigella sativa* seeds, this seed is composed of proteins (16-21%), carbohydrates (33-34%), dietary fibers (4.5-6.5%), mineral salts (3.7-7%), saponins (0.013%), water (6%) and lipids (30-35%); the latter contains phospholipids, essential oils and fixed oils which contain thymoquinone. Black seed has high thymoquinone content which could be responsible for hypolipidemia [18-21].

In a study, a team of researchers compared the effect of crushed seed and black seed oil with that of simvastatin (hypercholesterol lowering, HMG-Coa reductase inhibitor) on atherosclerosis of rabbits with induced hypercholesterolemia. A decrease in total cholesterol and LDL-cholesterol with an increase in HDL-cholesterol was observed in rabbits treated for 2 to 8 weeks [22]. Furthermore, a meta-analysis of human trials conducted by Hallajzadeh et *al.* [23] demonstrated the beneficial effects of *N. sativa* on fasting glucose, HbA1c, triglycerides, total cholesterol, VLDL-cholesterol and LDL-cholesterol levels.

For its part, atorvastatin is a statin that has been used as a cholesterol lowering drug [24]. According to studies by Andrew and McTavish [25], atorvastatin induced smaller reductions in triglycerides levels and more modest increases in high density lipoprotein (HDL) cholesterol levels than fenofibrate or nicotinic acid in patients with combined hyperlipidemia; it produced greater reductions in total cholesterol and LDL cholesterol. However, in the present study atorvastatin lowered total cholesterol and triglycerides, as well as LDL cholesterol with an increase of HDL levels.

5. Conclusion

The oral administration of the aqueous extract of *Nigella sativa* seeds alone to normal rabbits did not cause clinical, haematological or biochemical disturbances in the lipidemic profile.

The gavage of rabbits with egg yolk (group EY) caused a disturbance of the lipid balance resulting in a significant increase in total cholesterol (TC), LDL cholesterol as well as triglycerides (TG).

The two groups-fed with egg yolk and treated either with aqueous extract of black seed (EY+NS) or treated with atorvastatin (EY+ATV) demonstrated a clear prevention of hyperlipidemia caused by the egg yolk; this by lowering TC, LDL cholesterol and TG with an increase in HDL cholesterol.

The haematological results showed a significant decrease in hemoglobin, red blood cells and platelets number in EY and EY+ATV groups.

In light of these results, the study concludes that the aqueous extract of *Nigella sativa* L. seeds may exert an anti-hyperlipemic activity.

Declaration of Competing Interest

The authors declare that they have no competing interests that could influence the work reported in this paper.

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List of Abbreviations

CRL, Control; NS, *Nigella sativa* group; ATV, Atorvastatin; TG, Triglycerides; EY, egg yolk group; TC, total cholesterol.

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