

Article

Comparison of The Effects of Propolis and Royal Jelly Compared to Atorvastatin in Lowering The Levels of Lipids and Some Antioxidants in The Serum of Male Hyperlipidemic Rats

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Abstract: Hyperlipidemia negatively affects blood lipid and antioxidant levels due to the formation of free radicals. The accumulation of lipids, especially in endothelial structures, leads to the release of inflammatory substances, where the accumulation of triglycerides and oxidized cholesterol leads to increased oxidation, which exacerbates the production of free radicals. Free radicals are unstable molecules caused by metabolic reactions or external factors such as smoking and pollution. Their unbalanced increase leads to cell damage, and this damage affects fat cells and makes them more susceptible to hypertrophy and excessive fat storage. This study investigated the effect of propolis and royal jelly on hyperlipidemia and antioxidants due to the phenolic, sterolic, and antioxidant components of the two substances and compared them with atorvastatin, one of the most popular statins. 30 rats were used, divided into 6 groups, fed a high-fat diet, then treated with the above substances, and lipid levels and concentrations of some antioxidants were measured. After serological analysis, a clear effect of propolis and royal jelly on cholesterol and antioxidant levels was observed. Due to their effectiveness and ingredients. The research shows the importance of using natural products instead of medications in treating diseases. The research shows the importance of locally produced materials and their effective role in treating high fat. The research is also the first of its kind to study the synergistic effect of the alcoholic extract of propolis and royal jelly together.

Keywords: Propolis, Royal Jelly, Catalase, Malondialdehyde, Hyperlipidemia

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1. Introduction

Hyperlipidemia is defined as a disorder of lipid metabolism, resulting primarily from genetic predisposition, obesity, hypertension, diabetes, and unhealthy lifestyle habits [1]. It is known to be a significant negative prognostic factor for many diseases and contributes to millions of cardiovascular disease-related deaths annually worldwide [2]. There are two main types of hyperlipidemia. The first type is primary hyperlipidemia, which is usually familial and results from genetic abnormalities. The other type is called secondary hyperlipidemia, which results from precipitating factors such as obesity, thyroid dysfunction, alcohol and drug abuse (beta blockers), hypothyroidism, and chronic kidney failure [3]. Hyperlipidemia is characterized by disorders such as elevated total cholesterol (TC), cholesterol, triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), along with low levels of high-density lipoprotein cholesterol (HDL-C). These lipid disorders are major risk factors for cardiovascular disease, including coronary artery disease and stroke [4].

Propolis is a natural, viscous, and complex substance composed of a mixture of beeswax, resins, pollen, and essential oils collected by bees from tree buds. It is produced after being processed and mixed with enzymes by the bees [5]. Propolis contains a variety of secondary metabolites, such as flavonoids, phenolic compounds, alkaloids, and saponins, which contribute to its various effects, particularly its strong antioxidant activity [6].

Royal jelly is a secretion from the hypopharyngeal glands of young worker bees, known as nurse bees, aged between 5 and 15 days [7]. It is considered a rich source of essential nutrients crucial for the growth and differentiation of bees. Recent research has shown that the chemical composition of royal jelly consists of approximately 60–70% water, 9–18% proteins, 7–18% carbohydrates, and 3–8% fats, along with minerals and vitamins [8]. Additionally, royal jelly has demonstrated antioxidant and lipolytic activities against reactive oxygen species (ROS), including the reduction of oxidative damage to DNA [9].

2. Materials and Methods

Animals Used in the Study

Thirty male Wistar albino rats, weighing between 190 and 220 grams, were obtained from the animal house located in the Al-Owainat area of Tikrit province. The animals were housed in plastic cages under controlled conditions, including a temperature maintained at 25°C, a 12-hour light/dark cycle, and adequate ventilation. They were kept under these conditions for a duration of five weeks.

Materials and methods

Propolis was collected in solid form from the southwest region of Kirkuk (Al-Hawija) by a specialized office. It was then ground at home using an electric grinder and extracted with a Soxhlet apparatus at the Department of Chemical Sciences Laboratory, College of Education for Pure Sciences, University of Kirkuk, using a 1:9 ratio of ethanol to propolis.

Royal jelly was sourced from the Mount Karah area in Dohuk province, obtained through Dunia Bee Office, a specialized seller of bee products in Kirkuk.

The drug used in the study was Atorvastatin (ATOR), procured from a pharmacy, manufactured by the Indian company MICRO LABS LIMITED, and administered at a dose of 2.06 mg/kg according to reference [10].

Doses used in the experiment: 200 mg/kg was used as the effective dose for both propolis and royal jelly, as it recorded the highest effectiveness in lowering the glucose level in the experiment than the other doses.

Blood samples were collected after the animals had fasted from food and water for 12 hours. Following anesthesia with chloroform, approximately 3 ml of blood was drawn directly from the heart using the cardiac puncture method for each animal. The blood was then transferred into gel tubes and incubated at 37°C for 30 minutes. Serum was separated from the blood cells by centrifugation at 3000 rpm for 15 minutes. The collected serum samples were stored at -20°C until further analysis.

Statistical analysis: The statistical analysis was conducted using the SPSS program through the ANOVA test.

3. Results and Discussion

The results of the fat measurements showed clear and significant differences between the groups, as illustrated in the Figure and Tables below.

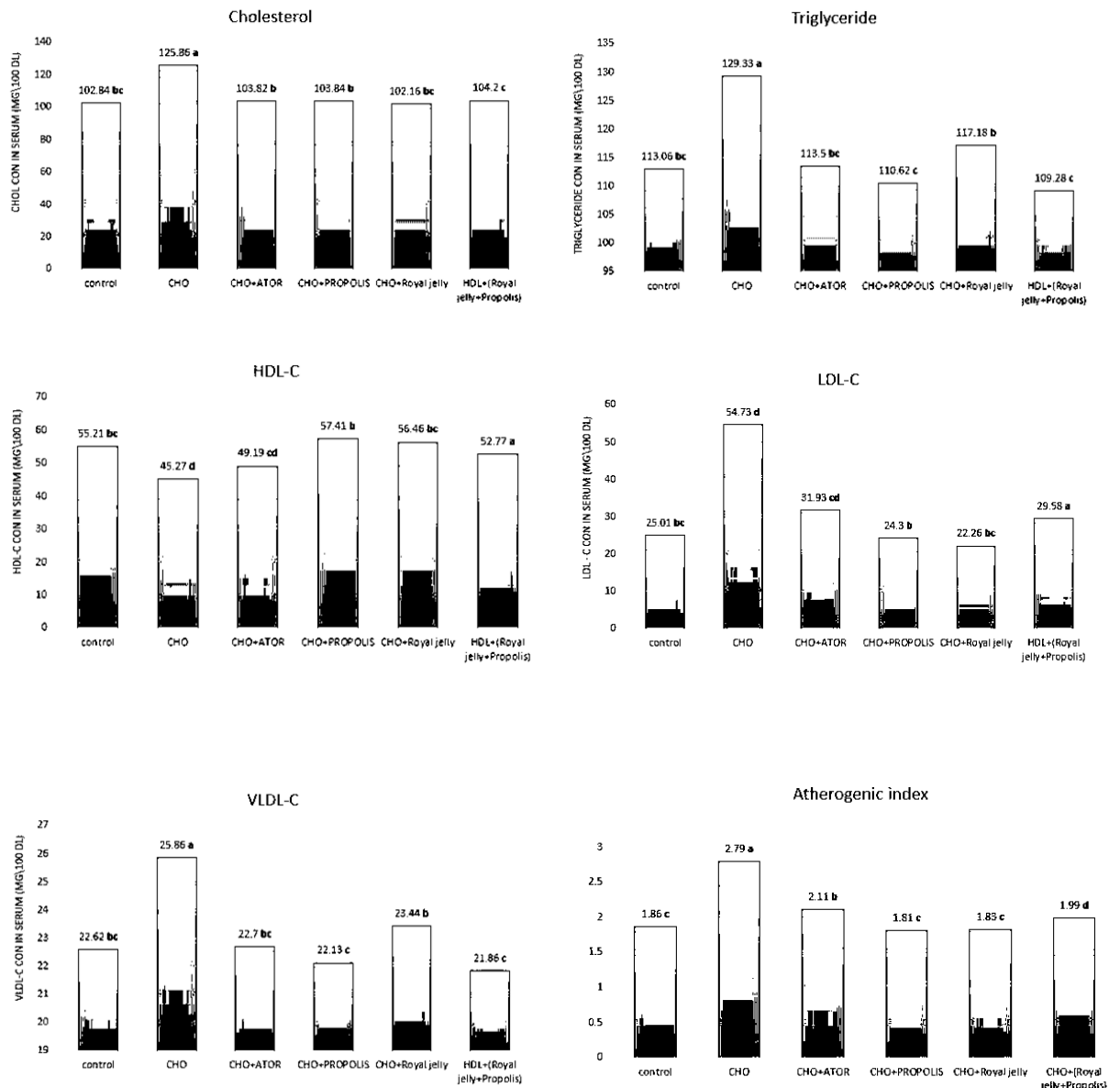


Figure 1. Shows the levels of fat plotted among the experimental groups.

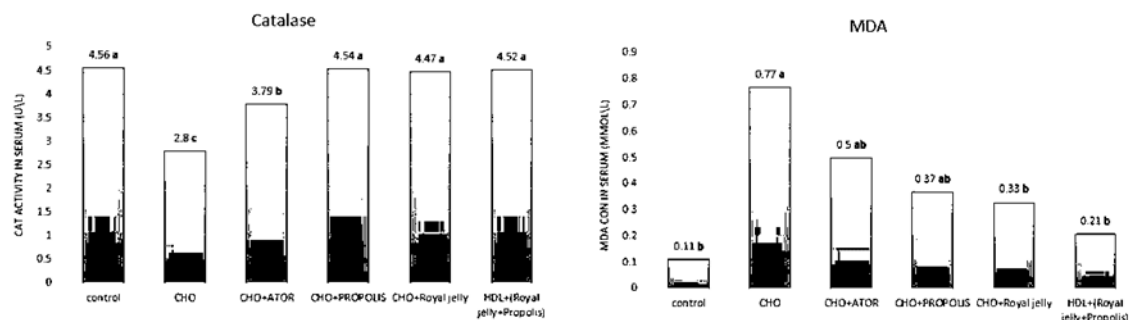


Figure 2. Shows some antioxidants between the experimental groups.

The study showed the effect of a high-fat diet in raising the levels of fat markers as well as antioxidants, while the drug and the natural substances used addressed the increase, as shown in the following tables.

Table 1. Effect of Diet and Statins on Fat Levels.

Parameters Groups	Total Cholesterol mg/dl	Triglyceride mg/dl	HDL-c mg/dl	LDL-c mg/dl	VLDL-c mg/dl	Atherogenic index
Control	102.84±2.01 ^{bc}	113.06±1.95 ^{bc}	55.21±0.87 ^{bc}	25.01±1.52 ^{bc}	22.62±0.39 ^{bc}	1.86±0.03 ^c
CHO	125.86±3.62 ^a	129.32±2.00 ^a	45.27±0.82 ^d	54.73±4.27 ^a	25.87±0.40 ^a	2.79±0.12 ^a
CHO+ ATOR	103.82±0.58 ^b	113.50±2.11 ^{bc}	49.19±1.13 ^{cd}	31.93±0.95 ^b	22.70±0.42 ^{bc}	2.12±0.04 ^b
CHO+ Propolis	103.84±1.23 ^b	110.62±1.79 ^c	57.41±1.07 ^b	24.30±1.47 ^{bc}	22.13±0.36 ^c	1.81±0.04 ^c
CHO+ Royal jelly	102.16±2.40 ^{bc}	117.18±1.45 ^b	56.46±3.23 ^{bc}	22.26±2.63 ^c	23.43±0.29 ^b	1.83±0.08 ^c
CHO+ (Propolis+ Royal jelly)	96.50±1.82 ^c	109.28±2.32 ^c	64.77±4.83 ^a	9.88±4.07 ^d	21.86±0.46 ^c	1.53±0.13 ^d

Table 1 shows in the above table the elevated fat levels in the group that was fed a high-fat diet with 2% cholesterol. Then, the drug group shows a decrease in fat levels due to the known effects of statins. The table also shows a reduction in fat levels in the other groups due to the effects of the substances used to repair liver cell damage.

Table 2. Impact of Diet and Treatment on Antioxidant Levels.

Parameters Groups	MDA (μmol/l)	CAT (IU/ml)
Control	0.11±0.01 ^b	4.56±0.11 ^a
CHO	0.77±0.24 ^a	2.80±0.07 ^c
CHO+ ATOR	0.50±0.21 ^{ab}	3.79±0.05 ^b
CHO+ Propolis	0.37±0.02 ^{ab}	4.54±0.14 ^a
CHO+ Royal jelly	0.33±0.05 ^b	4.47±0.04 ^a
CHO+ (Propolis+ Royal jelly)	0.21±0.02 ^b	4.52±0.04 ^a

Table 2 shows a clear change in the levels of antioxidants consumed in the study between the experimental groups, where the high-fat diet had a significant impact on them, while both the drug and the natural substances used altered their levels.

4. Discussions

Our results indicated that the experimental hyperlipidemia induced by adding 2% cholesterol with 100 grams of beef fat and 100 grams of food sugar to the rat diet led to an increase in total cholesterol, triglycerides, low-density lipoproteins, and very low-density lipoproteins, along with a decrease in high-density lipoproteins in serum. This is consistent with the study [11]. The reason for the increase in cholesterol concentration is due to a disturbance in fat metabolism, or a malfunction in the process of absorption and excretion of steroids, or perhaps due to a decrease in the concentration of bile salts. The increase in dietary cholesterol leads to greater reabsorption of bile acids, which are increasingly taken from the liver. The result is an inhibition of the conversion of cholesterol to bile acids by the liver due to the significant decrease in the concentrations of mRNA coding for the enzyme hydroxylase- $\alpha 7$, which in turn stimulates the rate-limiting step in bile acid synthesis. [12] The reason for the increase in triglyceride concentration is due to the reactive oxygen species (ROS) resulting from the elevated cholesterol levels, which led to the inhibition of the triglyceride lipase enzyme responsible for breaking down triglycerides, resulting in an increase in lipid metabolism and elevated concentrations in blood serum and liver extracts. The reason for the decrease in HDL-C

concentration may be attributed to the increased oxidation of LDL-C as well as the breakdown of the body's internal cholesterol due to reactive oxygen species (ROS), which causes a decrease in HDL-C concentration responsible for transporting cholesterol from body cells to the liver, resulting in a lowered concentration in the blood vessels. [13] The high-fat control group treated with ATOR showed a decrease in levels of TC, TG, LDL-C, and VLDL-C, along with a rise in HDL-C levels in the serum compared to the affected control group animals. The drug's role is to inhibit cholesterol production by regulating the formation of LDL-C receptors on the surface of liver cells, which leads to the removal of LDL-C from the bloodstream. [14] As for the role of propolis in reducing lipid levels in serum compared to the high-fat group, the results of the current study were consistent with the study. [15] Our results showed a positive improvement in the lipid profile in the serum of the group of animals treated with the alcoholic extract of propolis and royal jelly combined, significantly compared to the high-fat control group, as well as the groups treated individually with the ATOR drug, indicated by the decrease in levels of TC, TG, LDL-C, VLDL-C, and a significant increase in HDL-C levels. This positive change can be attributed to the fact that both propolis and local royal jelly contain many bioactive compounds that can regulate various mechanisms related to cholesterol metabolism and reduce cholesterol levels (Hypocholesterolemic).

The results of our current study showed a significant decrease ($p \leq 0.05$) in the enzyme antioxidant activity of catalase (CAT) and a significant increase in the concentration of malondialdehyde (MDA) in the cholesterol-treated group compared to the control group. These results are consistent with those of study [16], which indicated a significant decrease in some antioxidants, such as CAT, and a significant increase in the concentration of malondialdehyde (MDA) as a result of increased oxidative stress, which causes an increase in the formation of free radicals and depletion of antioxidants.

The study also clarified that CAT recorded a decrease in levels in the group treated with ATOR, which is consistent with previous studies that recorded a significant reduction in the enzyme level in catalase activity, indicating the occurrence of oxidative stress. Catalase is an iron-dependent enzyme that can function in a peroxidative or catalytic manner and is one of the most important antioxidant enzymes. Catalase acts as a key antioxidant enzyme that catalyzes the breakdown of H_2O_2 into H_2O . The accumulation of hydrogen peroxide leads to a decrease in catalase activity. [17] The study showed an increase in CAT levels in the propolis and royal jelly groups compared to the high-fat group. The significant increase observed after the administration of the alcoholic extract is attributed to the chemical composition of propolis, which is characterized by containing numerous biologically active chemical compounds (more than 850 compounds [18]). With diverse biological and therapeutic properties, particularly their ability as antioxidants, including flavonoids, phenolic compounds, phenyl acid, caffeine acid (CAPE), and coumaric acid [19].

The study showed an increase in the levels of MDA concentration in the high-fat group, where elevated fats lead to the production of a wide range of inflammatory products that cause disease progression, one of which is MDA, which is considered a common marker of oxidative stress [20]. While treatment with atorvastatin led to a decrease in MDA levels. The reduction in lipid peroxide levels may arise from the decrease in LDL-C levels as a result of atorvastatin treatment, as this drug may cause the removal of older LDL, which is more prone to oxidation [21]. The decrease in MDA concentration in the groups treated with propolis extracts is primarily attributed to the increased production of antioxidant enzymes and the inhibition of the enzymes that produce reactive oxygen species due to some biologically active compounds in propolis. This results in a reduction of lipid peroxidation and ultimately limits the production of malondialdehyde. Many studies have indicated the role of propolis supplements in affecting lipid metabolism and oxidative stress by decreasing the products of lipid oxidation, such as thiobarbituric acid reactive substances, and increasing the levels of

antioxidants [22]. As for the groups treated with Royal Jelly, there was a decrease in MDA levels due to the antioxidant components of Royal Jelly, and these results were consistent with [23]. Measuring degradation products, such as MDA, is one of the most common methods to determine the degree of induced lipid peroxidation. The elevated levels of cholesterol in the blood led to increased MDA levels compared to the control group, indicating an increase in oxidative stress in hypercholesterolemia [24].

5. Conclusion

In conclusion, this study demonstrated the significant effects of a high-fat diet on lipid profiles and oxidative stress markers in Wistar rats. The diet led to increased total cholesterol, triglycerides, LDL-C, and VLDL-C levels, alongside decreased HDL-C levels, confirming the role of hyperlipidemia in cardiovascular risk. Treatment with atorvastatin, propolis, and royal jelly reduced these lipid abnormalities, with the combination of propolis and royal jelly showing the most significant improvements in lipid profiles. Additionally, the antioxidant activity, indicated by catalase (CAT) and malondialdehyde (MDA) levels, was altered, with propolis and royal jelly helping to restore the antioxidant balance disrupted by the high-fat diet. These findings suggest that propolis and royal jelly possess hypolipidemic and antioxidant properties, potentially providing a natural alternative or adjunct to traditional statin therapy for managing hyperlipidemia and its associated oxidative stress. Further studies are needed to explore their long-term effectiveness and underlying mechanisms.

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