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The Antioxidant and Anti-inflammatory Effects of Lutein Supplementation and Physical Exercise in Heart Tissue of Obese Male Rats

Maha Alatawi, Wadiah Backer and Lina Baz*

Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21589, **Saudi Arabia**

***Correspondence:** lbaz@kau.edu.sa **Received: 16-05-2023, Revised: 09-06-2023, Accepted: 10-06-2023 e-Published: 11-06-2023**

Obesity is thought to be a major factor in increasing oxidative stress and inflammatory markers, which contribute to the development of cardiovascular diseases. Either lutein or exercise has been demonstrated to have anti-inflammatory and antioxidant properties. This study aims to assess the effects of lutein (L) in combination with physical exercise (Ph) on the hearts of rats fed a high-fat diet (HFD). There were 50 rats randomly divided into 2 groups, 40 rats were fed an HFD to induce obesity, while 10 rats were fed a standard diet for 17 weeks. After 12 weeks, 40 obese rats were randomly divided into the following 4 groups: high-fat diet (HFD), a high-fat diet plus lutein (HFD+L), a high-fat diet plus physical exercise (HFD+Ph), and a high-fat diet plus lutein plus physical exercise (HFD+L+Ph) for 5 weeks. Our data revealed that a combination of lutein and physical exercise decreases body weight gain and significantly reduces heart enzyme activity, oxidative stress markers, and inflammatory markers that were increased in HFD-fed rats. Also, the combination of lutein and physical exercise significantly increases antioxidant enzymes that were decreased in HFD-fed rats. Collectively, the effect of a combination of L and Ph is greater than the effect of either L or Ph alone, exhibiting that L and Ph are alternative approaches to improving heart health in HFD-fed rats by suppressing inflammation and reducing oxidative stress.

Keywords: lutein; exercise; heart; oxidative stress markers; inflammatory markers; high-fat diet; obesity

INTRODUCTION

Globally, obesity has emerged as a serious health issue that affects individuals of all ages and genders (Boutari & Mantzoros, 2022). Obesity is defined by the World Health Organization (WHO) and the National Institutes of Health (NIH) as a body mass index (BMI) of greater than 30.0 kg/m^2. BMI is the most widely used measure of obesity. A person's BMI is calculated by dividing their weight in kilograms (kg) by their height in meters squared (kg/m^2). An imbalance between calories ingested and calories burned is the primary factor in obesity. Obesity can increase morbidity and mortality of cardiovascular disease (CVD) directly and indirectly. Directly through changes in the structure and function of the cardiovascular system caused by excess body weight. Indirectly through concomitant CVD risk factors such as type 2 diabetes mellitus, hypertension, insulin resistance, and dyslipidemia, respiratory disorders like asthma, hypoventilation syndrome, and sleep apnea, and certain types of cancer like prostate, colorectal, endometrial, and breast (Bollapragada et al., 2017; Fock & Khoo, 2013).

A high-fat diet is a common choice for inducing obesity in a rat model due to its capacity to mimic the way of obesity in humans (Abdul Kadir et al., 2015). Numerous research revealed that consuming a high-fat diet for an extended period was linked to a considerable rise in body weight, oxidative stress, and inflammatory markers (Al-Thepyani et al., 2022; Baz et al., 2022). Obesity treatment typically entails using chemical compounds and/or surgical treatments, which carry significant health risks and are associated with a high likelihood of relapse. For this reason, recent studies have tended to use natural products and exercise as alternative and effective methods for treating obesity that is safer and at a lower cost (Karri et al., 2019; Oharomari et al., 2021).

Recently, carotenoids have become more relevant due to their well-known health benefits (Salem, 2015). Lutein is one of the most prevalent carotenoids commonly present in orange and yellow fruits and vegetables (Nwachukwu et al., 2016; Ouyang et al., 2019). Numerous pharmacological properties of lutein include antioxidant, anti-inflammatory, and anti-apoptotic properties as well as neuroprotective, hepatoprotective, and cardioprotective activities (Morell & Fiszman, 2017; Nwachukwu et al., 2016; Ouyang et al., 2019).

Among the most important aspects of a healthy lifestyle is a regular exercise routine, which has been shown to reduce body weight by increasing energy expenditure and reducing several risk factors for cardiovascular diseases (Zalaqi et al., 2022). Additionally, exercise enhances antioxidant activity as well as decreases inflammatory markers (Al-Thepyani et al., 2022; Kolieb et al., 2022).

Thus, by searching all relevant previous studies on the benefits of lutein in general, and its effects on heart health and obesity as well. There is no comprehensive study to assess the effect of lutein in combination with exercise in the same regime on heart health. Hence the importance of this research in evaluating heart function markers, antioxidant status, oxidative stress markers, and inflammatory biomarkers in the heart of obese rats.

MATERIALS AND METHODS

Chemicals and kits

Lutein (purity at 98%) extracted from Marigold flowers was obtained from Xi'an Tongze Biotech Co., Ltd., Xi'an (China). The standard diet was obtained from the King Fahd Medical Research Centre (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia. Casein protein was purchased from Xi'an Harmonious Natural Bio-Technology Co, Ltd, Xi'an (China). Avitone powder premix was purchased from Star, Lahore (Pakistan). Commercial cow butter and sunflower oil were purchased from a local market in Jeddah (KSA). Enzyme-linked immunosorbent assay (ELISA) Kits of tumor necrosis factor-α (TNF-α) (Cat. No: SEKR-0009), interleukin-6 (IL-6) (Cat.No: SEKR-0005), and spectrophotometer kits of superoxide dismutase (SOD) (Cat. No: BC0175), catalase (CAT) (Cat. No: BC0205), nitric oxide (NO) (Cat. No: BC1475), malondialdehyde (MDA) (Cat. No: BC0025), and DL-Methionine were purchased from Solarbio Science & Technology Co., Ltd., Beijing (China).

Animals

Fifty male Wistar albino rats (weight range 200 ± 20g, 7 weeks old) were obtained from the animal house unit of KFMRC. They were housed in polypropylene cages (5 rats/cage) under controlled laboratory conditions (temperature 23 ± 2 °C, humidity 65%, and a standard 12 h light/dark cycle) and had free access to standard diet and water. Before the beginning of the study, the rats were allowed to adapt to the laboratory conditions for one week. The study protocol was approved by the biomedical ethics research committee at King Abdulaziz University and followed the rules and regulations of the Animal Care and Use Committee at the KFMRC (Approval No. 548-20).

Diets

Two formulations of rats' experimental diets were used in this study, including a standard diet and a high-fat diet. The standard diet with a total caloric value of 387 kcal/100 g was made up of carbohydrates (67.8%), protein (20.0%), fat (4.0%), fiber (3.50%), mixed vitamins and minerals (4.50%), and choline chloride (0.25%) (Reeves, 1997). The high-fat diet with a total caloric value of 529 kcal/100g was made up of carbohydrates (40.4%), protein (19.4%), fat (32.2%), fiber (2.1%), mixed vitamins and minerals (5.6%), choline chloride (0.1%), and methionine (0.2%). The high-fat diet was prepared by mixing the following ingredients: powdered standard diet (59.60%), butter (29.79%), casein (7.45%), DL-methionine (0.2%), and mixed vitamins and minerals (2.97%). DL-methionine, mixed vitamins and minerals were added to overcome the limitation of casein and avoid vitamin and mineral dilution (Al-Thepyani et al. 2022; Gheibi et al. 2017).

Preparation of treatment

Lutein powder [50mg/kg (body weight)/day] was mixed with sunflower oil [~2ml/kg (body weight)/day] to obtain a uniform suspension and kept till usage in the dark at 4 °C. Before giving the mixture of lutein-sunflower oil to animals, it was stirred for 20 minutes at 30°C in a water bath (Pierine et al. 2014). Previously conducted studies were used to determine the lutein dosage (Han et al. 2015; Qiu et al. 2015).

Experimental design

After adaptation for one week, the fifty rats were divided into two groups, the first group (control, n = 10) and the second group (obese, n = 40). Over 17 weeks, the control group was fed a standard diet whereas the obese group was fed a high-fat diet. By the end of 12 weeks period, the mean body weight was compared between the control and obese groups to confirm the rat's obesity. The group with significantly higher body weight was considered obese (Abdul Kadir et al. 2015; Novelli et al. 2007). Then, to estimate the effect of lutein and physical exercise on those obese rats which are randomly divided into 4 groups, in addition to a control group of (10 rats/ group) as follows:

Group 1: Control group

Group 2: High-fat diet group (HFD)

Group 3: High-fat diet + Lutein group (HFD+L)

Group 4: High-fat diet + Physical exercise group (HFD+Ph)

Group 5: High-fat diet + Lutein + Physical exercise group (HFD+L+Ph)

On the 13th week, the control and HFD groups were treated with sunflower oil administered orally (2 ml/kg(body weight)/day), HFD+L group was treated with lutein administered orally (50 mg/kg (body weight)/day), and HFD+Ph group was treated with sunflower oil administered orally (2 mg/kg (body weight)/day) and did swimming as a physical exercise while the HFD+L+Ph group was treated with lutein administered orally (50 mg/kg/day) and did a swimming exercise for 5 weeks.

Exercise protocol

The swimming exercise was performed in the plastic swimming tank (dimensions: 78 cm × 56 cm × 480 cm) with a maintained water temperature of 32 ± 2 °C. Before the treatment period, the rats in HFD+Ph and HFD+L+Ph groups were acclimated to the water and exercised with a gradual increase in the swimming period for one week. In the next 5 weeks of treatment, the rats swam for one hour per day in the morning from 9:00 to 10:00 am. To prevent hypothermia, rats were dried after each exercise session and placed in a warm environment (Riahi & Riahi, 2016).

Collection of blood and heart samples

By the end of the experimental period, overnight fasted animals were anesthetized by inhalation of isoflurane. Blood samples were collected from the retro-orbital plexus of the eyes and centrifuged at 3000 rpm for 10 minutes. The clear supernatant serums were stored at -80°C until used in biochemical analysis. Hearts were removed, cleaned, and stored at -80°C until used in biochemical analysis.

Assessment of heart enzyme markers

The levels of heart enzyme [creatine kinase (CK-MB), lactate dehydrogenase (LDH), and cardiac troponin I (cTnI)] in serum were measured by using standard kit methods using Automated COBAS® 8000 modular analyzer series in the King Fahd Armed Forces Hospital, Jeddah, Saudi Arabia.

2.9. Assessment of oxidative stress and antioxidant markers

The levels of oxidative stress markers (MDA and NO) and antioxidant markers (SOD and CAT) in the heart were measured by using spectrophotometer kits according to the manufacturer's protocols.

2.10. Assessment of inflammatory markers

The levels of inflammatory markers (TNF-α and IL-6) in the heart were measured by using ELISA kits according to the manufacturer's instructions.

2.11. Statistical analysis

GraphPad Prism (version 9.4.1) was used to statistically evaluate all data, which were presented as the mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was performed, and then a Tukey post hoc test was conducted. At a P value of 0.05, the findings were deemed statistically significant.

RESULTS

3.1. Effect of lutein and physical exercise on body weight

Consuming a high-fat diet for 12 weeks resulted in a significant increase in the body weight of the HFD groups compared to the control group. After treatment with lutein supplementation and/or exercise performance for five weeks, a decrease in body weight was observed compared to the HFD group but this decrease was not significant (Figure 1). The body weight gain of all groups was viewed in Table 1, and it was found that the HFD group had a notably increased body weight gain than the control group (P < 0.0001). However, rats treated with lutein and/or exercise performance displayed a decline in body weight gain compared to the HFD group but didn't statistically differ (P > 0.05). Furthermore, there was no significant difference detected in body weight gain between all treated groups (HFD+L, HFD+Ph, and HFD+L+Ph) (P > 0.05).

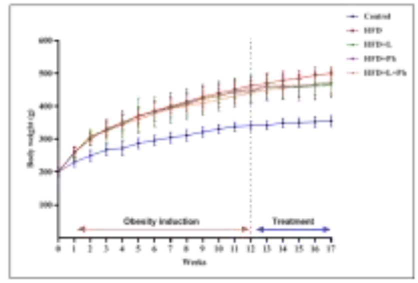

Figure 1: Body weight during 17 weeks of the experimental groups.

Table 1. Effect of lutein and physical exercise on body weight gain.

Parameters	Control	HFD	HFD+L	HFD+Ph	HFD+L+Ph
Initial body weight (g)	200.60±3.93	200.80 ±3.99	200.50 ±3.63	200.60 ±4.43	200.40 ±3.25
Final body weight (g)	354.60±4.91	500.90 ±6.50	473.50 ±10.21	468.40 ±13.37	464.60 ±8.60
Body weight gain (g)	154.10±4.81	300.20 ±5.88 **** [a]	273.00 ±11.03 ns [b]	267.80 ±12.99 ns [b] ns [c]	264.30 ±8.36 ns [b] ns [c] ns [d]

Data are presented as Mean ± SEM. HFD group, HFD+Ph group, HFD+L group, and HFD+L+Ph group. Significance **** at P < 0.0001, ns= non-significant. [a] Significant to the control group, [b] Significant to the HFD group, [c] Significant to the HFD+L group, and [d] Significant to the HFD+Ph group.

3.2. Effect of lutein and physical exercise on the heart enzymes

As presented in Figure 2 the results of serum cTnI, CK-MB, and LDH levels revealed that the HFD group exhibited a significant elevated (P < 0.0001) in cTnI, CK-MB, and LDH levels compared to the control group. However, rats treated with lutein and/or exercise performance in HFD+L, HFD+Ph, and HFD+L+Ph groups demonstrated a considerable reduction (P < 0.001) in cTnI, CK-MB, and LDH levels compared to the HFD group. Moreover, no significant difference was found in cTnI, CK-MB, and LDH levels between all treated groups (HFD+L, HFD+Ph, and HFD+L+Ph) (P > 0.05).

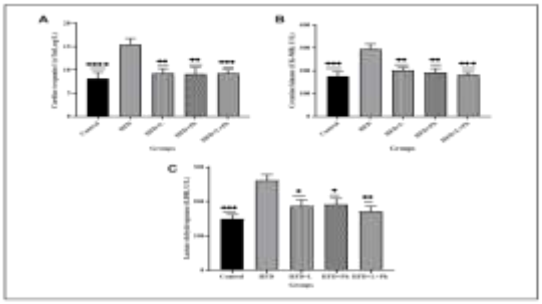

Figure 2: Cardiac troponin I (A), creatine kinase (B), and lactate dehydrogenase (C) levels in the serum of different experimental groups. Data are presented as Mean ± SEM. HFD group, HFD+L group, HFD+Ph group, and HFD+L+Ph group after 17 weeks (10 rats/group). Significant *, **, *, and **** at P < 0.05, P < 0.01, P < 0.001, and P < 0.0001; respectively versus HFD group.**

3.3. Effect of lutein and physical exercise on oxidative stress markers

The results of MDA and NO levels were viewed in Figure 3, and they showed that the HFD group had significantly raised MDA and NO levels than the control group (P < 0.0001). In contrast, rats treated with lutein and/or exercise performance (HFD+L, HFD+Ph, and HFD+L+Ph) displayed a significant decrease (P < 0.0001) in MDA and NO levels compared to the HFD group. Moreover, there was no significant decrease observed in MDA and NO levels between all treated groups (HFD+L, HFD+Ph, and HFD+L+Ph) (P > 0.05).

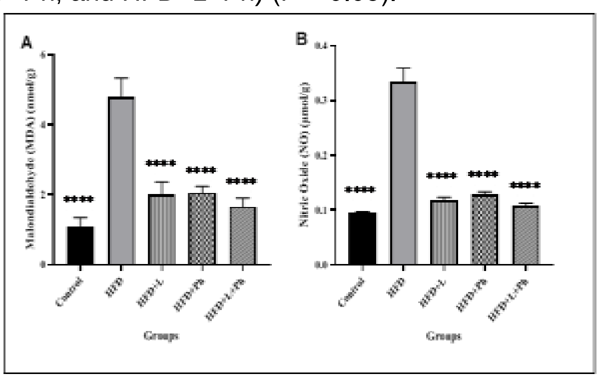

Figure 3: Malondialdehyde (A) and nitric oxide(B) levels in heart tissue of different experimental groups. Data are presented as Mean ± SEM. HFD group, HFD+L group, HFD+Ph group, and HFD+L+Ph group after 17 weeks (10 rats/group). Significance ** at P < 0.0001 versus HFD group.**

3.4. Effect of lutein and physical exercise on antioxidant markers

The results of the SOD and CAT levels were presented in Figure 4, and they indicated that the HFD group had markedly decreased SOD and CAT levels than the control group (P < 0.0001). Conversely, rats treated with lutein and/or exercise performance (HFD+L, HFD+Ph, and HFD+L+Ph) showed a substantial increase (P < 0.0001) in SOD and CAT levels compared to the HFD group. Furthermore, compared to the HFD+L and HFD+Ph groups, the HFD+L+Ph group demonstrated a significant increase (P ≤ 0.01) in SOD and CAT levels.

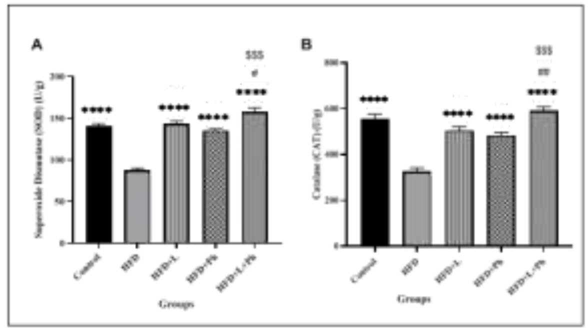

Figure 4: Superoxide dismutase levels (A) and catalase levels (B) in heart tissue of different experimental groups. Data are presented as Mean ± SEM. HFD group, HFD+L group, HFD+Ph group, and HFD+L+Ph group after 17 weeks (10 rats/group). Significant ** at P < 0.0001 versus HFD group; significant #, and ## at P < 0.05, P < 0.01; respectively versus HFD+Lgroup; significant $$$ at P < 0.001 versus HFD+Ph group.**

3.5. Effect of lutein and physical exercise on inflammatory markers

From Figure 5 the results of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels exhibited that the HFD group revealed a significant increase (P < 0.0001) in TNF-α and IL-6 levels compared to the control group. However, rats treated with lutein and/or exercise performance (HFD+L, HFD+Ph, and HFD+L+Ph) displayed a significant decrease (P ≤ 0.0001) in TNF-α and IL-6 levels compared to the HFD group. Moreover, no significant difference was revealed in TNF-α and IL-6 levels between all treated groups (HFD+L, HFD+Ph, and HFD+L+Ph) (P > 0.05).

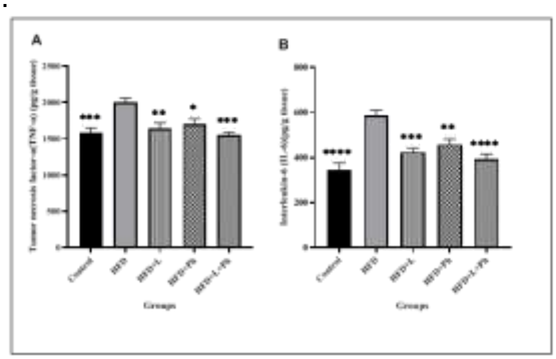

Figure 5: Tumor necrosis factor-α levels (A) and interleukin-6 levels (B) of heart tissue in different experimental groups. Data are presented as Mean ± SEM. HFD group, HFD+L group, HFD+Ph group, and HFD+L+Ph group after 17 weeks (10 rats/group). Significant *, **, *, and **** at P < 0.05, P < 0.01, P < 0.001, and P < 0.0001; respectively versus HFD group.**

DISCUSSION

Obesity is a chronic condition characterized by excessive accumulation of body fat that can lead to various health complications, including cardiovascular disease (CVD). physical inactivity and excessive high-energy food intake are considered the most important causes of obesity (Albaqami et al., 2023). Obesity treatment typically entails using chemical compounds and/or surgical treatments, which carry significant health risks and are associated with a high likelihood of relapse. For this reason, recent studies have tended to use natural products and exercise as alternative and effective methods for treating obesity that is safer and at a lower cost (Karri et al., 2019; Oharomari et al., 2021). This study aims to assess the effects of lutein (L) in combination with exercise (Ph) on the hearts of rats fed a high-fat diet.

This study found that feeding rats an HFD for 12 weeks caused a marked increase in body weight and weight gain when compared to the standard diet, and this finding is similar to a recent study by (Al-Thepyani et al., 2022; Li et al., 2020; Rufino et al., 2021; Sahraoui et al., 2020). After treatment with lutein and/or exercise performance for 5 weeks, body weight and weight gain were decreased in the HFD+L, HFD+Ph, and HFD+L+Ph groups, but with no significance compared to the HFD group. Recent research by Gopal et al. (2022) demonstrated that lutein administration for 4 weeks markedly reduced body weight and weight gain in HFD-induced obese mice. In addition, according to Wang et al. (2021) due to lutein's capacity to lower body weight, it may be possible to avoid excessive lipid production and obesity using this supplement. Weight gain was also reduced in the exercise performance groups (HFD+Ph and HFD+L+Ph) compared to the HFD+L group, indicating that regular exercise may help halt or slow the development of obesity. This finding is consistent with earlier studies showing that exercise can raise energy metabolism, aiding in weight control and lowering obesity rates (Kang et al., 2019; Yang et al., 2016).

A high-fat diet is one of the main risk factors for the emergence of cardiovascular diseases (Hamzeh et al., 2017). During this study, the rats fed with HFD exhibited a substantial rise in cardiac enzyme levels, including cTnI, CK-MB, and LDH as compared to the control group. This result agrees with Feriani et al. (2021) who found that rats fed an HFD exhibited a substantial increase in heart enzyme levels. However, after five weeks of treatment with lutein and/or exercise performance cTnI, CK-MB, and LDH levels were significantly decreased compared to the HFD group. In addition, the levels of cTnI, CK-MB, and LDH were the lowest in HFD+L+Ph compared to all treated groups (HFD+L and HFD+Ph). Similarly, Ouyang et al. (2019) found that lutein treatment reduced the levels of heart enzymes cTn I, CK-MB, and LDH due to potent anti-lipid peroxidation and antioxidant properties.

Numerous investigations have confirmed that oxidative stress plays a crucial role in the pathophysiology of heart failure which was apparent in our study. Our results revealed that HFD increased significantly oxidative stress markers (MDA and NO) in heart tissue and reduced the antioxidant activity (SOD and CAT) in heart tissue compared to the control group. Accordingly, Csige et al. (2018) and Feriani et al. (2021) indicated oxidative stress as a possible mechanism for obesity-induced cardiotoxicity. In this study, after 5 weeks of lutein administration and/or exercise performance, CAT and SOD levels elevated significantly, whereas MDA and NO levels reduced significantly compared to the HFD group. Furthermore, compared to the HFD+L and HFD+Ph groups, the HFD+ L+Ph had a notable rise in the SOD and CAT levels. On the other hand, it's important to note that lutein's effect was stronger than exercise's. Soundarya Priyadharsini et al. (2015) established that lutein has a strong antioxidant effect against hypercholesterolemia-induced oxidative damage in Wistar rats. The presence of conjugated double bonds and hydroxyl groups in lutein's structure is responsible for its anti-inflammatory and antioxidant properties (Ahn & Kim, 2021). In addition, following exercise, the body produces more endogenous antioxidants, which aid in the protection of cells from oxidative damage, demonstrating that exercise can lower oxidative stress (Neubauer & Yfanti, 2015).

In our study, TNF-α and IL-6 levels were considerably elevated in the HFD group compared to the control group. After administration of lutein and/or exercise performance for 5 weeks, the TNF-α and IL-6 levels were reduced significantly compared to the HFD group. Furthermore, compared to all treated groups (HFD+L and HFD+Ph), the HFD+L+Ph group had the lowest TNF- α and IL-6. A similar result had been reported by Tuzcu et al. (2017) and Kim et al. (2011) who found a noticeable decrease in the levels of inflammatory cytokines in animal models treated with lutein. The explanation for this result is that lutein attenuates the inflammatory state through inhibition of the nuclear factor-kappaB (NF-κB) pathway which is involved in cytokines production (Kim et al. 2011). In addition, exercise has been shown to have the capacity to reduce levels of TNF- α and IL-6 (Kizaki et al., 2011). Exercising regularly has been shown to reduce chronic inflammation in experimental animals and humans significantly (Suzuki, 2019; Teixeira et al., 2016).

CONCLUSION

The findings of the current study revealed that lutein supplementation and exercise performance considerably decreased heart enzyme activity, oxidative stress markers, and inflammatory markers that were increased in rats fed a high-fat diet. Lutein supplementation and exercise performance also drastically elevated antioxidant enzymes that declined in rats fed a high-fat diet. In summary, our findings proved that lutein and exercise are alternative approaches to reducing obesity and related complications through their antioxidant and anti-inflammatory properties. For future studies, we recommend increasing the duration of treatment to more than five weeks to determine the maximum synergetic effect that can be reached. Further studies on the effect of lutein and physical exercise on other HFD complications are recommended.

CONFLICT OF INTEREST

The authors declared that the present study was performed in the absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization, methodology, formal analysis W.B., L.B., and M.A.; software, investigation, data curation, writing—original draft preparation, M.A.; validation, writing—review and editing, supervision, W.B., and L.B. All authors have read and agreed to the published version of the manuscript.

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