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Original Article

# Comparative effects of garlic (*Allium sativum*) powder and atorvastatin in female reproductive system of hypercholesterolemic rats: A histological and biochemical evaluation

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**Abstract**

**Background and aims:** The abnormal increase in blood cholesterol can cause many problems. Statins have a cholesterol-lowering effect, but they also have adverse effects. Garlic prevents the formation of cholesterol due to its antibiotic properties. This study aimed to investigate the comparative effect of garlic powder and atorvastatin on hypercholesterolemia-induced reproductive failure in female rats.

**Methods:** In the present experimental study, 48 adult female Wistar rats were divided into eight groups (n=6), including control, atorvastatin (10 mg/kg/d; orally), atorvastatin (20 mg/kg/d; orally), garlic powder (100 mg/kg/d; orally), hypercholesterolemia (1.5 mg/kg/d of cholesterol; orally), hypercholesterolemia + atorvastatin (10 mg/kg/d), hypercholesterolemia + atorvastatin (20 mg/kg/d), and hypercholesterolemia + garlic powder. After 30 days, rats were euthanized and blood samples were obtained from their heart for serological assessments. The right ovary was transferred to 10% formalin for histological analyses, and the left ovary was transferred to a −80°C freezer for evaluation of oxidative stress markers. Data were statistically analyzed by ANOVA and Tukey's test using SPSS version 24.0 ($P<0.05$).

**Results:** The number of healthy primordial, primary, secondary, and antral follicles, catalase activity, total antioxidant capacity (TAOC) as well as estrogen and progesterone levels were lower in hypercholesterolemic rats compared to controls ($P<0.001$). Additionally, the number of the atretic primary, secondary, and antral follicles and malondialdehyde (MDA) levels were higher in hypercholesterolemic rats ($P<0.001$). However, garlic powder and atorvastatin 10 improved alterations in the mentioned parameters ($P=0.99$).

**Conclusion:** The results showed that hypercholesterolemia could have adverse effects on rat ovaries. However, the garlic powder improves ovarian toxicity in hypercholesterolemia rats better than atorvastatin.

**Keywords:** Atorvastatin, Garlic, Hypercholesterolemia, Rat, Ovary

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## Introduction

Nowadays, many factors are involved in infertility. One of the most important causes of infertility in women is a dysfunction of the reproductive system, which occurs in the form of ovarian or uterine disorders (1). Hypercholesterolemia is a form of hyperlipidemia that is characterized by high cholesterol levels in the blood. The cause of this disease can be environmental (such as morbid obesity and diet) or genetic (2). Evidence suggests that a high level of cholesterol also increases the risk of atherosclerosis, heart attack, transient ischemic attack, stroke, and peripheral vascular disease (3). Previous studies have shown that a high-cholesterol diet, in addition to increasing the number of atretic follicles and decreasing the number of antral follicles, significantly increases luteinizing hormone (LH) and decreases the response of LH to gonadotropin-releasing hormone (4). Studies on the rat models have shown ovarian disorder through an increase in lipid storage. This hypercholesterolemia significantly increases follicular atresia, apoptosis, steroidogenesis, oxidative stress, and caspase-3 expression (5,6). Many previous studies have shown the association between obesity and ovulation dysfunction (7) and pregnancy disorders (8). It has been hypothesized that obese women show cortisol stability and hypothalamic-pituitary-adrenal axis activation (9), which is a strong predictor of ovarian dysfunction and pathogenesis (10). Moreover, a high-fat diet impairs fertility and function of hypothalamic-pituitary-ovarian axis (11).

3-Hydroxy 3-methylglutaryl coenzyme A reductase (HMG-GA) enzyme inhibitors, known as statins, stimulate angiogenesis at low doses by increasing nitric oxide while at high doses, these compounds reduce protein prenylation and inhibit cell growth (12). Atorvastatin is a member of the statin family, which has fewer side effects and is more effective than other statins. Low-dose

atorvastatin has cholesterol-lowering effects as well as beneficial pleiotropic therapeutic effects. Previous studies have shown anti-inflammatory and antioxidant properties of atorvastatin (13). High doses of atorvastatin cause many complications such as reproductive dysfunction, nephrotoxicity, and testicular damage (14).

Garlic, scientifically known as *Allium sativum*, belongs to the genus *Asparagus*. The use of this plant is common as a medicinal plant and food flavoring (15). Garlic contains various compounds, including vitamins A, C, B1, B2, and B6, allicin, ajoene, sulfur, and antioxidant compounds. Allicin and ajoene are the most important compounds of this plant. Garlic also has antibiotic properties and prevents the formation of cholesterol. The pharmacological effects of garlic include its antioxidant and protective functions (16). The consumption of garlic extract in patients with high blood cholesterol significantly reduces cholesterol and serum lipids (17).

Therefore, this study aimed to investigate the comparative effect of garlic powder and atorvastatin on hypercholesterolemia-induced reproductive dysfunction in female rats.

## Materials and Methods

### *Animals and treatment*

In the present experimental study, 48 adult female Wistar rats weighing 220 ± 240 g were purchased from the Laboratory Animal Breeding Center of Urmia University. They were kept in polycarbonate cages under standard conditions (temperature of 22.00 ± 2.00°C; relative humidity of 50.00 ± 10.00%; light-dark cycles of 12:12 hours) with free access to food and water. Before experimenting, the rats were adapted to laboratory conditions for two weeks. The experimental protocol and procedures were in compliance with international guidelines for care and use of laboratory animals, and they were approved by Urmia University. After two weeks, the rats were divided into eight groups (n=6), including control, atorvastatin (10 mg/kg/d; orally), atorvastatin (20 mg/kg/d; orally), garlic powder (100 mg/kg/d), hypercholesterolemia (1.5 mg/kg/d of cholesterol; orally), hypercholesterolemia + atorvastatin (10 mg/kg/d), hypercholesterolemia + atorvastatin (20 mg/kg/d), and hypercholesterolemia + garlic powder. Atorvastatin (18) and garlic powder (Goldaru Pharmaceutical Company, Isfahan, Iran) (19) were dissolved in normal saline, and cholesterol was dissolved in sweet almond oil (Nourhan Company, Shiraz, Iran) (20).

### *Sampling*

After 30 days, the rats were euthanized by intraperitoneal injection of ketamine (75.00 mg $kg^{-1}$; Alfasan, Woerden, The Netherlands) and xylazine (10.00 mg $kg^{-1}$; Alfasan, Woerden, The Netherlands). Then, blood samples were taken from the heart for serological assessments. The right ovary was transferred to 10% formalin for histological analyses, and the left ovary was transferred to a −80°C freezer for evaluation of oxidative stress markers.

### *Histological analysis*

For histological studies, the ovaries were separated under high magnification using a stereomicroscope (Olympus, Japan). The samples were embedded in paraffin blocks, which were serially cut using a rotary microtome and stained with hematoxylin-eosin (H&E). For histomorphometric analyses, follicles were classified into primordial and primary (<70 µm), early preantral (70-110 µm), early antral (110-200 µm), and large antral (>200 µm). Follicular morphology was examined under a light microscope with ×200 magnification. Follicles with an intact layer of normal granulosa and flattened theca cells, oocytes with normal cytoplasm, and nuclei were considered normal follicles. Follicles were classified as abnormal if dissociation of granulosa cells, early antrum formation, luteinization of granulosa cells, and floatation in the antrum were observed. The follicular count was estimated by counting follicles in all serially prepared slides. Moreover, the atretic primordial and primary, preantral, and antral follicles were counted in serial sections for each sample and compared between groups (21).

### *Biochemical assay*

To measure lipid peroxidation, the concentration of malondialdehyde (MDA) in ovarian tissue was assessed using the reaction of thiobarbituric acid as previously described (22). Catalase (CAT) activity in homogeneous ovarian tissue was evaluated based on its ability to decompose $H_2O_2$ using Aebi's method (23). The amount of total antioxidant capacity (TAOC) of ovarian tissue was evaluated using the ferric reducing antioxidant power test (24).

To determine estrogen and progesterone levels, the serum concentrations of estrogen and progesterone were assessed by ELISA as described in the instructions provided by the manufacturer (Pars Azmun., Iran).

### *Statistical analysis*

For statistical analysis, the obtained data were analyzed by one-way ANOVA and Tukey's post-hoc test using SPSS version 24.0. All data were considered statistically significant at $P<0.05$.

## Results

### *Histopathological findings*

The mean number of healthy and atretic follicles at different stages is shown in Tables 1 and 2. Histological analysis showed that in the hypercholesterolemic group, the number of healthy primordial, primary, secondary, and antral follicles was significantly lower compared to the control group ($P<0.001$). The administration of atorvastatin 10 mg/kg significantly increased the mean number of the healthy primordial, primary, and secondary follicles ($P<0.001$, $P=0.007$, $P<0.001$,

**Table 1.** The effect of high and low doses of atorvastatin and garlic powder on healthy ovarian follicles in the experimental groups

| Groups | Number of healthy primordial follicles | Number of healthy primary follicles | Number of healthy secondary follicles | Number of healthy antral follicles |
|---|---|---|---|---|
| Control | 173.20±6.90 [a] | 80.40±4.50 [a] | 54.00±5.09 [a] | 5.40±0.89 [a] |
| Atro10 | 151.20±7.32 [b] | 78.20±4.43 [a] | 37.80±2.28 [b] | 4.20±0.83 [ab] |
| Atro20 | 138.20±5.93 [b] | 67.00±4.84 [b] | 36.80±1.64 [b] | 3.80±0.44 [ab] |
| Garlic | 173.80±8.16 [a] | 78.60±4.61 [a] | 51.40±5.31 [a] | 5.20±0.83 [a] |
| Hyper | 77.20±7.25 [c] | 36.20±2.58 [c] | 20.40±1.14 [c] | 2.20±0.83 [c] |
| Hyper + Atro10 | 117.20±10.49 [d] | 47.00±4.84 [d] | 36.00±2.12 [b] | 3.20±1.09 [bc] |
| Hyper + Atro20 | 109.40±11.14 [d] | 43.40±2.50 [dc] | 30.00±2.34 [d] | 3.00±0.70 [bc] |
| Hyper + Garlic | 166.60±9.93 [a] | 74.60±4.92 [a] | 48.20±4.60 [a] | 4.80±0.83 [a] |

[a,b,c,d] Different letters indicate a significant difference between groups in each column ($P<0.05$).

**Table 2.** The effect of hypercholesterolemia (Hyper), high and low doses of atorvastatin and garlic powder on the number of corpus luteum and atretic ovarian follicles in the experimental groups

| Groups | Number of atretic primary follicles | Number of atretic secondary follicles | Number of atretic antral follicles | Number of corpus luteum |
|---|---|---|---|---|
| Control | 8.20±1.64 [a] | 6.40±0.89 [a] | 4.80±0.83 [a] | 24.60±1.67 [a] |
| Atro10 | 13.20±0.83 [b] | 9.00±1.22 [b] | 5.20±0.83 [a] | 22.80±2.28 [a] |
| Atro20 | 14.60±1.14 [b] | 9.80±0.83 [b] | 6.40±1.14 [ab] | 17.80±0.83 [b] |
| Garlic | 8.80±1.64 [a] | 5.40±0.89 [a] | 5.40±1.14 [a] | 23.40±1.51 [a] |
| Hyper | 29.60±2.07 [c] | 18.40±2.30 [c] | 14.40±1.14 [c] | 11.60±1.14 [c] |
| Hyper + Atro10 | 22.20±1.30 [d] | 9.60±1.51 [b] | 8.00±1.22 [bd] | 22.20±1.64 [a] |
| Hyper + Atro20 | 26.80±1.78 [c] | 12.20±0.83 [d] | 9.40±1.14 [d] | 16.40±1.14 [b] |
| Hyper + Garlic | 12.80±2.58 [b] | 6.60±1.14 [a] | 6.40±1.14 [ab] | 21.80±1.92 [a] |

[a,b,c,d] Different letters indicate a significant difference between groups in each column ($P<0.05$).

respectively), but the number of antral follicles was not significantly different from the hypercholesterolemic group ($P=0.556$). Administration of atorvastatin 20 mg/kg significantly increased the mean number of healthy primordial and secondary follicles ($P<0.001$, $P=0.002$, respectively), however, the number of primary and antral follicles was not significantly different from the hypercholesterolemic group ($P=0.169$, $P=0.788$, respectively). Garlic powder could improve the mean number of healthy follicles in hypercholesterolemic rats better than atorvastatin. Thus, in the group receiving garlic powder with hypercholesterolemia, the mean number of healthy primordial, primary, secondary and antral follicles was not significantly different from the control group ($P=0.921$, $P=0.407$, $P=0.170$, $P=0.942$, respectively; Table 1, Figures 1 and 2).

The mean number of primary, secondary, and antral atretic follicles in hypercholesterolemic rats showed a significant increase compared to the control group ($P<0.001$). Low-dose administration of atorvastatin showed a significant reduction in the mean number of primary, secondary, and antral atretic follicles compared to the hypercholesterolemic group ($P<0.001$). However, high dose atorvastatin could not cause a significant difference in the mean number of primary atretic follicles compared to the hypercholesterolemic rats ($P=0.195$). The administration of garlic powder to hypercholesterolemic rats showed a reduction in the mean number of primary, secondary, and antral atretic follicles compared to the hypercholesterolemic group ($P<0.001$, $P<0.001$, $P=0.001$, respectively), so that the mean number of secondary and antral atretic follicles of hypercholesterolemic rats receiving garlic powder was not significantly different from the control rats ($P=0.99$, $P=0.307$, respectively; Table 2, Figures 1 and 2)

The mean number of corpus luteum in hypercholesterolemic rats showed a significant decrease compared to control groups ($P<0.001$). In the hypercholesterolemic group receiving low-dose atorvastatin, the mean number of corpus luteum was not significantly different from the control group ($P=0.275$). The administration of a high dose of atorvastatin to hypercholesterolemic rats showed an increase in the mean number of corpus luteum compared to hypercholesterolemia group ($P=0.001$). The administration of garlic powder could improve the mean number of corpus luteum in hypercholesterolemic rats, indicating no significant difference from the control rats ($P=0.131$; Table 2, Figures 1 and 2).

### *Biochemical study of ovarian tissues*

The estrogen level was significantly lower in hypercholesterolemic rats compared to the control group ($P<0.001$), while this value in the hypercholesterolemic + garlic powder group was nearly restored to the control group levels ($P=0.99$). However, the administration

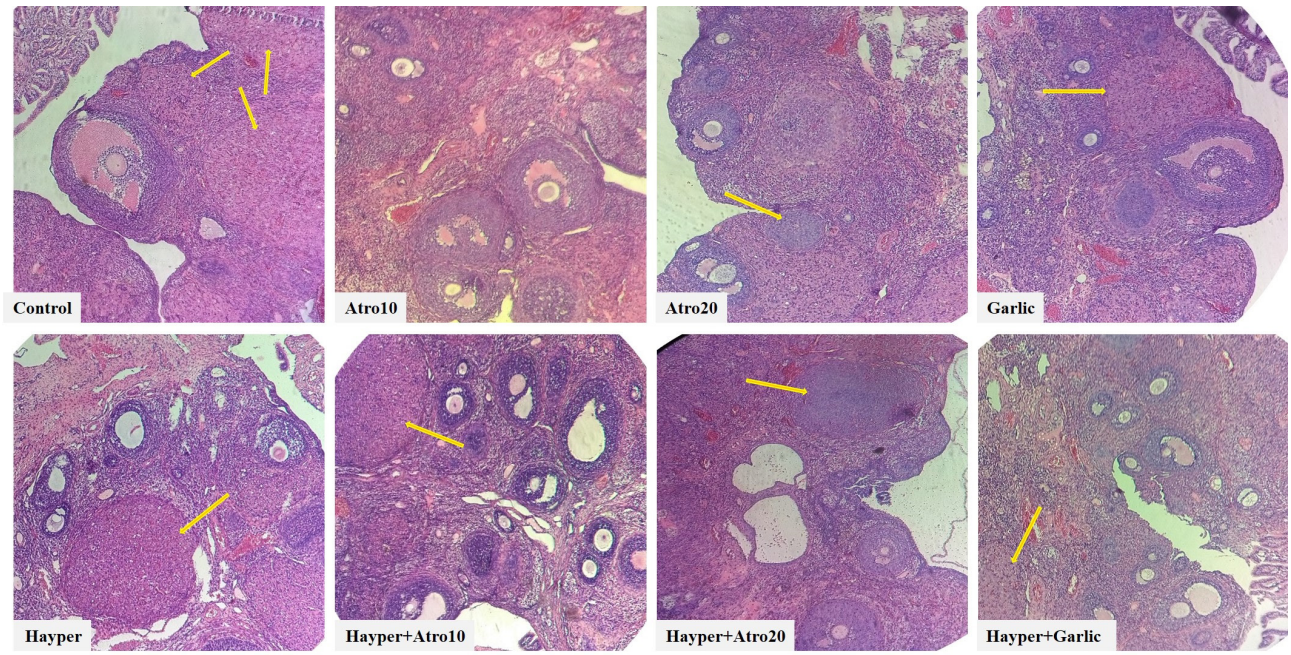


**Figure 1.** Cross-section of the ovary in different experimental groups with hematoxylin and eosin staining (×100 magnification). Arrow indicates the corpus luteum.

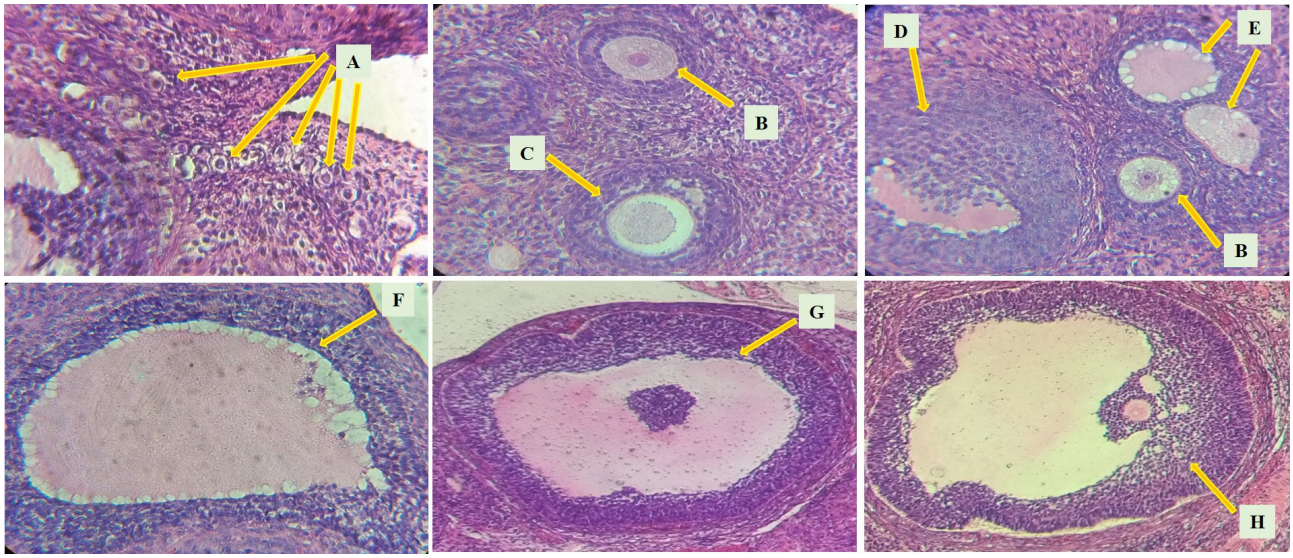


**Figure 2.** Cross-section of the ovary in different experimental groups (H&E staining, ×400 magnifications). **A:** Healthy Primordial Follicle, **B:** Healthy Primary Follicle, **C:** Healthy Secondary Follicle, **D:** Atretic Preantral Follicle, **E:** Secondary Atretic Follicle, **F:** Cystic Ovarian Follicle, **G:** Atretic Antral Follicle, **H:** Healthy Antral Follicle

of the low ($P=0.111$) and high ($P=0.99$) doses of atorvastatin to hypercholesterolemic rats did not show an increase in serum estrogen levels compared to the hypercholesterolemic group, indicating no significant difference (Figure 3).

The progesterone level was significantly lower in hypercholesterolemic rats compared to the controls ($P<0.001$). However, the progesterone level in hypercholesterolemic rats receiving high and low doses of atorvastatin groups showed a significant increase compared to the hypercholesterolemic rats ($P<0.001$). The level of this hormone in the hypercholesterolemic + garlic powder group was not significantly different from the control group ($P=0.662$; Figure 3).

The MDA concentration was significantly lower in hypercholesterolemic rats compared to controls ($P<0.001$), while there was no significant difference in hypercholesterolemic rats receiving low ($P=0.973$) and high ($P=0.857$) doses of atorvastatin and garlic powder ($P=0.99$) compared to the control group (Figure 4A).

CAT activity showed a significant decrease in the hypercholesterolemic group compared to the control rats ($P<0.001$). While in the hypercholesterolemic rats receiving low ($P=0.074$) and high ($P=0.061$) doses of atorvastatin and garlic powder ($P=0.008$), the activity of this enzyme had a significant increase compared to the

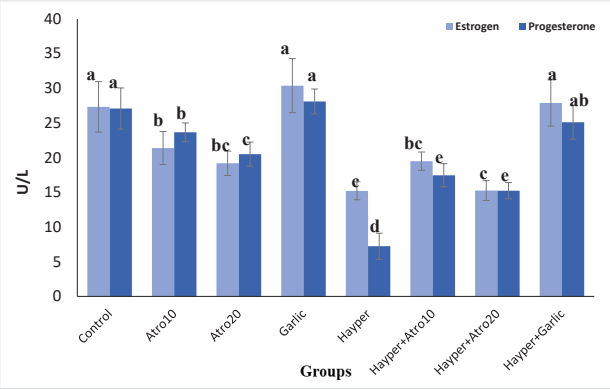


**Figure 3.** Comparative Effects of garlic powder and atorvastatin on estrogen and progesterone levels of hypercholesterolemia rats. All data are presented as mean ± SD. a, b, c, d, and e represent significant differences ($P<0.05$) between groups.

hypercholesterolemic rats (Figure 4B).

TAOC in hypercholesterolemic rats showed a significant decrease compared to controls ($P<0.001$). However, the administration of garlic powder to hypercholesterolemic rats showed an increase in the TAOC, so that it did not show a significant difference from the control rats ($P=0.976$). In the hypercholesterolemia groups receiving low ($P=0.002$) and high ($P=0.018$) doses of atorvastatin, the TAOC was significantly higher compared to the hypercholesterolemia group, however, it was significantly lower compared to the control rats ($P=0.001$; Figure 4C).

## Discussion

Genetic and environmental factors play important roles in obesity; a high-fat diet is the most important environmental factor that causes this disease. Studies have shown that hypercholesterolemia is associated with metabolic disorders, especially obesity and insulin resistance, infertility, menstrual irregularities, and anovulation (25). Increased body fat is associated with leptin hormone level and weight gain occurs due to changes in this hormone levels (26). Leptin hormone is made in fat cells and binds to leptin receptors, which are encoded by the LEPR gene. This hormone acts as a sensor of fat mass, and its concentration in the bloodstream is associated with the storage of body fat (27). It seems that in hypercholesterolemic rats, a disorder in the secretion of this hormone causes metabolic disorders and obesity due to an increase in cholesterol levels in the body. Cholesterol accumulation affects the fluidity and stability of cell membranes and causes cell damage (28). Moreover, the accumulation of fatty acids, including pristanic acid, causes Zellweger syndrome in the brain; and a cholesterol-rich diet can cause changes in pituitary gland function (29). Changes in pituitary gland function lead to disorder in the LH and FSH secretion, which causes large alterations in the menstrual cycle and reduces the number of healthy follicles (30).

In this study, the number of atretic follicles in the hypercholesterolemic group was significantly higher compared to the control and other groups. However, the administration of garlic powder and atorvastatin 10 significantly reduced the number of atretic follicles. Evidence suggests that apoptosis in granulosa cells is the principal mechanism of ovarian follicle atresia (31). Significant reduction in the percentage of atretic follicles in this study indicates the beneficial properties of these compounds in inhibiting the apoptosis process and reducing follicular atresia. Previous studies have shown that some factors, such as obesity, especially abdominal obesity, can cause oxidative stress (32). A high-fat diet leads to severe oxidative stress in cells by increasing reactive oxygen species (ROS) production. Additionally, a high concentration of androgens in follicular fluid is associated with high LH levels, which may cause follicular degeneration

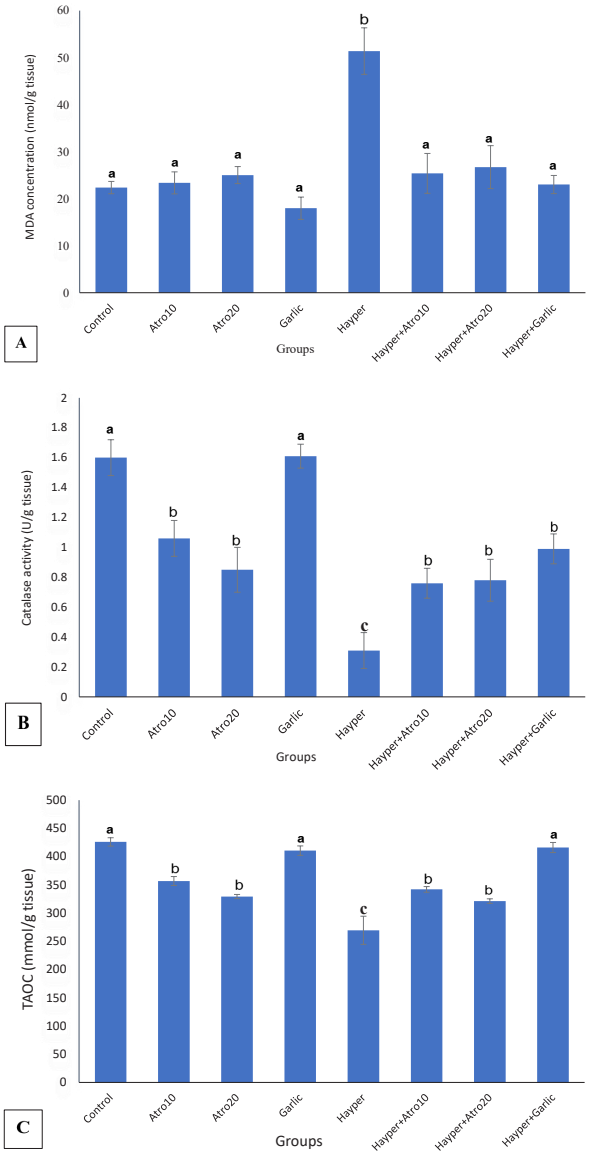


**Figure 4.** Effects of garlic powder and atorvastatin on MDA Concentration **(A)**, Catalase Activity **(B)**, and TAOC **(C)** in the Ovaries of Hypercholesterolemic Rats. a,b, and c represent significant differences ($P<0.05$) between groups. Results are presented as means ± standard deviation. MDA: Malondialdehyde; TAOC: Total antioxidant capacity.

and inhibit follicle development (33). Ovarian cysts can cause ROS production, high levels of oxidative stress such as MDA, and decreased total serum antioxidant capacity in patients with polycystic ovary syndrome (34). The level of oxidative stress is measured by various indicators, such as MDA level, catalase activity, and TAOC. In the present study, the MDA concentration significantly increased due to hypercholesterolemia. In addition, the catalase enzyme activity and TAOC significantly reduced due to hypercholesterolemia. This action indicates oxidative stress due to hypercholesterolemia, which may be due to an increase in the number of cystic follicles or atretic follicles due to hypercholesterolemia. The inhibitory effect of cholesterol accumulation in the pituitary gland causes unbalanced changes in LH secretion. A change in the amount of LH hormone leads to anovulation and consequently a decrease in corpus luteum. The increase in atretic follicles and decrease in healthy follicles decrease the number of follicles that have completed ovulation and cause a reduction in the number of corpus luteum (35).

Garlic has anti-diabetic properties that cause a change in the activity of the glutathione reductase enzyme and significantly reduces the activity of CAT and superoxide dismutase (36). Antioxidant and anti-inflammatory effects of atorvastatin have been demonstrated. Most researchers attribute these effects to the inhibition of the production of malonic acid, which is involved in the synthesis of isoprenoids. Malonic acid is a product of the HMG-COA reductase enzyme and the source of isoprenoids that play an important role in intracellular functions such as apoptosis, inflammation, coagulation, migration, and adhesion of leukocytes. Accordingly, the inhibition of isoprenoid synthesis by statins can be useful as a protective mechanism in cellular damage (37). Previous studies have shown that subcutaneous administration of atorvastatin prevents the production of anion superoxide by reducing NADPH oxidase activity (38). Therefore, the reduction in the number of atretic follicles due to the administration of garlic powder and atorvastatin can be due to the reduction of inflammatory factors, reduction of oxidative stress, and loss of ROS due to the antioxidant properties of garlic (39) and atorvastatin (40).

## Conclusion

According to the results of this study, it can be concluded that hypercholesterolemia causes changes in ovarian tissue, hormone balance, and oxidative stress, and the administration of garlic powder can have positive effects on the above-mentioned parameters. Further, the effects of atorvastatin are dose-dependent because atorvastatin 10 mg/kg improved hypercholesterolemia better than atorvastatin 20 mg/kg. Therefore, due to the adverse effects of atorvastatin on other organs, it is possible to use garlic powder to treat hypercholesterolemia instead of atorvastatin.

## Conflict of Interests

The authors declare no conflict of interest.

## Ethical Approval

All experiments were carried out according to Ethical Guidelines for the Care and Use of Laboratory Animals. This study was approved by the Ethics Committee of Urmia University (No: IR-UU-AEC-23/AD/3, 2021/4/7).

## Authors' Contributions

FF conceived and designed the experiment and was responsible for overall supervision. SJ performed laboratory tests and data collection. AS contributed to all experimental works and analysis of data. All authors read and approved the final manuscript.

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