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Effects of *Manna of Alhagi persarum (Taranjabin)* and *Portulaca oleracea (Khorfeh)* Seed Administration on Nickel-induced Allergic Contact Dermatitis: A Case Report

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ABSTRACT

A 19-year-old girl with documented nickel-induced allergic contact dermatitis was referred to the department of traditional Persian medicine five weeks after the sudden onset of bolus and erythematous skin rash to receive treatment based on complementary and alternative medicine. A detailed history of jewelry exposures, lifestyle, food intake, and dietary habits was obtained, as well as the history of other physicians' visits and administered treatments within the past eight weeks. Despite receiving treatment with corticosteroids, anti-histamines, and antibiotics, no satisfactory remission was achieved, and the patient discontinued the treatments. As the alternative medicine approach, treatment was started based on avoiding contact with nickel, administration of *Manna of Alhagi persarum* and *Portulaca oleracea* seeds, and avoidance of consuming tomato sauce, cocoa, coffee, and bitter chocolate. Within six weeks, a complete remission of the skin lesions and symptoms occurred. Moreover, normal clinical follow-up was observed after 12 months. Notably, the CARE (CAse REport) guidelines were used in the preparation of this case report.

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Introduction

Allergic contact dermatitis (ACD) is a prevalent allergic disease, which is characterized by a delayed hypersensitivity response to the external exposure to allergens (1, 2). Nickel is a widely known allergen, and the prevalence of allergic reactions to this element has increased recently. Nickel-induced ACD may occur due to the prolonged use of nickel-containing provokers of allergic reactions, such as costume jewelry, cosmetic products, and hand tools (2-4).

Despite avoidance strategies, promoting skin barrier regeneration, and topical/systematic symptomatic therapies, numerous patients consider complementary and alternative medicine (CAM) therapies (5). Traditional Persian medicine (TPM) is a branch of CAM, which mainly involves the use of medicinal foods and herbs, and the diagnosis and treatment of patients are accomplished by considering every aspect of their clinical condition (6, 7).

The present study aimed to describe the effectiveness of a TPM-based herbal compound in the treatment of a patient with ACD.

Ethical Considerations

Written informed consent was obtained from the patient for publishing the obtained data anonymously after the decision of the medical team to publish the TPM-based therapeutic process of the case.

Case Presentation

A 19-year-old female student with a history of atopy and documented nickel-induced ACD referred to the TPM department clinic of Qaem AJ Hospital affiliated to Mashhad University of Medical Sciences in Mashhad, Iran five weeks after the sudden onset of bolus and erythematous skin rash due to wearing a new wrist watch. The rash was initially located on the wrist and forearms and later spread to the entire body of the patient (mainly affecting both forearms and ears). The face, palms, soles, and tongue of the patient were unaffected. The rash

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was also accompanied by severe itching (Figure 1).

Before referral to the TPM clinic, the patient had undergone a four-week treatment course of avoiding contact with nickel and receiving valacyclovir, cephalexin, ethacridine lactate, triamcinolone, hydrocortisone, loratadine, adapalene, and fexofenadine. However, no satisfactory remission had been achieved. The patient had discontinued routine treatments for seven days before referral to the TPM clinic and did not accept continuing the routine treatments in the TPM-based treatment period.

Treatment was started with drinking a cup of steeped *Manna of Alhagi persarum (Taranjabin)* (5 gr) and *Portulaca oleracea (Khorfeh)* seeds (5 gr) twice a day. Two days after the onset of the treatment, the itching of the patient reduced significantly. The third visit of the patient was performed on the fifth day, with the patient stating that the itching had deteriorated, and the skin lesions had turned into dark, crusty patches (Figure 2).

Figure 1. Bolus and Erythematous Skin Rashes Focusing on Forearms of Reported Case

Figure 2. Dark Crusty Patches 5 Days after First Visit

Treatment continued with the prescription of similar doses for another week, and the patient was asked to avoid consuming tomato sauce, cocoa, coffee, and bitter chocolate. One week after the third visit, the severity and distribution of the skin lesions decreased, and the patient had complaints of no other complications than mild itching in the wrist (Figure 3).

The treatment resulted in the almost complete remission of the skin lesions and symptoms within six weeks, and only mild pruritus remained in the patient's wrist (Figure 4).

The clinical follow-up of the patient after 12 months was normal. In addition, the patient **completed the prescriptions with no reports of adverse effects at the end of the study period.** Figure 5 depicts the timeline of the case study.

Figure 3. Improved Skin Lesions 12 Days after First Visit

Figure 4. Almost Complete Remission of Skin Lesions 38 Days after First Visit

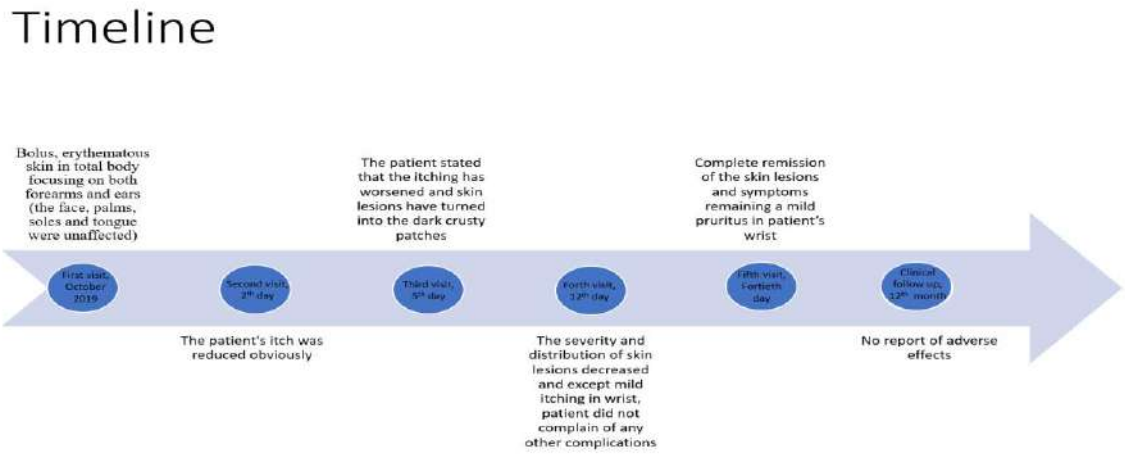

Figure 5. Timeline of Case Study

Discussion

Nickel-induced ACD is a common disorder affecting 28% of the Iranians experiencing allergies mostly in the case of young female patients. It is considered to be the most common ACD in Iran (3, 8, 9). Although allergen avoidance is the major preventive strategy against nickel-induced ACD, numerous patients opt for CAM strategies (especially the use of herbal medicines) for the management of acute flares. Although several studies have assessed the effectiveness of various medicinal plants such as *Impatiens biflora, Viola odorata, Althea officinalis,* and *Avena sativa* in the treatment of ACDs (9), few studies have investigated the effects of *Alhagi persarum* and *Portulaca oleracea* in this regard.

In the present study, no satisfactory remission occurred following the administration of valaciclovir, cephalexin, ethacridine lactate, triamcinolone, hydrocortisone, loratadine, adapalene, and fexofenadine and avoiding contact with nickel within a four-week treatment course. The TPM-based treatment was started seven days after the routine treatments had been discontinued by the patient, and she did not agree to continue the routine treatments during the TPM-based treatment course.

From the perspective of TPM, *Manna of Alhagi persarum* (*Taranjabin*) and *Portulaca oleracea* (*Khorfeh*) seeds have anti-inflammatory, wound-healing, and regenerative/moisturizing properties (10-12). These two therapeutic agents were primarily prescribed to our patient, and the observed effects could be attributed to their bioactive agents.

Portulaca oleracea exerts its effects through anti-inflammatory properties and balancing the adaptive and innate immune system depending on the conditions of the patient. *Portulaca oleracea* also acts as an immunomodulatory and antioxidant agent in both inflammatory states through dominating the Th2 response (e.g., asthma, cancer, atopic dermatitis) and evokes Th1 disorders such as hepatitis and multiple sclerosis (13). According to the literature, *Portulaca oleracea* contains omega-3 fatty acids, flavonoids, tannin, saponins, and terpenoids, which could suppress the inflammatory response pathways and reduce the inflammatory cytokines in the acute phase of nickel-induced ACD flare (12). Additionally, *Portulaca oleracea* may cool the skin-related temperament in inflammatory lesions (14). The seeds of this plant are an abundant source of omega-3 fatty acids, which has been reported to play a pivotal role in controlling the symptoms of dermatitis (15). The activity of catecholamines such as dopamine and L-methyl-adrenalin, which are found in *Portulaca oleracea* seeds, could also explain the anti-allergic effects of this plant (16).

Previous findings have indicated that phenolic compounds have potent antipruritic effects (17). *Taranjabin* and *Khorfeh* are abundant sources of polyphenolic compounds, especially flavonoids (18). Other plants that have shown positive effects on ACD are also reported to be rich sources of polyphenols (19, 20). Therefore, it could be inferred that the improvement in the symptoms of our patient could be partly attributed to the activity of the polyphenolic compounds in the herbal medicines used in the treatment.

The macromolecules found in the water-soluble portion of *Alhagi persarum* may exert immunomodulatory effects (11). Polysaccharides, alkaloids, steroids, and flavonoids in *Alhagi persarum* are also considered to be the sources of the anti-inflammatory, antimicrobial, anti-ulcer, and analgesic effects of this plant, which could be highly effective in the treatment of dermatitis as well (21, 22).

The use of herbs in the treatment of ACD may be challenging. Some reports have indicated the induction of ACD by some plants or herbal preparations (23, 24). However, some patients may not respond to conventional treatments, and complementary or alternative therapies may be required in such cases. Basic studies, especially those examining the anti-inflammatory and anti-allergic effects of natural substances, could shed light on the choice of complementary therapies for these patients. Moreover, herbal treatments should be carefully monitored in terms of safety and their possible short-term/long-term complications.

The results of the present study indicated that the administration of two natural drugs for 40 days may significantly suppress the signs and symptoms of ACD. Furthermore, the one-year follow-up of the patients showed that the therapeutic effects of the treatment were not temporary, and no local or systemic side-effects were observed in the patient. Although increased pruritus was observed during the course of the

treatment, it could not be attributed to the treatment given the history of consuming spicy sauces by the patient and the control of itching when the consumption was discontinued. In addition to the clinical improvement of the disease symptoms, the treatment adherence of the patient was satisfactory.

The main limitation of this study was that the therapeutic effects of the two herbs were not investigated separately. Therefore, the obtained therapeutic outcomes cannot be attributed to one or both of the herbs. It is recommended that further investigations in this regard be focused on interventional factors such as the patient's diet although the dietary abstinence of the patients was noted in our study as well. Additional controlled studies are also essential to determining the exact role of these medicinal herbs as a potential option in the treatment of nickel-induced ACD.

Conclusion

According to the results, avoiding continuous contact with nickel and some allergenic foods and consuming *Manna of Alhagi persarum (Taranjabin)* and *Portulaca oleracea (Khorfeh)* seeds as the main therapeutic agents could be effective in the medical management of nickel-induced ACD. However, further studies are required to investigate the exact role of these medicinal herbs as potential treatment options for nickel-induced ACD.

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Conflicts of Interest

None declared.

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A Systematic Review of the Nutritional Consequences of the 2012 East Azerbaijan Earthquake, Iran

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ABSTRACT

Introduction: Concerns about nutrition and food constantly affect different communities following natural disasters. The present study aimed to investigate the nutritional consequences after the East Azerbaijan earthquake in Iran in 2012.

Methods: This systematic review was conducted via searching for relevant surveys in databases such as PubMed, Scopus, Google Scholar, Cochrane Library, ScienceDirect, Web of Science(English studies), Google, Yahoo, and Persian information and library resources (Persian studies) using various keywords. The collected data were analyzed, summarized, and reported manually by content analysis.

Results: Out of 975 studies identified in the scientific databases, 21 were reviewed in order to address the research subject. The consequences of the earthquake were classified into three categories of food safety, food security, and nutritional consequences.

Conclusion: Despite the fulfilled efforts after the 2012 East Azerbaijan earthquake, diverse nutritional problems and consequences have arisen in the community, especially among women and children.

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Introduction

Iran has experienced about 181 disasters, which have led to approximately 160,000 deaths and over 170,000 injuries, affecting more than 44 million people. Iran is one of the most seismically active countries in the world. Over the past century, Iran has undergone more than 20 devastating earthquakes with the overall magnitude of ≥6 on the Richter scale, which have led to nearly 500,000 deaths(1).

One of the most destructive earthquakes in Iran during the past decade was the twin earthquakes occurring on 11 August2012 in East Azerbaijan province, in which 272 villages and nearly 50% of rural healthcare centers were destroyed according to official reports. In addition, 300 people were killed in this disaster, and the number of the injured victims was estimated to be more than 3,000 (2).

Every natural disaster affects the nutritional status of the people, such as flood, landslides, cyclone, tsunami, hurricane, and earthquake(3). Earthquakes intensely affect the nutritional status of vulnerable groups through direct impacts such as physical injuries and economic problems and indirect impacts such as mental and health difficulties, water resource scarcity, food supply disruption, limited nutrition access (4-7),

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livestock losses, and food storage mass destruction. Newborns may even suffer from restricted breastfeeding. Therefore, the lack of timely nutritional interventions could lead to extremely detrimental nutritional consequences with short-term and long-term effects on the community (8).

Malnutrition has been reported to account for 23% of the pediatric mortalities in displaced populations(9). For instance, formula-fed infants may be exposed to infectious agents due to the consumption of contaminated water to prepare milk(10).Several studies have globally indicated that natural disasters such as earthquakes adversely affect children's growth (11-14). After the2003 Bam earthquake on Friday December 26, weight and height loss and various nutritional difficulties were observed in children(15).

Several studies were conducted after the 2008 Wenchuan earthquake, indicating that the affected urban and rural areas had more nutritional problems(16, 17). Most of these cross-sectional studies were performed on the small groups that were available in the affected areas, and none extensively evaluated nutritional consequences using comprehensive and systematic approaches. After any incident, a proper understanding of the subsequent nutritional problems is essential as it could contribute to designing effective interventions to prevent disastrous events in the future.

The present study aimed to combine the results of quantitative and qualitative studies using a coherent and integrated approach and identify the consequences of the East Azerbaijan earthquake in terms of the nutritional status of the affected population. We have also provided a comprehensive and clear view of the nutritional consequences of the East Azerbaijan earthquake by comparing and summarizing the results of valid studies and reports in this regard.

Materials and Methods

This study was designed and conducted in 2019 in the form of a systematic review (18).

Literature Search Strategy

Data were collected directly from databases such as PubMed, Scopus, Google Scholar, Cochrane Library, ScienceDirect, Web of Science(English studies), Google, Yahoo, and other Persian information and library resources (Persian studies), such as Magiran, Civilica, SID, and Iran Medex using various English/Persian keywords based on the objective of the study, including East Azarbaijan OR East Azerbaijan OR Azerbaijan Sharghi OR Ahar OR Haris ORHeris ORVarzequan OR Varzegan OR Varzagan AND Earthquake OR Quake OR Shake. In order to ensure the identification and review of all the published articles in this regard, the references of the relevant articles were also searched. In addition, letters to the editor, conferences papers, and evidenced department reports were selected for the review.

Inclusion Criteria

The inclusion criteria of the study were the organizational reports and papers published during 2012-2019 based on the verified documents on the post-earthquake consequences and malnutrition problems of the affected community.

Exclusion Criteria

The exclusion criteria of the study were the articles focused on the other aspects of the natural disaster, such as geological, social, economic, and physical aspects.

Papers Reporting Quality Evaluation

The quality of the article reports was evaluated after extraction from the searched databases using the mentioned keywords. Following that, the articles were evaluated using in accordance with the checklist of the Strengthening the Reporting of Observational Studies in Epidemiology(STROBE)(18). The checklist consists of 22 items, and we eliminated the items regarding case studies and cohorts. In total, 18 items remained in the survey checklist, which were scored zero, one, and two to show the lack of relevant content to the checklist criteria, intermediate agreement, and complete agreement with the checklist criteria, respectively. The score range of the entire checklist was 0-36, with scores 0-24, 12-24, and 24-36 interpreted as weak, moderate, and favorable, respectively. The primary evaluation process was conducted by two experts on five articles in order to reach consensus. After reaching high consensus, the remaining articles were evaluated by one researcher. Notably, the unclear problems that had to be removed were referred to a second expert with greater skills about the methodology of the research.

The reporting quality of the qualitative articles was assessed by two evaluators in accordance with the Critical Appraisal Skills Program checklist (CASP) with 10 items; the first two items were intended for screening (Yes/No). Article evaluation would continue if both answers were affirmative. The next eight item should be answered as Yes/No/Not Known based on the opinion of the evaluator (19). Scores three, two, and one were assigned to the Yes, Not Known, and No answers, respectively, and the score range of the checklist was8-24. Studies without affirmative screening answers were also selected for the review. In case of disagreement between the two evaluators, a third party would judge.

Data Extraction

A checklist layout was designed manually in Microsoft Word for data extraction, which included data on the authors' names, publication year, sample size, subjects, and study design. Initially, data were extracted from three studies in apilot study. Afterwards, the observed problems and shortcomings were eliminated from the primary checklist.

Table 1. Data Analysis and Encoding

Code	Parameter
1	Studying article texts (deliberating study results)
2	Primary context recognition and extraction (food safety, food security, nutritional consequences)
3	Article categorization in determined contexts
4	Over viewing and completing final results in each context based on study results in each context
5	Checking contexts and reliability of extracted results in each context (by debating and removing controversies between two researchers during article encoding)

DataAnalysis

After data extraction using the designed checklist, data analysis was performed using content analysis, and the data were summarized and reported manually. In addition, thematic analysis was usedto recognize, analyze, and report the patterns (themes)investigated in the texts. This methodology has been frequently applied in qualitative data analysis(20-23).In our study, data analysis was carried out by two researchers in order to decrease selection bias. The main stages of data analysis were reading the extracted texts several times (data immersion), identifying and extracting the basic codes, creating primary themes (classifying the initially extracted code in the created themes), completing and revising the themes, and ensuring the reliability of the codes and themes (reaching agreement between the two coders). Table 1 shows the analysis and data encoding processes.

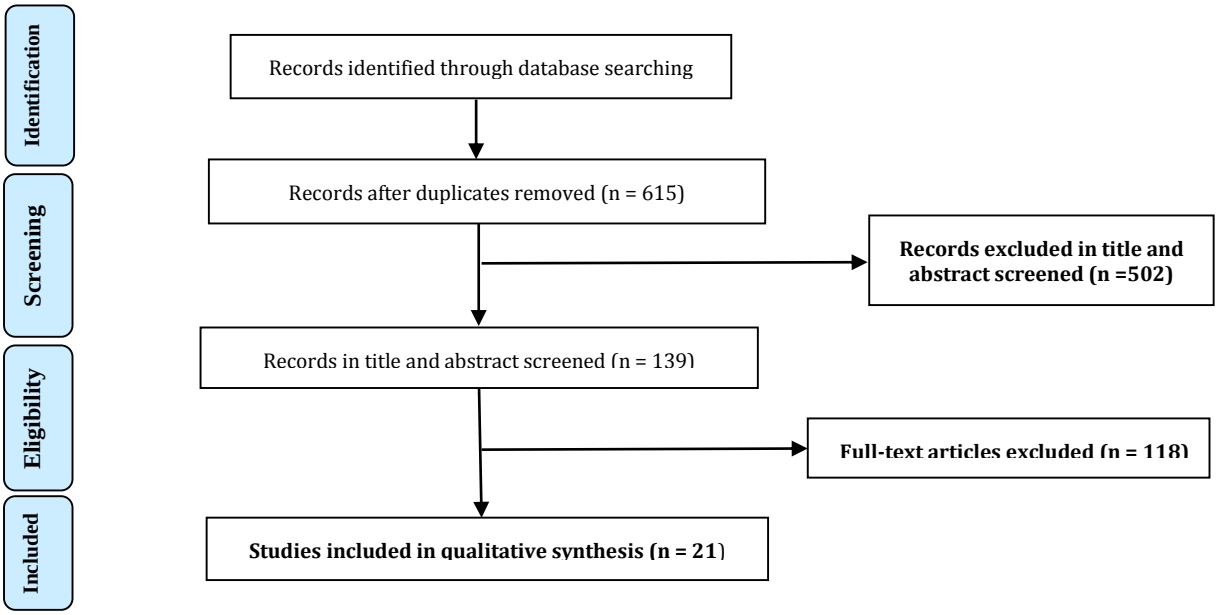

Figure 1. Flow diagram of the search and selection of studies

Results

Figure 1 depicts the PRISMA flow diagram of the article selection process in the current review. As can be seen, 975 articles and reports were identified via the database search, and 360 papers with shared records were eliminated. After investigating the remaining papers (titles and abstracts only), 139 papers were selected for the final review. The full text of these articles was studied, and 118 records were excluded. At the end of the process, 21 papers were screened in order to address the research complications. These papers were also eliminated since they were focused on other aspects of health (mental, physical, social, vulnerable groups) and management problems without reporting any data on nutritional status.

Among the selected articles, only four were related to post-earthquake nutritional consequences. In addition, nutritional problems and consequences were mentioned indirectly in 18 studies. Table 2 shows the characteristics of the reviewed articles. Three contexts were also surveyed based on the research objectives regarding food safety, food security, and nutritional consequences.

Food security is defined as food adequacy for all the people in order to live a healthy life, which is measured based on three main factors, including food availability, food access, and sustainable food supply(24). Food safety refers to the reliability of food health in the production-consumption cycle(25). In this regard, food safety surveys the risk of waterborne outbreaks and food with various causes, including inappropriate food storage in terms of temperature and maintenance, limited cooking time, and the microbial contamination of food and cookware (26).

Food Security

With regard to food security in the East Azerbaijan earthquake, 15 surveys were reviewed; six studies investigated inappropriate food and drinking water access and their incorrect distribution by the relief forces in the affected regions (27-31). Furthermore, post-incident economic problems were identified as the key contributing factor to the development of nutritional consequences in two studies. The study by Sohrabizadeh et al. (2017) revealed that the incident led to disturbances and job losses among the regional residents who were involved in agriculture and livestock farming. Consequently, a high poverty rate was reported in this affected population (32).

In another study, Fallahi et al. (2013) reported some of the factors affecting the regional people, including job losses, wasted food storages, and decreased income(33).Two other studies also reported the damage caused by the incident to the drinking water resources of the affected areas. Babaee et al. (2014) investigated the status of drinking water in the earthquake-tricken stricken regions(34), while Zeinalzadeh et al. (2017) studied health status in 95 destroyed rural regions after the earthquake (35), reporting that drinking water resources and water distribution networks underwent serious destruction in the affected regions, there by leading to disruptions in food and drinking water security.

Bahman-Janbeh et al. (2017) stated that women's involvement in regeneration and finding solutions to the post-incident problems would result in their low self-care as they would forget to follow their supplement therapy in adherence to the prescribed and delivered medical medication due to their mental occupation(36). Three studies were also focused on vulnerable populations with limited or no access to food and drinking water due to potential disability. Ahmadi et al. (2018) designed a qualitative study on18 elderly subjects(37), Sohrabizadeh et al. (2016) studied pregnant women(38), and Pakjouei et al. (2018)evaluated physically disabled subjects in this regard (39). The need assessments of vulnerable and poor population sare also recognized as the contributing factors to decreased food and drinking water access in these affected groups in the afore mentioned studies. On the other hand, Babaee et al. (2014) investigated the post-earthquake need assessment in Azerbaijan incident and the lack of accelerated need assessments, which led to the low awareness of individual nutritional requirements in the affected regions(2).

Table 2. The specification of studies mentioned to the nutritional consequences

Study	Sample size and Subjects	Study type	Post-accident nutritional dimensions	Summary results
Nader Oveisi 2014	Relief and rescue workers	qualitative	Food security	Inappropriate food access and distribution(27)
Aghaamiri, et al. (2012)	491 persons of the affected people	descriptive-analytical	Food security	Inappropriate food and drinking water access and distribution (31)
Bahman-Janbeh, et al. (2017)	Fertile Women	qualitative	Food security	Forgotten food supplements consumption due to high mind occupation and running a busy life after the accident (36)
Sohrabzadeh, et al. (2017)	11 women, 2 men recruited from the affected residents	qualitative	Food security	Disturbance of agriculture and livestock farming in the region, farmers' low-incomes, job losses, and increased poverty in the region (32)
Golzari, et al. (2012)	-	A descriptive report	Food security	Inappropriate food access and distribution(28)
Fallahi, et al. (2013)	-	A descriptive report	Food security	Job losses, wasted food resources, decreased household income(33)
Golzari, et al. (2012)	-	A descriptive report	Food security	Concerns about the contaminated water and foods in the affected regions and occurrence of diseases(57)
Mosaferi, et al. (2012)	Healthcare workers	A descriptive-qualitative study	Food security	Inappropriate food and drinking water distribution, lack of state surveillance on public support (58)
Sheghaghi, et al. (2012)	-	A descriptive report	Food security	Inappropriate distribution, decreased level of access (30)
Babaee, et al. (2014)	31 persons recruited from the affected region	qualitative	Food security	Damaged drinking water resources and reservoirs, the destructed water distribution network (34)
Pakjouei, et al., 2018	18 affected disable persons	qualitative	Food security	Decrease access to the required food and water among the vulnerable people (with movement problem) (59)
Sohrabzadeh, et al. (2016)	Pregnant women	qualitative	Food security	Decrease access to the required food and water among the vulnerable people (with movement problem) (38)
Bahman-Janbeh, et al. (2016)	Recently labored women	Descriptive-qualitative	Nutritional consequences	The increased consumption of baby formula compared to pre-accident years (43)
Dolatkhah, et al. (2013)	Consumed Meat specimens	descriptive	Food security	The consumption of contaminated meat with Yersinia enterocolitica in the affected regions (40)
Mokhtari, et al. (2016)	169 children aged 6-48 months recruited from the affected regions	Descriptive-qualitative	Nutritional consequences	The raised prevalence of slimness, low-weight, and stunting among children aged below 5 years old, the increased malnutrition prevalence(10)
Esfandyari, et al. (2018)	486 medical records belonged to women in the low-damaged region, 94 medical records belonged to the high-damaged region	Retrospective descriptive	Nutritional consequences	The decreased access to the required foods amongvulnerable people (pregnant women) in the first days, decreased BMIs in pregnant women in the affected regions, mild malnutrition (slimness and low-weight) in children aged below one years old and pregnant women, stunting (according to age) in children aged below two years old (60)
Ahmadi, et al. (2018)	An interview with 18 old subjects	qualitative	Food security	The decreased access to drinking water among the vulnerable people (the elderly), because the relief services were not provided according to the elderly's needs (37)
Kohan, et al. (2016)	Healthcare workers and the affected women	Qualitative	Nutritional consequences	The psychological stresses due to the accident with the decreased willingness to eating food, the decreased breast milk, the decreased baby weight at the birth (61)
Kusha, et al. (2012)	128 children aged below 5 years old	Descriptive-analytical	Nutritional consequences	The raised developmental disorders (developmental retardation and depression) due to nutrition(9)
Mosaferi, et al. (2013)	Healthcare workers	Descriptive-analytical	Food security	Over-distribution of mineral water drinking and their undesirable storage(62)
Zeinalzadeh, et al. (2017)	95 earthquake-affected villages	Descriptive-analytical	Food security, food safety	Damaged drinking water resources and reservoirs, the destructed water distribution network, the microbial water sampling in the affected regions due to water contamination in many villages(35)

Food Safety

Two of the reviewed articles evaluated food safety. Dolatkhah et al. (2013) assessed the consumed meat specimens by the people of the earthquake-stricken regions, reporting that 13% of the studied meat specimens were contaminated with Yersinia bacteria, which was associated with a potentially high risk of gastroenteritis and other gastrointestinal diseases in the affected residents(40). Moreover, Zeinalzadeh et al. (2017) observed that drinking water resources had microbial contamination cases even for 200 days after the incident in numerous rural places, and only 50% of the affected villages had access to healthy water. The researchers also claimed that the discrepancy of the rural sites caused major difficulties for reconstruction workers to access the water distribution systems of villages(35).

Nutritional Consequences

Five studies evaluated post-earthquake nutritional consequences, and three studies investigated incident-induced nutritional consequences in pregnant women and those with recent childbirth. Esfandyari et al. (2018) designed a retrospective study on pregnant, recently labored women, and their newborns after the earthquake(41, 42). According to the findings, low access to warm foods among the pregnant women within the first days after the earthquake, low access to fruits and milk during pregnancy, undesirable nutritional status during pregnancy, and lack of awareness regarding proper nutrition in the pregnant women led to reduced weights and BMI in the residents of the affected regions, which justified the high rates of low-birth-weight newborns and premature infants.

In another research, Bahman-Janbeh et al. (2016) investigated pregnant and recently labored women as vulnerable groups, observing that formula was consumed by the newborns with a rising trend(43). Moreover, Kohan et al. (2016) evaluated the post-earthquake consequences in pregnant women, recommending that post-incident psychological effects were responsible for the reduction of breast milk in the recently labored women. Another psychological aspect was the increased number of low-birth-weight infants(44). Additionally, Esfandyari et al. (2018) assessed the newborns and infants aged less than two years, reporting that the prevalence of malnutrition, age-related stunting, slimness, and low birth weight increased among the infants aged less than one and two years compared to the pre-incident statistics in this regard.

According to the findings of Kusha et al. (2012), the rate of developmental disorders (developmental retardation and depression) increased among children due nutritional factors after the earthquake(9). Furthermore, Mokhtari et al. (2016) proposed that the prevalence of slimness, low birth weight, stunting, and malnutrition increased among the children aged less than five years after the incident (10).

Discussion

According to the results of the present study, the consequences of this natural disaster regarding the nutritional status of the earthquake-stricken areas have been widely assessed by numerous researchers. In total, 21 reports and articles have been published in various journals, and some of these articles have discussed the nutritional consequences affecting the residents of the earthquake-stricken areas. Moreover, several studies around the world have reported the nutritional consequences after an earthquake, including the Bam earthquake. Accordingly, the most important nutritional problems of the infants affected by the Bam earthquake were the increased prevalence of low body weight, stunting, and slimness (45, 46). In addition, the nutritional problems and significant prevalence of malnutrition in vulnerable populations(e.g., infants and children) were reported to increase after the Wenchuan earthquake in China(47).

In the Haiti earthquake, the health and nutritional conditions of the affected areas were reported to be poor(48); nevertheless, timely actions prevented a nutritional disaster. Appropriate actions after natural disasters could reduce malnutrition, low body weight, and increased health indicators in children. Our findings in this regard are consistent with the results of the previous studies regarding the research subject, which demonstrated that residents of the affected areas by natural disasters experience grave nutritional consequences. Water and food security after the East Azerbaijan earthquake were reported to declined due to reduced food access, lack of self-care and proper nutrition, inappropriate food distribution, inattention to nutrition of the victims and those around, increased economic

problems (e.g., low income, job loss), and waste water and food storage.

Monitoring the food security of children after the Haitian earthquake indicated that post-disaster food insecurity was intensified in children due to the declined household income as a major influential factor in this regard (49).Self-neglect is one of the examined dimensions of food security, which has been identified in the self-care behaviors of women due to their mental occupation and co-involvement with men in seeking solutions for post-disaster regeneration, and nutritional status is considered to be largely responsible for this issues (36). After the 2008 Sichuan earthquake, the affected women paid more attention to their health, dietary status, and water and food safety(50). Therefore, it could be inferred that potentially vulnerable groups with special nutritional needs should be identified in post-disaster periods. If vulnerable people pay special attention to their food requirements, the nutritional status of the entire affected populations may be positively influenced (51).

Although different relief teams are involved in the recovery of the needs of the residents of affected areas, needs assessment sare not performed effectively, and this issue leads to poor access to essential foods, thereby further increasing food insecurity. Therefore, it is crucial to develop appropriate nutrition monitoring systems during the post-disaster phase(52). Economic issues have been established as another aspect of food security, which were investigated in the current research as well. The mentioned natural disaster in Iran occurred in the harvest season during the summer when the rural residents of the affected areas were occupied with their crop and livestock products. Consequently, the earthquake prompted the recognition of the subsequent economic problems, such as the reduced access of the regional people to various resources and demolish of food products and storage in the region. Therefore, needs assessment and plans also depend on the time of the incident and the agricultural condition of the affected areas(53).

One of the dietary issues occurring after natural disasters is food contamination with hazardous microbiological and chemical agents during food transport, maintenance, and storage, which could increase the incidences of waterborne and food borne diseases. The Great East Japan Earthquake was one of the earthquakes after which food safety was extensively investigated(54, 55). Our findings indicated that few studies have been focused on water and food safety after the East Azerbaijan earthquake, most of which have only evaluated food security. Therefore, more investigations are required about water and food safety after this earthquake. On the other hand, no incidents and challenging flooding were reported in the affected areas due to the care and control quality delivered by the healthcare workers after the incident. In addition, the efficacy of concentrated care was high in controlling food safety after this earthquake(56).

Conclusion

Evidence overview, analysis, and data collection from relevant studies indicated that poor dietary status was observed in different populations after the2012 East Azerbaijan earthquakes, especially in children and women. Although various teams and volunteers oriented their efforts toward the overall improvement of the situation for the people affected by the earthquake, food safety and security indicators were observed to decline after the incident. Therefore, EOP programs must be developed and revised with the aim of nutrition interventions in emergencies in line with designated functions in order to maintain food safety and security indicators. These issues are mostly preventable in case of natural disasters. Further studies should be conducted in this regard to select other indicators of nutritional problems by expert judgment, and the studies regarding nutritional status in emergencies should be promoted as well. By assessing the nutritional status in different regions of Iran using such designated tools, appropriate data could be obtained by health managers and decision-makers for effective policymaking and developing the required regulations to solve nutritional problems in natural disasters and emergencies.

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Effects of Acupuncture on the Glutathione System in Overweight and Obese Individuals

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ABSTRACT

Introduction: Obesity is the fifth leading cause of death worldwide. Several approaches are used for the treatment of obesity, and acupuncture has attracted attention globally in this regard. However, the therapeutic effects of acupuncture at a molecular level remain unclear. Obesity is reported to cause oxidative stress through various mechanisms. The glutathione system is one of the main antioxidant defense mechanism. The present study aimed to evaluate the effects of acupuncture on the glutathione system as the most abundant intracellular antioxidant mechanism in overweight and obese individuals.

Methods: This study was conducted on 40 obese and overweight individuals with the BMI of ≥24.9 kg/m^2 who were selected randomly. The participants received authentic acupuncture (case) or sham acupuncture (control) for six weeks combined with a low-calorie diet. Before and after the intervention, the activity of glutathione peroxidase, glutathione reductase, and reduced/oxidized glutathione levels were measured.

Results: Higher glutathione peroxidase activity was observed in both groups after the treatment, while the increase was more significant in the case group compared to the control group (P=0.005). In addition, reduced glutathione levels were observed to increase in both groups after the treatment. Similarly, the rate of increase in the case group was more significant than the control group (P=0.02). A significant increase was also denoted in the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio in the case group compared to the control group (P=0.02).

Conclusion: According to the results, the combination of acupuncture with a standard obesity diet could increase antioxidant activity in the overweight individuals. Furthermore, the approach could further prevent oxidative damage through increasing reduced glutathione and improving the GSH/GSSG ratio.

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Introduction

Obesity is a major health issue in the modern era, which is associated with numerous diseases, including cancer, asthma, renal dysfunction, infertility, hepatic dysfunction, sleep disorders, diabetes, and cardiovascular diseases (1, 2). Body mass index (BMI) is an internationally accepted criterion for assessing the severity of obesity. According to the World Health Organization (WHO), BMI of 25-29.9 kg/m^2 is defined as overweight, and BMI of ≥30 kg/m^2 is defined as obese (11, 15). In 2016, more than 1.9 billion adults were overweight worldwide, 650 million of which were obese. In Iran, the prevalence of obesity in the population aged more than 18 years has been reported to be 21.7% (3). In addition, models predict the prevalence of obesity to reach 33% by 2030 (4). Several therapeutic approaches are applied clinically for obesity management, including lifestyle modification/diets, pharmacotherapy, surgery, and complementary medicine. Recently, complementary therapies have been increasingly used as a promising approach to obesity

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management, and acupuncture has attracted special attention in this regard (5, 6).

Oxidative stress refers to the imbalance between oxidants and antioxidants. Physiologically, several factors are involved in maintaining the balance between produced reactive oxygen species (ROS) and antioxidant defense components. Oxidative stress occurs in obesity and contributes to outcomes such as metabolic syndrome (7). The biochemical mechanisms that cause oxidative stress in obesity include free fatty acid accumulation in plasma (8, 9), oxidative phosphorylation, hyperglycemia, and high-fat diets (7, 10-13). In addition, the increased consumption of cellular oxygen due to mitochondrial dysfunction in obesity may be associated with increased ROS production (14).

According to the literature, acupuncture combined with dietary regimen could decrease the serum pro-oxidant/antioxidant balance (PAB) values in overweight and obese individuals (15). Glutathione is the most abundant antioxidant within cells (16), and the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio is considered to be the main determinant of oxidative stress (17).

The present study aimed to investigate the effects of acupuncture on the glutathione system in obesity, as well as the concentration of glutathione-dependent enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), and blood glutathione (reduced: GSH, oxidized: GSSG) as well as GSH/GSSG ratio.

Materials and Methods

Study Design

This study was performed on the remaining disposable serum samples of a recorded randomized clinical trial (RCT code: IRCT201706107265N9), which was conducted on participants with the BMI of ≥24.9 kg/m^2 (unpublished under review data). Briefly, the participants of the mentioned trial were selected from the obese and overweight volunteers referring to an acupuncture clinic affiliated to Mashhad University of Medical Sciences.

The present study was conducted on a case group, which received acupuncture with a standard diet treatment as prescribed by a nutritionist (n=20), and a control group, which received sham acupuncture with the same standard regimen (n=20). In the original RCT, all the patients received this treatment and had restricted physical activity, the data of which was recorded and monitored weekly during the study period.

The case and control groups in our study were age-, gender- and BMI-matched. The patients, statistical analyst, and treatment team (except the acupuncturist) were blinded to the research procedure. Serum samples were collected before and six weeks after the intervention. The sera were kept frozen until the measurement assays.

Ethical Considerations

This study was performed on the remaining disposable serum samples of a recorded randomized clinical trial (RCT code: IRCT201706107265N9). The study aimed to assess completely distinct factors associated with oxidative stress. The study protocol on the remaining sera was reviewed and approved by the Ethics Committee of Mashhad University of Medical Sciences (ethics code: IR.MUMS.fm.REC.1397.133). In addition, written informed consent was obtained from the subjects prior to enrollment. All the procedures were in accordance with the human research ethics of the Declaration of Helsinki.

Electroacupuncture and Sham Electroacupuncture

The electroacupuncture process has been explained in detail in the original RCT. Accordingly, all the subjects participated in 18 sessions of acupuncture therapy based on standard procedures for six weeks. In the intervention, six bilaterally acupuncture points were defined on the abdomen, including ST21, ST25, SP15, CV4, CV6, and CV12 as well as three bilateral points on the lower extremities including ST44, ST37, and LR3. In the case group, the abdominal acupoints were at the depth of 15-30 millimeters, and the needles in the lower extremities were punctured bilaterally until the participant stated the sensation of de-qi (soreness, numbness, distention or heaviness). Following that, the inserted abdominal needles were connected to three electrode pairs. The electroacupuncture process was performed with sterile, disposable needles (0.25x40 mm; Suzhou, China) using the Great Wall multi-purpose health electroacupuncture device (KWD 808, China) at the frequency of 2 Hz and intensity of 1-5 mA for 30 minutes.

To implement the sham acupuncture approach in the control group, sterile, disposable needles (0.25x25 mm; Suzhou, China) were used. The

needles were inserted through the skin to the defined depths considering the de-qi sensation. In this protocol, no electrical current was applied. The acupuncture process was performed by an experienced acupuncturist in accordance with the revised STRICTA guidelines (18).

Measurement of GSH/GSSG Ratio, GPX, and GR

In this study, the kits used for determining the antioxidant markers were purchased from the ZellBio GmbH Company (Germany).

In reaction with 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), GSH generated 2-nitro 5-thiobenzoic acid, which was yellow. The absorbance of the yellow product was measured at 412 nanometers, and the GSH concentration was calculated based on the kit manual. The difference between total glutathione and GSH indicated the amount of GSSG.

GR activity was assessed using NADPH as the substrate. The decreased absorbance at 340 nanometers indicated NADPH oxidation, which was proportional to the reduction of GSSG to GSH. GR activity was calculated in units per liter (U/L). GPX activity was determined on the basis of a colorimetric assay at 412 nanometers. GPX has a selenocysteine at its active site, which directly contributed to the reduction of the peroxide substrate. The enzyme also regenerated the reduced form of selenocysteine by adding excessive GSH as the ultimate reductant. Afterwards, GSH was converted into GSSG by the GPX enzyme, and the remaining GSH reacted with DTNB to produce a yellow product, which was measured calorimetrically at 412 nanometers. The production of the yellow color indirectly indicated the activity of GPX.

$$2\ GSH + H_2O_2 \longrightarrow GS\text{–}SG + 2\ H_2O$$

Statistical Analysis

Data analysis was performed in SPSS version 22 (Chicago, IBM, USA). Quantitative data were assessed in terms of normal distribution using the Kolmogorov-Smirnov test. Wilcoxon test was applied to compare the quantitative variables in each group before and after the intervention. Moreover, the Mann-Whitney test was used to compare the variables between the case and control groups. In all the statistical analyses, the P-value of less than 0.05 was considered significant.

Table 1. Demographic and clinical characteristics of participants.

Group	N	Gender		Age (year)	BMI (kg/m^2)
		Male	Female	(mean ± standard error)	(mean ± standard error)
Case	20	5	15	38.75 ± 9.7	32.6 ± 3.6
Control	20	1	19	38.33 ± 11.6	32.2 ± 4.2
p-value			0.06	0.9	0.7

BMI= body mass index

Table 2. Oxidative stress markers before and after 6 weeks of intervention in two groups as case (treatment) and control (sham group).

	Case (N=20) Median (IQR)	Control (N=20) Median (IQR)	Mann Whitney test
GR (U/ml)			
Before	56.2 (24-96.1)	88.4 (72.3-140.6)	$p = 0.02$
After	112.5 (50.2-136.6)	96.4 (72.3-120.5)	$p = 0.5$
Wilcoxon test	$p = 0.3$	$p = 0.7$	
GPX (U/ml)			
Before	160 (115-267)	-8.9 ((-160)-240)	$p = 0.6$
After	329 (189-509)	485 (262-770)	$p = 0.1$
Wilcoxon test	$p = 0.001$	$p = 0.13$	
GSH (nmol/L)			
Before	0.14 (0.13-0.16)	0.15 (0.14-0.17)	$p = 0.3$
After	0.18 (0.17-0.19)	0.17 (0.16-0.18)	$p = 0.1$
Wilcoxon test	$p = 0.001$	$p < 0.001$	
GSSG (nmol/L)			
Before	0.58 (0.55-0.65)	0.58 (0.56-0.62)	$p = 0.9$
After	0.59 (0.57-0.63)	0.59 (0.57-0.65)	$p = 0.7$
Wilcoxon test	$p = 0.62$	$p = 0.2$	
GSH/GSSG (ratio)			
Before	0.24 (0.2-0.27)	0.26 (0.24-0.28)	$p = 0.2$
After	0.29 (0.27-0.31)	0.28 (0.26-0.3)	$p = 0.4$
Wilcoxon test	$p = 0.001$	$p = 0.004$	

Results

In total, 40 overweight and obese subjects were enrolled in the study. The characteristics of the participants are presented in Table 1. According to the information in Table 2, GR activity and GSSG concentration had no significant differences before and after the intervention in the case group. However, GPX activity and GSH concentration increased significantly after the intervention. In the control group (sham acupuncture), a significant increase was observed in GSH concentration (P<0.001).

According to the obtained results, the GSH/GSSG ratio had a significant difference during the course of the treatment (P=0.02) and increased in both groups after the treatment, which was considered more significant in the case group compared to the control group (P=0.001). After six weeks of treatment, the difference in GPX before and after the intervention (Δ GPX) was more significant in the control group compared to the case group (P=0.005). Furthermore, GSH concentration increased after the treatment in both groups, which was more significant in the case group compared to the control group (P=0.001). No significant differences were observed in the other variables before and after the treatment between the case and control groups.

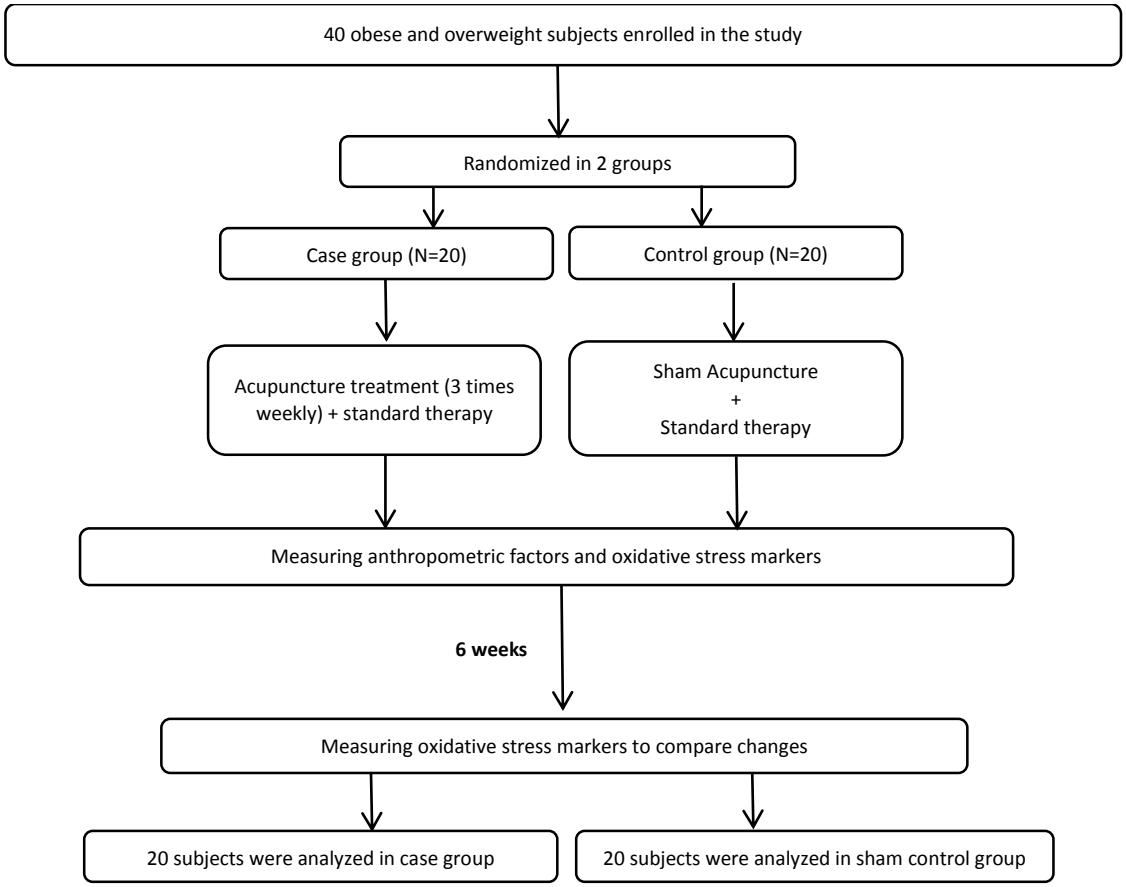

Figure 1. Flow diagram of the study selection procedure.

Discussion

Data are scarce regarding the mechanism of action of acupuncture against oxidative stress, especially in humans. Animal studies have proposed contradictory results in this regard, and the subject remains controversial. The present study aimed to investigate the possible changes in oxidative stress induced by acupuncture. To the best of our knowledge, this

was the first English report on the human glutathione system.

According to our findings, acupuncture increased GPX enzyme activity and GSH concentration in the obese and overweight subjects, which is consistent with the previous studies, indicating that acupuncture could reduce the PAB values in overweight and obese individuals (19). However, previous studies have evaluated the balance in general, while we extended the study to explore the glutathione system as a major component of this balance.

Several animal studies have demonstrated the increased activity of other antioxidant enzymes, such as GPX, catalase (CAT), and super oxide dismutase (SOD) through the acupuncture procedure (20-24). To justify, it could be stated that acupuncture signals are transferred to the central nervous system (CNS) and alter the antioxidant defense system. The effects of psychological conditions or CNS signals on oxidative stress have been previously elucidated. For instance, Jinata et al. evaluated the role of acupuncture stimulation in the regulation of oxidative stress in cerebral ischemic rats, concluding that acupuncture could significantly decrease malondialdehyde (MDA), while elevating CAT, GPX, and SOD activity in the animal model (20).

In another research, Zhang et al. examined rats with multi-infarct dementia, reporting that acupuncture could regulate the GSH/GSSG ratio, increase the activity of SOD, and decrease serum MDA, superoxide anion, and subsequently oxidative stress in the mitochondria. The authors of mentioned study attributed these effects to increased glucose and oxygen sources following the improvement of the cerebral blood flow (24). Similarly, Liu et al. observed that acupuncture could enhance antioxidant effects by increasing the activity of SOD and GPX in the hippocampus of rats with multi-infarction, thereby maintaining the oxidant-antioxidant balance properly (21).

Other studies have proposed inconsistent results in this regard. The findings of Wang et al. indicated that acupuncture exerted antioxidant effects on a mouse model of Parkinson's disease by increasing glutathione concentration and SOD activity, while GPX activity was observed to decrease, which is inconsistent with the results of the present study (25). The discrepancy could be due to the difference in the study conditions (animal/human) and various interfering factors. As such, summative data are required to reach a definite conclusion. Due to increasing application of acupuncture, it seems that more human studies must be performed in this regard in order to bridge the knowledge gap.

Some of the limitations of the present study were the small sample size and remaining confounders (e.g., socioeconomic data). This is one of the few reports to explore the effects of acupuncture on oxidative stress markers in humans. It is recommended that studies on other acupuncture sites, in addition of vast list of other molecules that are involved in the oxidative stress network, be performed to obtain more accurate results regarding the association of acupuncture and oxidative stress.

Conclusion

According to the results, GPX activity significantly increased in the case group, which suggested that acupuncture may enhance the antioxidant defense system. Furthermore, acupuncture could regulate the GSH/GSSG ratio by increasing GSH concentration. Therefore, it seems that acupuncture is a viable option for the complementary treatment of obesity and overweight, while it could also decrease oxidative damage in these individuals.

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The Effect for Eight Weeks of Resistance Training with Royal Jelly Consumption on Anxiety and Depression in A Rat Model for Alzheimer's Disease

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ABSTRACT

Introduction: Today, due to the prevalence and spread of Alzheimer's disease (AD) and negative impact on life and health, effective methods have been considered for treating the disease. This study aimed to investigate the effect of eight weeks for resistance training (RT) with royal jelly (RJ) on anxiety and depression in rats with AD.

Methods: In the experimental study, 56 male Sprague-Dawley rats with AD (induced by 8 mg/kg Trimethyltin chloride) divided into seven groups of eight rats including (1) Alzheimer's control (AD), (2) sham (Sh), (3) RT, (4) RT+ 100 mg/kg RJ (RT+RJ100), (5) RT+ 200 mg/RJ (RT+RJ200), (6) RJ100, and (7) RJ200. Eight rats were assigned into the healthy control (HC) group to investigate the effect of AD induction on research variables. The groups of 3, 4 and 5, performed RT with an intensity of 30 to 100% of body weight three sessions per week for eight weeks, and the groups of 4-7 received the selected doses of RJ peritoneally daily. Anxiety-like behaviors and depression were measured by the elevated plus-maze test and forced swim test respectively. To analyze the findings, one-way ANOVA was used with Tukey's *post- hoc* test, and two-way ANOVA with Bonferroni's *post- hoc* test ($p \leq 0.05$).

Results: Eight weeks of RT ($p \leq 0.05$) and RJ ($p \leq 0.05$) could significantly reduce anxiety and depression in rats with AD. 200 mg/kg RJ had a more favorable effect on reducing anxiety ($p \leq 0.05$) and depression ($p \leq 0.05$) than 100 mg/kg RJ. In addition, RT and RJ had an interactive effect on reducing depression in rats with AD ($p \leq 0.05$).

Conclusion: Apparently, the RT and RJ consumption have interactive effects on reducing anxiety; Besides, the anti-anxiety and anti-depression effects of RJ can be dependent on dosage.

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Introduction

The human brain is a collection and organ to store, recall, and process all the necessary information. Its destruction by Alzheimer's disease (AD) is very similar to a computer memory, which has lost its information (1,2). The damage process in AD begins in the hippocampus of the brain (3). The hippocampus, the part of the brain that is responsible for storing information about short-term memory, plays an important role in learning. By interfering with other parts of the brain, AD usually begins in the area, it also interferes with speaking, reading, calculating, making decisions, and coordinating body movements (3). As the levels of serotonin in the brain in AD patients decreased, it leads to forgetfulness, learning disabilities, and aging (4). Most prescribed drugs in each part of the world are drugs to change the level of serotonin. These drugs are used in depression, general anxiety disorder, and social phobia (2).

Exercise is proved to have beneficial effects on physical and mental health as it reduces the prevalence of diseases, promotes neuroprotection, neuroplasticity, increases cognitive function, and has anti-anxiety and anti-depressant properties (3). Moreover, Exercise reduces the symptoms of anxiety and depression and improves mood and the feeling of well-being. positive effects on fatigue and anger could be gained by only one session of aerobic and resistance training (RT) (5). The researchers mentioned that regular aerobic physical activity is a good tool to prevent or treat many diseases, reduce the baseline risk factors for stroke and heart attack, prevent neurological disorders such as anxiety, depression, degenerative and weakening diseases of the central nervous system including Parkinson's, AD and improving

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brain function (6). Rashidi *et al.,* (2017) compared the effect of aerobic and anaerobic exercise on students' depression and anxiety. In the end, the results showed that both aerobic or anaerobic exercises have a significant effect on reducing of depression and anxiety (7). Exercise causes brain cells to proliferate, especially in the hippocampus. The structure (hippocampus) is involved in the transfer of information from short-term to long-term memory. Choosing an effective and appropriate exercise for the treatment and prevention of depression, AD and other diseases for the nervous system is vital (8). Besides, royal jelly (RJ), or its compounds have been reported to facilitate neurogenesis in the hippocampus and to differentiate different types of brain cells such as neurons and neuroglial cells from precursor stem cells in the area of the dentate gyrus (9). One of the compounds that have been proven to have protective and anti-apoptotic effects on nerve cells is RJ. RJ is a light yellowish-white gelatinous substance, colloidal, and sticky with a special odor and a bitter taste (10). This jelly is a viscous substance which have been produced by young worker bees in the genus Apis mellifera. It helps the hive larvae to be fed to become queens (11). In recent decades, several researches investigated the role of physical activity in reducing anxiety and stress and indicate the importance and benefits of physical activity on brain function (12). Considering the importance of the role of exercise in AD and the uncertain effects of RJ on improving the symptoms of this disease, the current study was conducted to investigate the effect of eight weeks of RT with RJ consumption on anxiety and depression in rats with AD.

Methods

In this experimental study, 64 Sprague-Dawley rats were purchased from the animal lab in Marvdasht branch of Islamic Azad University and as for the adaptation to a new environment, all rats kept in sport physiology lab of the noted university for one week in the standard situation (22- 24 C temperature, 12 hours light and darkness cycle and 55- 60 percent relative humidity) with free access to food and water. In all of the research periods, ethical considerations were according to guidelines of the animal ethics committee. Then 56 rats were induced with AD with 8 mg/kg Trimethyltin chloride (TMT) (Sigma- Aldrich, MERK Company, CAS Number: 1065-45-1). After two weeks, to diagnose AD disease, in addition to physical examination such as aggression, bleeding around the eyes, tail twisting, the memory, and learning tests were performed using a shuttle box device (13). WhenAD induction was confirmed, rats were randomly divided into seven groups of eight rats including (1) Alzheimer's control (AD), (2) sham (Sh), (3) RT, (4) RT+ 100 mg/kg RJ (RT+RJ100), (5) RT+ 200 mg/RJ (RT+RJ200), (6) RJ100, and (7) RJ200. It is noteworthy that to investigate the effects of AD induction on the research variables, 8 rats were assigned to the healthy control group (HC). Groups of 3, 4 and 5, performed RT with an intensity of 30 to 100% of body weight three sessions per week for eight weeks (14), and the groups of 4-7 received 100 and 200 mg/kg RJ dissolved in physiological saline peritoneally for eight weeks (12).

Resistance training protocol

The training in each session consisted of four sets (first set 50%, second set 75%, third set 90%, and fourth set 100% weight set for that week) and two repetitions (climbing stairs twice). The interval between each set was 2 to 3 minutes and the interval between each repetition was 40 to 60 seconds (14).

Measurement of variables

A forced swimming test was used to measure depression. The forced swimming test is among most valid and common tests for rodent depression. The time of this test is 5 minutes and the behavior of rats is recorded during the period. Conventionally, the cessation of the movement for the rat's limbs and its floating is considered as immobility and its duration is considered as immobility time. To gain the experience of forced swimming, , the animal is placed in water for 15 minutes in twenty- four hours before the test (15).

Elevated plus-maze was used to measure behavioral model anxiety. The evaluation was based on a model first proposed by Ploo *et al.,* The number of times the animal moved freely in different parts of the plus-maze during 5 minutes; the number of times the animal entered different parts of the open arm; the number of times the animal entered the closed arm; the length of time the animal stayed in the open arm; and finally, the length of time the animal remained in the closed arm of the plus-maze were measured by video recording. Only when all four legs of the animal were in the arm meant

they enter an open or closed arm. The time spent in each arm was calculated accordingly. For each animal, the percentage of entry into the open arm and the percentage of time spent in the open arm was calculated (3,16).

Data analysis procedure

To evaluate the normality in data distribution, the Shapiro-Wilk test was used, and to evaluate the effect of TMT on the research variables, one-way ANOVA with Tukey's *post- hoc* tests were used. Also, to evaluate the effect of RJ supplement and RT on the research variables two-way ANOVA with Bonferroni's *post- hoc* tests in SPSS 22 were used ($p \leq 0.05$).

Findings

Table 1 presents the mean and standard deviation for anxiety-like behaviors and depression in rats in the study groups.

Table 1. The mean and standard deviation of research variables in rats in the research groups

Group	Percentage of the number of open arm entries	Percentage of time spent in the open arm	Movement time (seconds)
HC	41.40±3.34	30.43±2.72	152.85±18.89
AD	21.37±6.94	15.40±4.67	37.79±6.17
Sham	30.53±4.44	13.05±2.17	44.00±7.37
RT	38.14±3.08	23.19±2.41	79.02±5.81
RT+RJ100	38.14±3.08	27.29±3.26	109.28±9.67
RT+RJ200	41.91±5.77	29.10±2.64	144.00±10.45
RJ100	29.00±2.88	19.44±1.35	80.54±8.57
RJ200	29.78±2.76	20.45±1.28	100.42±8.57

The findings of the present study showed that AD induction significantly increased anxiety-like behaviors and depression in rats (P= 0.001). Eight weeks of RT significantly reduced anxiety-like behaviors (P = 0.001) and depression (P = 0.001) in rats with AD. Eight weeks of RJ consumption significantly reduced anxiety-like behaviors (P = 0.001) and depression (P = 0.001) in rats with AD.

Consumption of 200 mg / kg RJ had a more favorable effect on reducing anxiety (P = 0.001) and depression (P = 0.001) than 100 mg/kg RJ. RT and RJ consumption had significant interactive effects in reducing depression in rats with AD (P = 0.007). RT and RJ consumption did not have significant interactive effects in reducing anxiety-like behaviors in rats with AD (P = 0.61).

Discussion

The obtained results in the current study showed that AD induction significantly increased anxiety and depression in rats. AD is the leading cause of dementia and characterized by progressive loss of memory and other cognitive functions. Evidently oxidative stress plays a significant role in depression and anxiety disorders in AD patients. Anxiety is one of the behavioral symptoms of AD (17). In depression and anxiety, the individual's neurotransmitters of the brain are reduced, which the individuals feel depressed and lonely due to the decline of their physical and mental abilities, (13). The use of TMT in laboratory animals is known to induce severe and selective neuronal death associated with microglial and astroglial activity, selectively in the limbic region and especially in the hippocampus; as a result, it provides a valuable tool for studying the function of the hippocampus during neurodegenerative events (18).

The results showed that eight weeks of RT and RJ consumption significantly decreased anxiety-like behaviors and depression in rats with AD. The previous studies show that regular exercise balanced sympathetic nerve activity; in other words, modulates the hypothalamic-pituitary-adrenal (APH) axial response (19). It is observed that physical activity can exert its effects to improve depression and anxiety by affecting the release of beta-endorphins and monoamines, reducing cortisol levels, and increasing self-efficacy in the individual (20). Researches have shown that exercise for 9 weeks and three 20-minute sessions per week reduces depression and increases adaptation mechanisms (21). Besides, it has been observed that the activity of the hypothalamic-pituitary-adrenal axis decreases in some patients with depression, and therefore , improving the function of the hypothalamic-pituitary-adrenal axis as a result of physical activity also plays a useful role in improving depression (19).

It seems that balance levels in serotonin and norepinephrine during exercise can reduce depression and moderate anxiety. In other

words, exercise can affect the human spirit in two ways, which are increasing the release of endorphins and reducing the levels of cortisol that are released into the blood due to stress (22). Consistent with the present study, researchers reported that 10 weeks, three sessions per week, and each session of 60 to 90 minutes of incremental exercise had a significant effect on reducing stress, anxiety, depression, and improving systolic blood pressure in kidney transplant patients (22); 12 weeks, three sessions per week of exercise in 5 sets reduced anxiety and depression for the patients with metabolic syndrome (23); eight weeks, three sessions per week and 60 minutes of water exercise per session had a significant effect on reducing anxiety and stress in older men (16). As could be seen on the literature review, most of the studies were in line with the present study, so it seems that a variety of long-term sports activities with different intensities reduce anxiety.

RJ, as the main food of the queen, is known as the richest biological nutrient and seems that have been used in traditional medicine to treat many diseases(24,25). The results of several studies show that RJ significantly enhanced the memory and learning of AD patients (25). The substance is absorbed into the blood after consumption and reaches the gray cells of the brain (26). RJ neutralizes free radicals by interrupting the oxidation chain reaction (27), which can prevent the destruction of nerve cells from this pathway. 10 days Consumption of food containing RJ (3% by weight) significantly improved memory and learning indices in the diabetic rats (28). It seems that differences in the amount of RJ consumption can have different effects on cognitive function and the central nervous system, as the results of this study are more favorable in a significant reduction in depression at a dose of 200 mg compared to a dose of 100 mg RJ.

Conclusion

It seems that RT and RJ consumption can reduce depression and anxiety in rats with AD. Therefore, it is suggested to use the RT and RJ in the diet plan to reduce the harmful effects of AD such as anxiety and depression.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Effects of Ramadan Fasting on the Resilience and Psychological Hardiness of Students

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ABSTRACT

Introduction: Psychological hardiness and resilience are new psychological factors that have attracted the attention of psychologists in various fields. These factors are speculated to moderate stressors and their adverse effects on life. The present study aimed to investigate the effects of Ramadan fasting on the resilience and psychological hardiness of students.

Methods: This study was conducted on 300 students of the Islamic Azad University, Khoy Branch in Khoy, Iran in 2019. The sample population included 150 female and 150 male students who were selected via convenience sampling. One week before the holy month of Ramadan (pretest), data were collected using Connor-Davidson resilience questionnaire and Ahvaz psychological hardiness questionnaire, and posttest was performed one week after Ramadan. Data analysis was performed using one-way analysis of covariance.

Results: Significant differences were observed in the posttest scores of resilience and psychological hardiness. The effect size of fasting on the posttest scores of resilience and psychological hardiness was 0.73 and 0.78, respectively (P=0.001).

Conclusion: Practicing religious beliefs largely influences psychological issues and enhances mental health. The individuals who are more inclined to spirituality are less prone to mental disorders and abnormalities. According to the results, religious rituals (especially Ramadan fasting) could improve resilience and psychological hardiness.

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Introduction

In the holy month of Ramadan, Muslims fast and abstain from immoral actions and attempt for soul purification. As the most important religious month in the Islamic calendar, Ramadan is associated with special features, customs, traditions, and duties. A large collection of religious rituals are performed during Ramadan, which are considered to be the longest in terms of time and the most extensive rituals in Islam.

Ramadan fasting necessitates extensive changes in individual and social life, the most important of which are altered dietary habits, resting time, sleep patterns, and leisure time, as well as the changes in work hours, commuting, business, and social interactions. According to the literature, Ramadan fasting has numerous health benefits. In the Islamic viewpoint, fasting is a spiritual practice and an effort to promote mental and physical health (1).

In the past two decades, the use of religion in psychotherapy has attracted the attention of researchers. Religious studies in psychotherapy across the world are considered fundamental, and a growing number of scholars are inclined toward these matters in many countries. Religion could be effectively applied to promote social support, adaptation, and compliance in matters of health and wellness (2).

Psychological hardiness and resilience are new psychological factors, which have attracted the attention of psychologists in various fields. These factors are speculated to moderate stressors and their adverse effects on life. Humans differ in terms of inner strength, flexibility, and tolerance for hardship (1).

Resilience is defined as the process, ability or consequence of successful adaptation to a threatening situation. In other words, positive adaptation in response to adverse life conditions and self-healing with positive emotional, cognitive, and cognitive consequences form the concept of resilience (3-6). Resilience also refers to the ability of an individual to establish psychological and biological balance in perilous

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conditions and foster successful adaptation, which is manifested in the face of stress and debilitating power. The best definition of resilience is successful adaptation to adverse conditions (7-9).

The term 'hardiness' was first used by Kubasa to refer to a set of personality traits that acted as a protective shield in the face of stressful life events. Accordingly, the individuals with this trait are those who can effectively cope with the challenges and pressures of life (10). In addition, Kubasa (1983) defines psychological toughness as a set of beliefs about oneself and the world in terms of the three components of commitment, control, and struggle. This concept is also a single structure that is related to the integrated and coordinated action of these components originating together (11).

According to the literature, stubbornness is positively correlated with physical and mental health and, as a source of internal resistance, reduces the adverse effects of stress and prevents physical and mental disorders (10-14). Resilience could act as a shield against disaster and the debilitative effects of exposure to risk factors. A resilient individual processes an unfortunate situation in a more positive manner and considers themselves capable of coping with the events (15).

The studies regarding human resilience are particularly focused on the recognition of the individual differences in relation to adverse experiences (16). For instance, Reef and Singer believe that resilient individuals are generally able to maintain their mental and physical health and recover easily from stressful life events (17). Therefore, it could be concluded that resilience improves and promotes psychological growth in negative situations. Quoting Kubasa et al., Funk and Houston have stated that toughness is a single structure that encompasses cognitive, emotional, and practical components. Toughness also plays a pivotal role in maintaining the survival and continuity of individual generation and is essential to being productive and achieving growth and excellence (18).

Psychological stubbornness acts as a shield against stress in various life situations (19, 20) and reduces the risk of stress-related physical illness, mental disorders, and behavioral and functional impairments (15). Furthermore, stubbornness could significantly predict the psychological wellbeing and success of individuals in occupational choices (21, 22).

Given the effects of religious rites on mental health and the importance of Ramadan to Muslims and Iranians, the present study aimed to investigate the effects of Ramadan fasting on a new population and determine whether Ramadan fasting could be effective in the improvement of resilience and psychological hardiness in students.

Materials and Methods

This study was conducted on the students of the Islamic Azad University of Khoy Branch, Iran. In total, 300 students (150 males and 150 females) were selected as the sample population using Morgan's table. The participants completed three questionnaires in the pretest stage one week before Ramadan in 2019. Notably, six students were excluded from the study. After 45 days and one week after Ramadan, 294 students had completed the questionnaires at the posttest stage. Informed consent was obtained from the students for enrollment in the study.

Research Tools

Resilience Questionnaire (Connor and Davidson, 2003)

This tool has 25 items that are scored based on a six-point Likert scale (Completely Incorrect=0, Always Correct=5). The score range of the scale is 0-100, and the minimum and maximum scores are 25 and 125, respectively; the higher scores indicate more resilience in the subject. The instrument is divided into five dimensions, including the perception of individual competence, trust in individual instincts/negative emotion tolerance, positive acceptance of change and safe relationships, control, and spiritual effects. Connor and Davidson have reported the Cronbach's alpha coefficient of 0.89 for the resilience scale. In addition, the reliability coefficient obtained from the retest method with a four-week interval in the present study was estimated at 0.87. The scale has been standardized for the Iranian population, and its reliability coefficient has been estimated at the Cronbach's alpha of 0.89.

Ahvaz Hardiness Questionnaire

This instrument has 27 items, and each item has four options of never, rarely, sometimes, and most of the time, which are scored within the range of 1-4, respectively. Notably, phrases six,

seven, 10, 13, 17, and 21 in this instrument have a negative factor load and are scored in a reverse manner. The score range of this tool is 0-81, with the higher scores indicating high psychological toughness. The reliability coefficients of the questionnaire have been determined by retest at the Cronbach's alpha of 0.84 and 0.76.

Researcher-made Questionnaire

This questionnaire consists of two sections; the first section covers demographic characteristics such as age, gender, and education level, and the second section has one question regarding the amount of the religious duties performed by the respondent during Ramadan. This question is answered by two phrases (I fasted on all/most days of Ramadan; group I, I did not fast at all during Ramadan; group II). In the present study, this scale was completed by the subjects at the posttest and after the completion of the resilience and psychological hardiness instruments.

The subjects completed the resilience and psychological hardiness questionnaires one week before and one week after Ramadan. Data analysis was performed using one-way analysis of covariance (ANCOVA).

Results

The mean age of the male and female participants was 22.88 and 21.71 years, respectively. In order to investigate the effect size of Ramadan fasting on resilience and psychological hardiness, the subjects completed two questionnaires one week before (pretest) and one week after (posttest) Ramadan. The results of ANCOVA indicated significant differences between the pretest and posttest scores of the resilience and psychological hardiness of the subjects (P=0.05).

Table 1. Mean and standard deviation of two variables in pre-test and post-test of groups 1 and 2

Variable		Stage			
		Pre-test		Post-test	
		Group 1 M±SD	Group 2 M±SD	Group 1 M±SD	Group 2 M±SD
Subscales of Resilience	Competence/ Personal strength	19.42±2.75	20.21±2.94	33.67±3.65	20.52±2.71
	Trust in personal instincts/ Tolerating negative emotions	18.27±2.87	19.54±2.94	29.76±3.71	20.21±3.10
	Accepting positive emotions/ Safe emotions	9.74±1.46	9.53±1.38	15.65±2.07	9.44±1.19
	Inhibition	6.48±0.92	6.88±0.81	11.47±1.39	6.75±0.76
	Spirituality	4.35±0.83	4.52±0.73	7.83±1.32	4.61±0.95
Resilience		58.26±7.15	60.68±7.62	98.38±8.84	61.53±7.15
Psychological Hardiness		52.68±6.74	53.56±6.43	65.83±7.61	53.89±6.45

Table 2. Results of post-test (ANCOVA) of groups

Source of change	Sum of Squres (SS)	Mean of Squres (MS)	Fisher's F-test (1,291)	P-value	Partial π^2 (PES)
Resilience	64.39	64.39	17.53	0.001	0.46
Group	357.48	357.48	193.61	0.001	0.73
Error	52.74	0.42			
Psychological Hardiness	147.28	147.28	24.38	0.001	0.51
Group	628.57	628.57	241.82	0.001	0.78
Error	118.53	0.84			

(*P=0.05)

The posttest results of ANCOVA regarding the scores of resilience and psychological hardiness showed that the posttest scores significantly differed between the groups. In addition, the results of posttest ANCOVA regarding resilience indicated the effect size of 0.73 (π^2=0.73; P=0.001); in other words, 73% of the difference in the posttest scores of resilience could be attributed to the effect of fasting. As for psychological hardiness, the obtained results showed the effect size of 0.78 (π^2=0.78; P=0.001); similarly, 78% of the difference in the psychological hardiness posttest scores was attributed to the effect of fasting.

Discussion

Our findings indicated that the total scores of resilience and psychological hardiness were higher in the fasting group compared to the non-fasting group. Several studies have confirmed

that religious rites could act as a functional resource through creating a support system for individuals (23); some of these studies have been performed by Fajin and Pragment (24), Jamshidi Solklo (25), Garmzi and Mastan (3), Rabiee Khojin et al. (26), Karimi (27), and Khazaei et al. (28).

To date, several studies have evaluated the correlation of resilience and psychological hardiness with religiosity, such as the studies by Dehghani et al. (29), Hamid et al. (30), and Yasemi Nejad et al. (31). Our findings in this regard are consistent with the previous studies. Imanifar et al. argues that religion is a source of support for individuals in the face of adversity through prayer, trust, and recourse to God (32).

Stubborn individuals hope for successful and efficient coping with tensions despite adverse events, while they are also able to find meaning in disturbing experiences and believe in their role as a valuable and important person. A strong personality (commitment, restraint, and struggle) requires mental health, and those with mental imbalance experience despair and depression in the face of negative life situations. Therefore, it could be inferred that the variables of stubbornness and hope are complementary (33); unless man is hopeful, he cannot create positive or negative life situations that require adaptation. He merely considers negative events as a threat to his security and comfort, exhausts his ability to endure vague and uncomfortable situations in life, and disrupts the normal routine of life. An individual with a hopeful and tenacious personality believes that experiences and events could be predicted and controlled, and flexibility helps them adapt and evaluate even the most uncoordinated events (32).

Religious activities directly and indirectly predict hardiness and resilience (34). Performing religious duties could explain resilience and stubbornness without intermediaries, which may be due to the fact that religion encompasses various cognitive, value-based, and behavioral elements to create resilience and resilience (34). These cognitive patterns mainly serve as a supportive factor, as well as an organizer in the face of crisis.

All the major religions of the world offer tasks to allow the survivors of adversity to adapt and create meaning for hardships and enrich their belief system and values (35). In addition, many spiritual-religious duties and behaviors (e.g., fasting) intend to prepare humans for voluntarily dealing with hardships and difficulties, so that they could basically view these challenges as tests sent from God (36).

Conclusion

According to the results, practicing religious beliefs plays a key role in relieving psychological issues and improving mental health. Religious rituals (especially Ramadan fasting) are associated with enhanced resilience and psychological hardiness.

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Effects of a Healthy Diet plus Peanut Consumption on the Fasting Lipid Profile of HIV-infected Adults in Nyeri County, Kenya: A Randomized Crossover Study

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ABSTRACT

Introduction: Dyslipidemia is a key modifiable cardiovascular risk factor and a major clinical feature in the patients infected with the human immunodeficiency virus (HIV) in the current era of highly active antiretroviral therapy. Peanuts could reduce the risk of cardiovascular diseases as an abundant source of fiber, α-tocopherol, copper, arginine, magnesium, folate, and resveratrol. The present study aimed to evaluate the impact of supplementing peanut and counseling in the form of a healthy diet on the fasting lipid profile of HIV-infected adults.

Methods: This randomized crossover clinical trial was conducted on the eligible participants who were randomly assigned to a two-arm study. In treatment I, the participants consumed 80 grams of peanuts plus their regular diet. In treatment II, the participants were provided with nutrition counseling on a healthy diet and consumed 80 grams of peanuts. Each treatment continued for eight weeks with a six-week washout interval.

Results: A 3.07% reduction was observed in the total cholesterol of the subjects receiving treatment I, while the reduction rate was 5.39% in treatment II. In addition, a 12.8% decrease was observed in the triglycerides of the subjects receiving treatment 1I, as well as a 17% reduction in treatment II. A significant increase was reported in the high-density lipoprotein cholesterol in treatments I and II, with the rate estimated at 7.38% and 5.1%, respectively. Furthermore, low-density lipoprotein cholesterol decreased by 5.56% in treatment I and 4.32% in treatment II. The estimated 10-year risk of contracting coronary heart disease reduced significantly between the baseline and end of the study (P=0.03).

Conclusion: According to the results, regular consumption of peanuts could improve the fasting lipid profile of HIV-infected patients and reduce the risk of coronary heart disease.

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Introduction

The burden of cardiometabolic diseases is growing in Sub-Saharan Africa (SSA) as the landscape of the human immunodeficiency virus (HIV) care changes, [1] and the cardiovascular disease mortality rate is expected to double to 2.4 million by 2030 relative to reports in 2000. [2] Evidence suggests that cardiometabolic diseases would become a key health concern in SSA, competing for limited health resources with infectious diseases. [3]

Cardiovascular diseases (CVDs) are currently the second most common cause of death after cancer in the populations living with HIV in the regions of the world where highly active antiretroviral therapy (HAART) is widely available. [4]

Dyslipidemia associated with HAART is a prevalent condition in the patients living with HIV. Above 85% of the patients infected with HIV who receive HAART currently survive for more than 10 years after acquiring the infection. [5] On the other hand, the significant increase in life expectancy coupled with the reduction of morbidity and mortality as a result of HAART have been accompanied by the increased rate of clinical and metabolic complications. Some of these metabolic complications include dyslipidemia, hyperinsulinemia, and adipose tissue distribution. [6]

Dyslipidemia is a key modifiable cardiovascular risk factor and a major clinical feature of HIV-infected patients in the current era of HAART. [7] Non-pharmaceutical interventions such as diet

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and physical exercise should be the first-line intervention for the management of dyslipidemia. [8] Other interventions include reduced calorie intake, achieving an ideal bodyweight, and increasing physical activity. These first steps may yield added health benefits in HIV-related dyslipidemia. [9] In an HIV-uninfected population, dietary counseling may respectively result in 11% and 22% reduction of cholesterol and triglyceride (TG), while in HIV-infected individuals, the reduction rate may be 4-17% and 21-26%, respectively. [10] A study conducted by Barrios et al. [11] aimed to prospectively evaluate the impact of a low-fat diet on the reduction of cholesterol and TG in 230 HIV-infected individuals, and it was reported that proper dietary adherence resulted in 11% and 10% reduction of the total serum cholesterol and 12% and 23% in the serum TG after three and six months, respectively.

Peanuts could reduce the risk of CVDs as an abundant source of fiber, α-tocopherol, copper, arginine, magnesium, folate, and resveratrol. Therefore, regular consumption of peanuts might benefit high-risk individuals for CVDs. The available studies on the effects of peanuts have only been performed on healthy adults or patients with diabetic dyslipidemia based on a fat-restricted diet.

The present study aimed to investigate the effects of dietary counseling and peanut supplementation on the serum lipid profile of normal HIV-infected adults and those with hyperlipidemia referring to the comprehensive care clinics in Nyeri Level-5 Hospital.

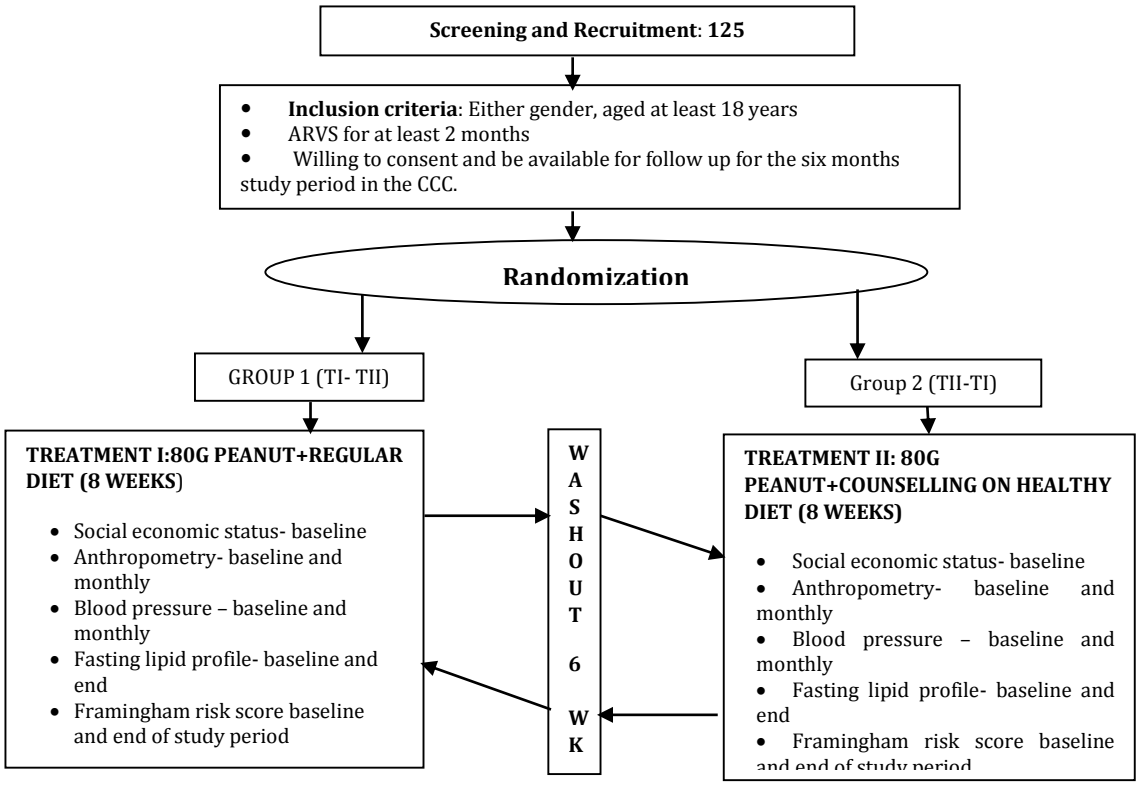

Figure 1. Intervention procedure

Materials and Methods

This randomized crossover clinical trial was conducted on the eligible participants who were randomly assigned to a two-arm study. In treatment I, the participants consumed 80 grams of peanuts plus their regular diet. In treatment II, the participants were provided with nutrition counseling on a healthy diet and consumed 80 grams of peanuts. Each treatment continued for eight weeks with a six-week washout interval.

The sample population included male and female outpatients aged at least 18 years with a normal

lipid profile or hyperlipidemia. In addition, the patients had to have been receiving antiretroviral drugs for a minimum of two months and willing to be available for a six-month follow-up at the comprehensive care center. The exclusion criteria were as follows: 1) known hypersensitivity to peanuts; 2) allergic reactions to peanuts after a skin test performed by a clinician; 3) pregnant and breastfeeding women; 4) receiving lipid-lowering therapies; 5) performing rigorous exercise; 6) history of diverticulitis or irritable bowel disease that could be deteriorated by daily peanut intake; 7) habitual peanut/tree nut consumers who were unwilling to discontinue the intake of peanut and/or tree nuts for six weeks prior to their first scheduled clinic visit and 8) renal and liver diseases and/or severe dyslipidemia (TG>4.52 mmol/l or TC>7.77 mmol/l).

Sample size was determined using the Fischer equation adopted by Chow Shao and Wang. [12] The sample size had a balanced, crossover design analyzed by t-test, and the probability of type I error (significance level) was estimated at 1.96, while the probability of type II error (test power) was 1.282.

In addition, the expected variance for LDL- C (σ_m^2) was 0.074. [19] When these values were substituted in the formula, the sample size was approximately 38 for each group considering the attrition rate of 20% and a total of 91 participants.

Randomization Procedure

Out of 125 screened subjects, 95 patients provided written informed consent for enrollment. However, only 85 patients were followed-up for the six months, and the dropout rate was 10.5%. The recruited participants were randomized into two groups. Group one started with TI and crossed over to TII, and group two started with TII and crossed over to TI after the washout period (Figure 1)

Freshly roasted unsalted peanuts were packaged in separate bags of 80 grams each as the daily serving (variety: Red Valencia). Each package contained a thirty-day serving and provided to the participants to carry home and consume every day for four weeks after which they would refer for another batch for the following four weeks. The peanuts were consumed as part of the participants' snack or with their main meals. Dietary intake was obtained by a 24-hour recall from 20% of the samples.

Fasting blood samples were collected at 7.00-8.00 AM. Approximately five milliliters of venous blood was collected to measure the fasting lipid profile, transferred to heparinized tubes, and centrifuged at 3,000 grams for three minutes. Serum and plasma were separated using an automatic pipette and transferred into specific labeled tubes in a rack for analysis. Lipid profile assays were routinely analyzed on the Mindray BS series autoanalyzer (Mindray-Bio Medical GmbH, Hamburg, and Germany) using established techniques.

Ethical clearance was sought from Kenyatta University Ethical Review Committee (REF:KU.R/COMM/51/273), the required permit was obtained from NACOSTI (REF: NCST/RCD/12A/013/4), and informed consent was obtained from the participants.

Statistical Analysis

Data analysis was performed in MS Excel spread sheet, and the analyzed data were exported to SPSS version 20 for analysis. Student's t-test was used to assess the significant difference in the lipid profile of the two treatment arms from baseline. In all the tests, the level of significance was set at $P<0.05$. In addition, data analysis for the 24-hour dietary recall was focused on the total energy and fat intake, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), protein, carbohydrate, vitamin E, folate, magnesium, and dietary fiber.

Nutrient composition was analyzed using the Nutrition Survey (2014) program, and one-way analysis of variance (ANOVA) was used to compare nutrient intake between the three random 24-hour recalls. In addition, Chi-square was applied for categorical variables such as the socio-demographic data, and paired student's t-test was used to compare the differences of the subjects in terms of various outcomes at baseline and the end of each treatment period. The Framingham risk score was also calculated for each individual based on variables such as age, gender, smoking habits, diabetes status, systolic blood pressure, total cholesterol, and high-density lipoprotein (HDL)-cholesterol. The Framingham score was derived using the approximated 10-year added risk of CVD and classified as low (<10%), medium (10-20%), high (20-30%), and very high (>30%). [13]

Table 1. Baseline socio economic and demographic characteristics

	Category	Period of treatment N=85		P value χ
		GROUP1 (TI- TII) n=45	GROUP2 (TII- TI) n=40	
Age	18-29	0(0.0)	1(1.2)	0.592
	30-39	8(9.4)	10(11.8)	
	40-49	23(27.1)	18(21.2)	
	>50	14(16.5)	11(12.9)	
Sex	Male	10(11.8)	8(9.4)	0.802
	Female	35(41.2)	32(37.6)	
Marital status	Single parent	12(14.1)	10(11.8)	0.405
	Married/Living together	19(22.4)	14(16.5)	
	Divorced/Separated	7(8.2)	10(11.8)	
	Widowed	7(8.2)	4(4.7)	
	Single	0(0.0)	2(2.4)	
Level of education	Lower primary	3(3.5)	2(2.4)	0.892
	Upper primary	13(15.3)	15(17.6)	
	Secondary	22(25.9)	19(22.4)	
	College	3(3.5)	2(2.4)	
	University	1(1.2)	0(0.0)	
	No formal education	2(2.4)	2(2.4)	
Occupation	Agricultural labor	12(11.1)	15(17.6	0.515
	Employed(Salaried)	7(8.2)	7(8.2)	
	Merchant/ Trader	18(21.2)	11(12.9)	
	Housewife	3(3.5)	2(2.5)	
	Waged labor	5(5.9)	5(5.9)	

Results

The obtained results only apply to the participants who completed the two treatments. Group one included 48 participants, and group two included 47 participants. Three participants dropped out of the study before the completion of the first treatment. In group two, seven participants dropped out of the study, and three participants dropped out before the completion of treatment II since they could not attend the clinic monthly. In addition, three participants dropped out after the first treatment, and one patient died. Group one started with TI and crossed over to TII, while group two started with TII and crossed over to TI after the washout period. The baseline socioeconomic and demographic characteristics of the subjects were analyzed, and no significant differences were observed between the participants in the two treatment arms at baseline.

Table 2. Mean daily energy and nutrient intakes from three random-day 24-h recall

	Baseline	T1	T2
Energy (kcal/day)	1937.10±309.98 [a]	2056.02±224.12[a]	2091.99±307.47[a]
Fat (%energy)	21.82±6.22[a]	32.05±7.64[b]	32.76±6.91 [b]
SFA	14.61±9.33[a]	19.39±4.51 [a]	19.39±5.72 [a]
MUFA	16.33±7.95[a]	32.53±6.17 [b]	33.19±5.84 [b]
PUFA	8.89±3.61[a]	17.86±2.66 [b]	19.16±2.96 [b]
Cholesterol (mg)	118.91±157.18[a]	118.12±211.06[a]	103.34±206.23 [a]
Protein(% energy)	12.23±2.56[a]	13.88±3.19[a]	13.35±2.47 [a]
Carbohydrate (% energy)	66.00±7.77[a]	54.23±9.71 [b]	53.64±7.58 [b]
Vitamin E	3.25±2.70[a]	8.87±2.13 [b]	8.80±1.48 [b]
Folate (mg/day)	313.89±188.11[a]	387.40±229.22 [a]	395.12±230.52 [a]
Magnesium (mg/day)	489.68±102.89[a]	592.53±142.34 [a]	618.22±248.03 [a]
Carotene	456.32±1103.60[a]	2149.58±4765.67 [a]	1892.89±4173.92 [a]
Dietary fibre (g/day)	24.61±8.76[a]	29.57±10.78 [a]	31.87±9.11 [a]

Values presented as the mean±/standard deviation; n=17. Means with different superscript letters are statistically significant at (P< 0.05). ONE WAY ANOVA. PUFA- poly unsaturated fatty acid, MUFA- mono unsaturated fatty acids, SFA – saturated fatty acid. TI- Treatment I TII- Treatment II

Dietary intake

The baseline comparison of the estimated nutrient and energy intake between the study

groups indicated no significant differences in this regard. ANOVA was also used to compare the nutrient intake of the three random 24-hour recalls between the two groups. The 24-hour dietary recall was administered to 20% of the participants at three random times. Table 2 shows the mean change in the dietary intake after the addition of peanuts to the regular diet of the subjects (TI) and after the provision of counseling in the healthy diet plus 80 grams of peanuts (TII). The obtained results indicated a significant difference in fat intake between the baseline and the two treatments ($F[2, 48]=13.185$; $P<0.05$). Similar findings were observed for carbohydrate intake ($F[2, 48]=11.664$; $P<0.05$), PUFA intake ($F[2, 48]=55.091$; $P<0.05$), vitamin E intake ($F[2, 48]=37.614$; $P<0.05$) and MUFA intake ($F[2, 48]=34.328$; $P<0.05$).

Compared to baseline, energy intake from fats significantly increased in the TI and TII groups ($P< 0.05$). Furthermore, MUFA and PUFA intake increased significantly during TI and TII ($P<0.05$), while no significant change was observed in the SFA intake. A significant decrease was also observed in carbohydrate intake during TI and TII ($P<0.05$). On the other hand, the dietary intake of vitamin E ($P<0.05$) increased significantly compared to baseline in both treatments ($P<0.001$). These changes could be attributed to addition of peanuts to the diet of the subjects. Notably, folate and magnesium did not change significantly in the two treatments compared to baseline, and no significant difference was observed between the dietary intakes of the two treatment.

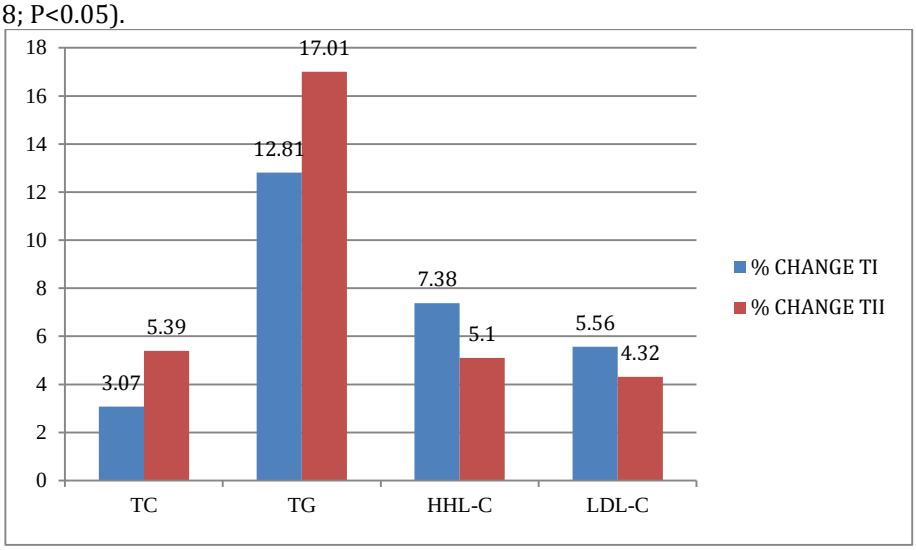

Figure 2. Percentage change in lipid profile in the two treatments Changes in Framingham's risk scores

C Changes in the Lipid Profile

Figure 2 shows the percentages of the changes in total serum cholesterol, serum TG, HDL-C, and low-density lipoprotein-cholesterol (LDL-C). A 3.07% decrease was observed in the total cholesterol of TI, while the reduction rate in TII was estimated at 5.39%. The reduction was considered significant in both treatments ($P<0.001$), and the mean change of the two treatments was also significant ($P<0.001$) (Table 3).

According to the findings, TG decreased in TI (12.81%), and the rate was estimated at 17.01% in TII. However, the mean change in the two treatments was not considered significant ($P=0.121$). A slight, significant increase was denoted in HDL-C in TI and II (7.38% and 5.1%, respectively) (Figure 1), and the mean change was considered significant in the two treatments ($P=0.012$) (Table 3).

In the present study, LDL-C reduced by 5.56% in TI and 4.32% in TII, and the change in this regard was significant in both treatments ($P<0.001$). However, the mean change in the two treatments was not considered significant ($P=0.242$). The mean reduction in the total cholesterol was more significant in the subjects with the total cholesterol of >5.1 mmol/l compared to those with the total cholesterol of <5.1 mmol/l.

Table 3. Mean change in serum lipid profile

	Treatment I				Treatment II				D1 &D2
	Baseline	End	D1	P value(t-test)	Baseline	end	D2	P value (t-test)	P value(t-test)
TC (mmol/L)	5.17±1.18	5.01±1.07	-.1589±.38	0.001	5.17±1.13	4.89±1.08	-0.27±0.20	0.001	0.001
TG (mmol/L)	1.88±.85	1.64±.83	-.24±.24	0.001	1.89±.90	1.57±.94	-0.32±0.48	0.001	0.121
HDL- C (mmol/L)	1.40±.41	1.51±.42	.10±.11	0.001	1.42±.42	1.49±.42	-0.07±0.09	0.001	0.012
LDL-C (mmol/L)	3.31±1.01	3.12±.92	-.18±.28	0.001	3.25±1.00	3.11±.99	.-14±25	0001	0.242

Values presented as the mean±/standard deviation; n=85. Means are statistically significantly different at (P< 0.05). TC- total cholesterol, TG- triglycerides, HDL-C- high density lipoprotein cholesterol, LDL-C- Low density lipoprotein cholesterol, D1- delta change in treatment I, D2- delta change in treatment II. Independent t-test was used to analyze the difference between the means.

Table 4. Change in Framingham's risk scores

	Baseline			End			P value (baseline& end)
Framingham's risk	Male	Female	P value (chi)	male	female	P value (chi)	
<10%	9(10.6)	42(49.4)	0.08	11(12.9)	44(51.8)	.356	0.03
10-20%	7(8.2)	25(29.4)		5(5.9)	23(27.1)		
20-30%	2(2.4)	0(0.0)		2(2.4)	0(0.0)		
>30%	0(0.0)	0(0.0)		0(0.0)	0(0.0)		

Approximated 10-years added risk of cardiovascular disease: low (< 10%), medium (10-20%), high (20-30%), and very high (> 30%). [1]

According to the current research, the mean reduction in TG was more significant in the participants with the TG of >2.25 compared to those with the TG of <2.25. The mean reduction in LDL-C was more significant in the participants with the LDL-C of >4.2 mmol/l compared to those with the LDL-C of <4.2 mmol/l. The mean increase in HDL-C was more significant in the participants with normal HDL-C levels (1.03-1.55), while the lowest value was observed in the participants with high HDL-C levels (>1.55). Total cholesterol and HDL-C were significantly different between the two treatments (P<0.001 and P<0.012, respectively), indicating that nutrition counseling on healthy diets increased HDL-C and decreased total cholesterol. On the other hand, regression analysis showed no correlations between the changes in the lipid profile of the two treatments and the changes in the PUFA, MUFA, and SFA intake as predicted.

The Framingham risk score was used to determine the 10-year risk of developing a CAD. Table 4 shows the changes in the Framingham risk score between the baseline and end of the study. The majority of the participants (60% and 64.7%) were at a low 10-year risk of CADs (<10%) at baseline and the end of the study, respectively. Meanwhile, 2.4% of the participants were at a moderate 10-year risk of CADs (20-30%). The male and female subjects had no significant difference in terms of the 10-year risk at baseline and the end of the study period (P>0.05). However, a significant reduction was observed in the 10-year risk of CADs between the baseline and end of the study (P=0.03).

Discussion

The present study aimed to investigate the impact of peanut consumption and nutrition counseling regarding healthy eating on the serum lipid profile in HIV-infected patients. Three random 24-hour recalls were used to assess the dietary intake of the participants. According to the obtained results, energy intake did not change significantly when the regular diet of the subjects was supplemented with 80 grams of peanuts (TI) and when the patients were counseled on a healthy diet (TII). This could be due to the significant reduction of the carbohydrate intake in both treatments and the increased fiber intake, which might have led to early satiety and a non-significant increase in energy intake. These findings are consistent with the study conducted by Alper CM et al. [14], which was a 30-week crossover study in which the

subjects were provided with 500±136 kilocalories worth of peanuts for an eight-week free feeding (FF) diet. In another research, Mckiernan F. et al. [15] reported no significant changes in energy intake within a four-week randomized clinical trial. Compared to the baseline, energy intake from fats increased significantly during TI and TII ($P<0.05$) in the present study, and the MUFA and PUFA intakes also increased significantly during TI and TII ($P<0.05$). On the other hand, the SFA intake remained unchanged. Similar findings have been reported by Alper CM et al., McKiernan F. et al., and Lokko et al. [14-16].

In the current research, a significant decrease was denoted in carbohydrate intake during TI and TII ($P<0.05$), which is in line with the study by Alper CM et al., [14] while inconsistent with the study by McKiernan F et al. [15], which indicated no reduction in carbohydrate sources when peanut was added to a regular diet. Dietary intakes of vitamin E ($P<0.05$) increased significantly from the baseline in both treatments in the present study ($P<0.05$).

Our findings indicated a significant reduction in the total serum cholesterol in both treatments (3% and 5.3%, respectively). In addition, the change in TG was considered significant in both treatments (12.8% and 17.1%, respectively). On the other hand, LDL-C decreased significantly in both treatments (5.5% and 4.3%, respectively). Epidemiological studies and clinical trials have demonstrated the benefits of nuts and peanut consumption on the CAD risk and the associated risk factors [17, 18]. Our findings in this regard are consistent with the study by Lokko P. et al. [16], which demonstrated a total cholesterol decrease of 7.2%, while a 20% decline was also reported in TG after adding 500 kcal/day of peanuts to the daily diet of the subjects for eight weeks.

A recent four-week study conducted by McKiernan F. et al. [15] on hyperlipidemic patients indicated a significant reduction in the total serum cholesterol, LDL-C, and TG after consuming 56 grams of unprocessed whole raw peanuts, roasted unsalted/salted peanuts, honey grazed roasted peanuts, or peanut butter daily. However, HDL-C concentrations increased significantly compared to the baseline. Recently, a pooled analysis of 1,284 observations has been proposed from 583 unique participants in 25 clinical studies conducted in seven different countries using different nuts, including peanuts. [19] The results of the mentioned pool showed that the exerted cholesterol-lowering effects were dose-dependent. Furthermore, the analysis indicated that 67 grams of the average daily intake of nuts could lead to the mean estimated reduction of 5% in total cholesterol and 7% in LDL-C. However, nuts intake had no significant effects on HDL-C and TG, while the effects were reported to be significant on the participants with serum TG of >150 mg/dl (10.2 mg/dl reduction observed).

In the present study, the mean reduction in the total serum cholesterol, LDL, and TG was higher but not significantly higher in the participants with high serum levels compared to those with normal serum levels. This is inconsistent with an interventional study with peanuts, which demonstrated a reduction in total cholesterol (12%) and LDL-C (10%) in the normocholesterolaemic patients consuming whole peanuts and peanut butter for 24 days. [20] (Kris-Etherton et al., 1999).

The current research indicated no significant correlation between the changes in the dietary PUFA, MUFA, fat, and fiber individually or together and the changes in the lipid profile of the patients based on linear regression analysis. This is because there are other components in nuts (e.g., fiber and phytosterols) along with unsaturated fatty acids, which are likely to contribute to the favorable effects of nuts on the plasma lipid. [21-23]

In the present study, the reduction of TG may have resulted from the decreased carbohydrate intake of the subjects following the addition of peanuts to their diet. According to the literature, TG concentration decreases with reduced carbohydrate intake. [24] Therefore, the reduction of carbohydrate intake might have exerted an independent effect on the lipid profile of our participants. It is estimated that the reduction of total cholesterol and LDL-C by 1 mmol/l may result in a 24-28% decrease in the relative mortality risk of coronary heart disease. In addition, TG reduction by 1.0 mmol/l may result in a 14-37% decrease in the total risk of CVDs. [25]

The present study had a crossover clinical trial design, which reduced interpersonal variations. However, it was performed on free living individuals under the assumption that the provided peanuts were consumed daily and not shared with other family members.

Conclusion

According to the results, the consumption of peanuts with and without counseling on a healthy diet could improve the lipid profile of the subjects living with HIV infection, thereby reducing the 10-year risk of developing coronary heart disease.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Effects of Ramadan Fasting on Hepatic Steatosis and Liver Volume in Individuals without Chronic Liver Conditions

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ABSTRACT

Introduction: Ramadan fasting (especially in summer) provides an opportunity to evaluate the effects of eating and drinking variances on the metabolism. The present study aimed to assess the effects of Ramadan fasting on hepatic steatosis and liver volume by magnetic resonance imaging (MRI) in overweight and obese individuals and determine the impact of the fasting period on liver function tests and serum lipid profile.

Methods: This study was conducted on 34 individuals (28 males and six females) without chronic liver or systemic diseases who did not use alcohol, had the body mass index (BMI) of ≥25, and were committed to Ramadan fast. Abdominal MRI and blood analysis were performed twice one week before and after Ramadan. In addition, liver fat fractions and volumes were calculated based on the MRI. Data were statistically analyzed and compared.

Results: The mean age of the subjects was 44.5 years (age range: 19-68 years). Before Ramadan, the mean weight and mean BMI were 86.76 kilograms and 30.29 kg/m^2, respectively. Although the liver fat fraction increased (2.92±7.99% vs. 3.44±8.11%; P>0.05) and the liver volume decreased (1,555.37±316.92 vs. 1,546.63±339.82; P>0.05) after Ramadan, the differences were not significant. However, significant, positive changes were observed in the serum lipid profile and liver function tests.

Conclusion: Excess food consumption in the evening and at night and a sedentary lifestyle may have affected our findings. Nonetheless, the prolonged avoidance of a predetermined amount of food and drinks could lead to statistical changes in the measured variances. As such, further longitudinal studies on larger sample sizes could be performed to examine individuals with the BMI of less than 25 kg/m^2 for more accurate results.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) refers to a fatty liver occurring secondary to nonalcoholic causes [1]. NAFLD could potentially progress into cirrhosis due to hepatic damage and increase mortality and morbidity. The incidence of NAFLD has recently been on the rise parallel to the increased obesity. In addition, NAFLD is ranked second in terms of the etiology of chronic liver diseases in Turkey. In the current research, we performed a quantitative analysis to investigate the effects of 29 days of Ramadan fasting with the approximate duration of 16 hours and 50 minutes per day in June 2016 in Van, Turkey. The main objective was to evaluate the effects of fasting on hepatic steatosis and liver volume based on chemical-shift magnetic resonance imaging (MRI) in overweight and obese individuals with NAFLD and those without underlying diseases. Additionally, the effects on the liver function and serum lipid profile were assessed.

Ultrasonography (US) and computed tomography (CT) could be employed for the diagnostic imaging and follow-up of NAFLD patients. However, the recently defined chemical-shift MRI has come forefront for the diagnosis and quantitative assessment of the disease. The key advantage of MRI over US and CT is that this imaging modality involves no radiation exposure and detected the presence of fats at a cellular level through a chemical shift [2].

Ramadan fasting is compulsory for healthy adult Muslims, and the duration of fasting is 29-30 days in the 9th month of the lunar calendar. According to the Islamic calendar (Hijri

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calendar), Muslims must refrain from eating and drinking for 13-14 hours per day (from dawn to sunset). However, ill individuals, pregnant/lactating and menstruating women, the elderly, and travelers are exempt from Ramadan fasting. In this month, eating and drinking is allowed during the night from sunset to approximately 1.5-2 hours before sunrise. In many countries, one meal is traditionally taken at *Sahur* (before sunrise) as breakfast, and the other meal is taken at *Iftar* (after sunset) as dinner. In some countries, more than two meals could be taken from *Iftar* to *Sahur*.

Previous studies have shown that nutrients ingested at unusual times may exert different metabolic effects [3-5]. Ramadan fasting (especially in summer) provides an opportunity to evaluate the effects of eating and drinking variances on the metabolism. NAFLD has been reported to be a complication and sequela of overweight and obesity, with a higher prevalence among obese individuals [6, 7]. In the present study, we hypothesized that the prolonged daytime fasting in Ramadan in June may have positive metabolic effects on the lipid profile (especially on the liver metabolism) in overweight and obese individuals with NAFLD. Lipid profile [3, 8-12] and liver function [5, 13, 14] have been previously analyzed during Ramadan using blood tests.

The present study aimed to investigate the effects of Ramadan fasting on hepatic steatosis and liver volume through blood tests and MRI. To the best of our knowledge no prior studies have measured hepatic steatosis and volume by MRI in Ramadan.

Materials and Methods

Patient Selection

This study was conducted on 34 out of 85 patients aged more than 18 years who referred to the clinics of the medical centers of our institute, presenting with hepatic steatosis, obesity, and impaired serum lipid profile. The body mass index (BMI) of the patients was calculated, and those with the BMI of ≥25 kg/m^2 were enrolled in the study. On the other hand, 22 patients who were not able to fast during Ramadan were excluded for different reasons, including pregnancy (n=5), menstruation (n=8), severe diseases (n=4), and chronic drug use (n=5). In addition, 16 patients with the BMI of <25 kg/m^2 and 13 patients with chronic liver disease, systemic disease, history of malignancies, and chronic medication use affecting the liver were excluded.

The required permit for the study was obtained from the Clinical Research Ethics Committee of our institute (05.05.2016; No. 03), and the study was conducted on 34 patients, including 28 males (age: 18-70 years) and six menopausal females (age: 45-70 years), who committed to Ramadan fasting during June 2016. None of the participants had a known chronic liver disease or alcohol use.

Imaging Technique

MRI was carried out using a 1.5 Tesla MRI device (Syngo MR B17, Siemens MAGNETOM Symphony, Germany, 2009) with an abdominal coil. The upper abdominal MRI of the patients was obtained twice, with the first process performed in the week before Ramadan fasting (BR) and the second in the week after Ramadan (AR). The interval between the BR and AR tests was planned to be at least 30 days, with the maximum of 34 days (including the 29-day fasting period). The breath-hold T1-weighted gradient echo dixon sequences were used for the imaging. In this sequence, the voxel dimensions were determined as 1.9×1.2×3.0 (SNR:1.00), with the section slice thickness of three millimeters (TR:7.71; TE:2.38), flip angle of 10, and bandwidth of 340 HZ/Px. In each examination, 64 three-dimensional (3D) section images were also obtained in four different sequences, including in-phase (IP), out-phase (OP), water (W), and fat (F).

Laboratory Serum Values

Simultaneously with the imaging studies and after at least eight hours of fasting, biochemical tests were carried out to measure triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TCL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and direct bilirubin (DB) twice at the BR and AR.

MRI-based Hepatic Steatosis Measurement

The MRI images were examined by radiologists with 15 and seven years of clinical experience. Considering the craniocaudal size of the liver, the measurements were performed at the upper, middle, and lower levels by division into three equal segments in order to ensure homogeneity. In addition, the region of interest

(ROI) was drawn polygonal to the liver in the IP and OP images using the picture archiving communication system, including the entire extent of the liver parenchyma. The mean ROI values were measured in the IP and OP images separately at the same level in each region (Figure 1). The IP and OP values at the upper, middle, and lower levels of the BR and AR were also recorded separately. The values were applied to the fat signal fraction (FF) formulas (FF=[IP – OP]/[2×IP] or FF=F/[W+F]) for the BR and AR regions separately, and the values were obtained as percentages. The mean FF of each region was calculated, and the values were recorded.

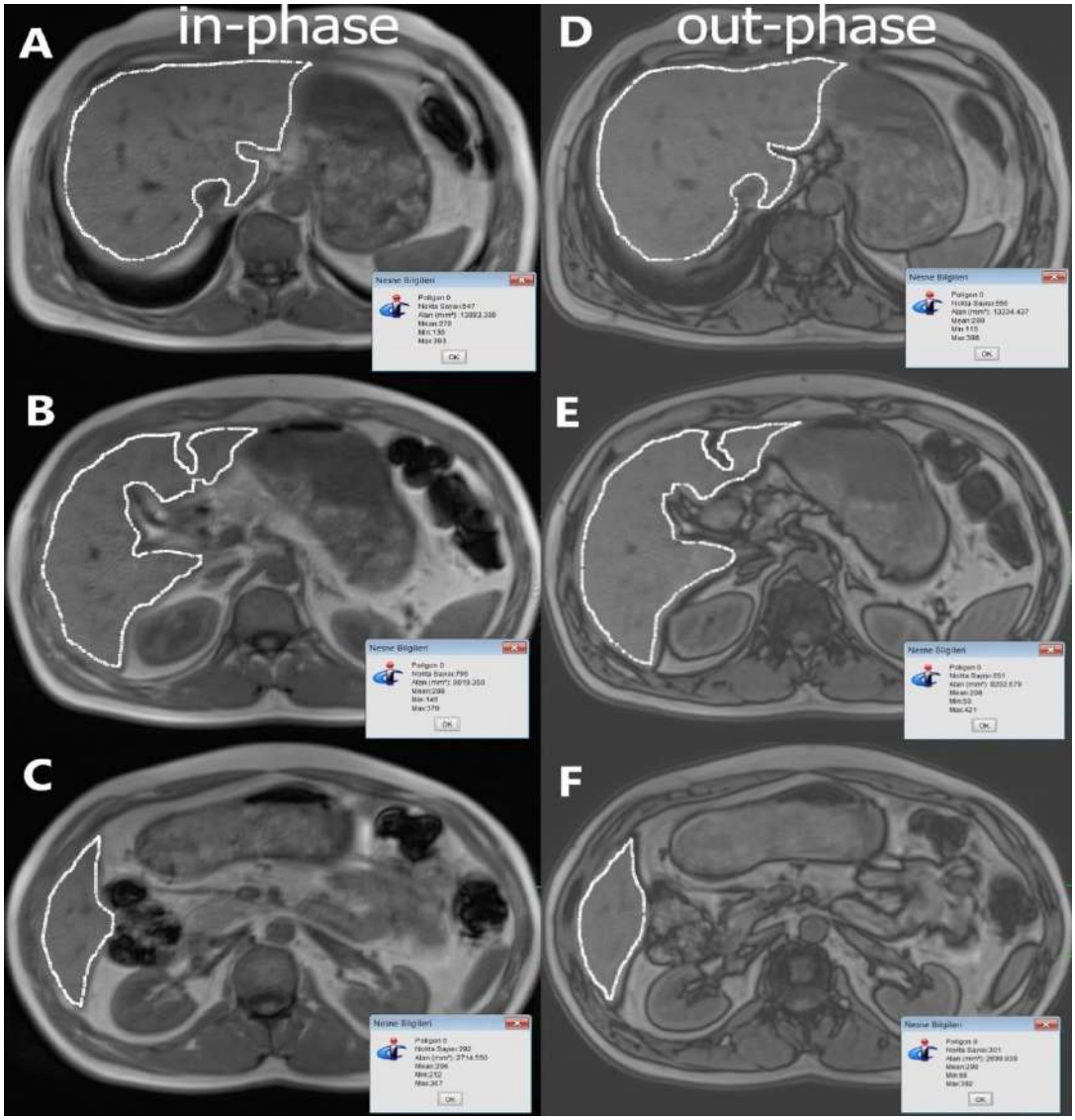

Figure 1. Polygon-shaped region of interest (ROI) drawing of the liver contours in the in-phase (IP) (A, B, C) and out-phase (OP) (D, E, F) images using the Picture Archiving Communication Systems **(**PACS) in three separate areas of the liver, upper (A, D), middle (B, E) and lower (C, F), and calculating the average ROI values**.**

MRI-based Liver Volume Measurement

The obtained 3D images were uploaded into a Linux operating system-based XIO treatment planning system program (version 4.34; CMS Software, St. Louis, MO). The visible outer contours in each section of the liver were drawn manually using the program, and the areas within the drawn contours were reconstructed by the program to calculate the liver volume

(Figure 2). Moreover, the BR and AR liver volume values were recorded separately.

Statistical Analysis

Data analysis was performed in SPSS version 20.0 (IBM Corporation, Armonk, NY) using descriptive statistics for the continuous variables, which were expressed as mean, standard deviation, minimum, and maximum. The categorical variables were expressed as numbers and percentages. The within-group comparisons of the continuous variables were also performed using dependent t-test, and the level of statistical significance was considered to be 5%.

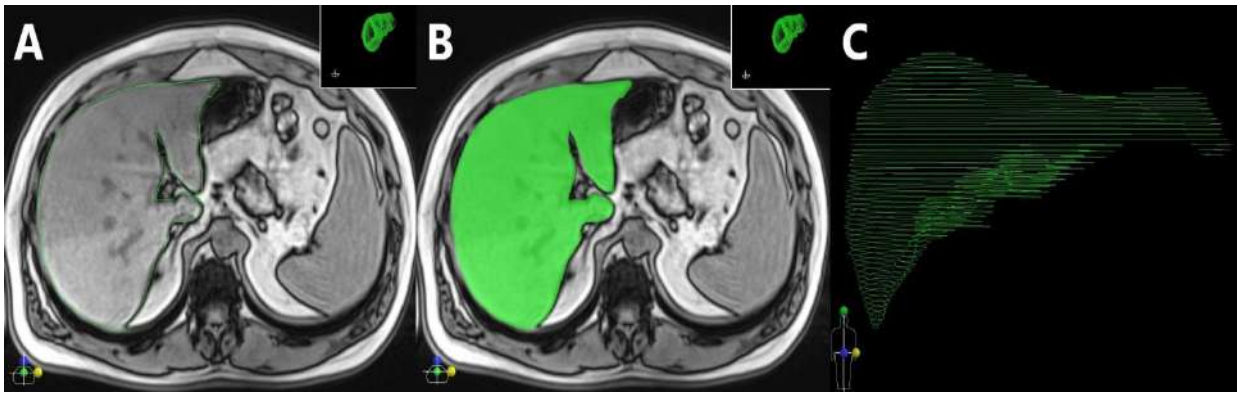

Figure 2. Considering the craniocaudal size of the liver, manual drawing of all external contours seen in all sections of the liver through the XIO (v4.34, CMS Software, St. Louis, MO) Treatment Planning System program (A, B). Obtaining liver volumes by reconstructing the areas within the contours that are drawn through the program (C).

Results

Baseline Characteristics

In total, 34 patients were enrolled in the study, including 28 males (82.4%) and six females (17.6%) with the mean age of 44.5±10.81 years (age range: 19-68 years). The mean weight of the patients was 86.76±12.82 kilograms (range: 63-111 kilograms), and the mean BMI was 30.29±3.40 kg/m^2 (range: 25.46-38.06 kg/m^2).

Outcomes of Serum Lipid Profile

The mean values of TG, LDL, TCL, and HDL BR were 232±167.76, 102.38±35.37, 190.06±39.89, and 42.44±8.66 mg/dl, respectively, while they were estimated at 156.85±134.45, 129.65±41.82, 200.65±36.42, and 39.09±7.13 mg/dl AR, respectively. The comparison of the measured values BR and AR indicated a very significant reduction in the TG and HDL (P<0.001), as well as a very significant increase in the LDL (P<0.001), and a significant increase in the TCL (P=0.01) (Table 1).

Outcomes of Liver Function Tests

The mean values of DB, AST, and ALT BR were 0.22±0.10 mg/dl, 22.97±10.27 IU/l, and 26.15±13.48 IU/l, respectively, while they were estimated at 0.28±0.10 mg/dl, 21.74±6.92 IU/l, and 21.85±10.80 IU/l AR, respectively. The comparison of the measured values BR and AR indicated a significant decrease in ALT (P=0.01), as well as a significant increase in DB (P<0.001), and an insignificant reduction in AST (P=0.20) (Table 1).

Outcomes of Hepatic Steatosis Measurement

The mean liver fat fractions measured BR and AR at the upper, middle, and lower levels of the liver were calculated to be 2.47±7.67%, 2.77±8.04%, 3.51±8.42%, and 3.08±7.9%, 3.31±8.25%, and 3.93±8.33%, respectively. In addition, the mean values of fat fraction measured at these three levels of the liver BR and AR were estimated at 2.92±7.99% and 3.44±8.11%, respectively. The comparison of the fat fraction values measured BR and AR indicated that despite the increase in the rate of fat fractions at all the levels, the obtained values had no statistically significant difference in this regard (P>0.05) (Table 1).

Outcomes of Liver Volume Measurement

The mean values of liver volume BR and AR were 1,555.37±316.92 and 1,546.63±339.82 cc, respectively. The comparison of the values measured BR and AR indicated that despite the decreased liver volume, the obtained values had no statistically significant difference in this regard (P>0.05) (Table 1).

Table 1. Average and comparison of the values before and after Ramadan

Variables	Before Ramadan (mean ± std. deviation)	After Ramadan (mean ± std. deviation)	Paired Samples Test				*p values
			Paired Differences				
			Mean Difference	Std. Deviation	95% Confidence Interval of the Difference		
					Lower	Upper	
Fat Fraction 1 (%)	2.47 ± 7.67	3.08 ± 7.9	-0,61	2,73	-1,56	0,35	,204
Fat Fraction 2 (%)	2.77 ± 8.04	3.31 ± 8.25	-0,54	2,68	-1,47	0,40	,252
Fat Fraction 3 (%)	3.51 ± 8.42	3.93 ± 8.33	-0,42	3,21	-1,54	0,70	,447
Mean Fat Fraction (%)	2.92 ± 7.99	3.44 ± 8.11	-0,52	2,72	-1,47	0,43	,272
Liver Volume (cc)	1555.37 ± 316.92	1546.63 ± 339.82	8,75	151,02	-43,95	61,44	,738
Triglyceride (mg/dl)	232 ± 167.76	156.85 ± 134.45	75,15	104,05	38,84	111,45	,000
Low Density Lipoprotein (mg/dl)	102.38 ± 35.37	129.65 ± 41.82	-27,26	28,24	-37,12	-17,41	,000
Total Cholesterol (mg/dl)	190.06 ± 39.89	200.65 ± 36.42	-10,59	23,53	-18,80	-2,38	,013
High Density Lipoprotein (mg/dl)	42.44 ± 8.66	39.09 ± 7.13	3,35	4,44	1,80	4,90	,000
Direct Bilirubin (mg/dl)	0.22 ± 0.10	0.28 ± 0.10	-0,06	0,07	-0,08	-0,04	,000
Aspartat Aminotransferaz (IU/L)	22.97 ± 10.27	21.74 ± 6.92	1,24	5,55	-0,70	3,17	,204
Alanin Aminotransferaz (IU/L)	26.15 ± 13.48	21.85 ± 10.80	4,29	9,63	0,93	7,66	,014

*p <0.05 statistically significant.

Discussion

Several studies have investigated the epidemiology, pathogenesis, natural course, and treatment of NAFLD, and prevalence studies have been carried out in the community and on various risk groups. However, the evaluation of these studies may be technically challenging. The lack of a standardized definition for NAFLD, growing number of the asymptomatic cases, unavailability of specific diagnostic tests, and impracticality of liver biopsy as a screening method are among the main limitations in regards to the disease.

The prevalence of NAFLD has been reported to be 20% with US is used as the screening tool, while the rate increase 2-3 fold with liver biopsy [15]. This discrepancy is mainly due to the fact that invasive liver biopsy is only used under special circumstances when it is unavoidable.

The prevalence of NAFLD has also been reported to be higher in specific risk groups, such as patients with type II diabetes mellitus, obesity, and hyperlipidemia, compared to the general population [7, 16-18]. As such, we focused on overweight and obese cases in the present study.

Currently, liver biopsy is considered to be the 'gold standard' for NAFLD diagnosis and determining the level of steatosis. The procedure could be affected even by inconsequential factors, such as the angle of the liver biopsy needle upon insertion, length of the sample, and differences in number, while there is also the possibility of numerous minor and major complications due to its invasive nature [19, 20]. Therefore, the diagnosis and follow-up of the patients are primarily performed using US, CT, and MRI [21]. Liver stiffness could be measured by transabdominal US with

elastography, which is a novel and accurate method for evaluating the severity of NAFLD [22, 23]. Notably, the available MRI methods are chemical-shift imaging modalities and MR spectroscopy (MRS) [24].

The main advantage of MRI is its direct chemical specificity for TG, the accumulation of which is a histopathological sign of steatosis. Furthermore, MRI has been reported to have high sensitivity and specificity in the detection of mild steatosis [21, 24, 25]. In a meta-analysis, MRI and MRS were proven superior in the differentiation of various mild types of the disease [26].

In the current research, chemical-shift MRI was employed for determining the level of hepatic steatosis as a noninvasive approach involving no radiation, with an easier application and examination compared to MRS. MRS has some other limitations as well; for instance, it is only available in academic centers, the process is time-consuming, the evaluation requires specialty training, and only the area of examination could be evaluated rather than the entire liver.

Variable results have been reported regarding the effects of Ramadan fasting on the lipid profile. In a study of 30 premenopausal women, a significant decrease was observed in TG and LDL, and a significant increase was reported in the HDL values, with no significant difference in the TCL values [8]. In another study conducted on 81 healthy subjects, the reported values demonstrated a significant increase in the TG of the subjects with the BMI of less than 25 kg/m^2, while a significant decrease was denoted in TG, a significant increase was reported in LDL, and a significant decrease was observed in HDL. In the mentioned study, the TCL levels remained unchanged in the subjects with the BMI of ≥25 kg/m^2 [3].

According to the findings of Shehab A. et al., TG significantly increased in women and decreased in men, and they also reported the significant reduction of LDL, the significant increase of HDL, and increased TCL in the women and decreased TCL in men, while no significance was denoted in 65 healthy volunteers with the mean BMI of 27.5 kg/m^2 [12]. In another study in this regard, a significant decrease was observed TG and LDL, a significant increase was reported in HDL, and TCL was also observed to increase in 33 healthy volunteers [10]. Another research conducted on 28 healthy women also demonstrated increased TG, as well as the significant reduction of LDL and TCL and the significant increase of HDL [9].

In the study by Vardarli MC et al., a significant increase was observed in LDL, and a significant decrease was reported in HDL [11]. In the present study, TG significantly decreased, which is consistent with the previous studies in this regard. On the other hand, we observed a significant increase in LDL and TCL and a significant reduction in HDL, which is inconsistent with the previous findings. Table 2 shows the complex variables observed in the serum lipid profile and a comparison with the literature in this regard. Previous findings on the effects of Ramadan fasting on serum lipid profile are inconsistent and often contradictory, which could be due to variables such as the genetic structure, seasonal differences, duration of Ramadan fasting, type of consumed foods, socioeconomic status of fasting individuals, and frequency of physical activity.

Table 2. Comparison of our study with the variable data observed in the blood-lipid profile in the literature

	Our Study (n=34)	Culha C et al. (n=30)	Ziaee V et al. (n=81)	Shehab A et al. (n=65)	Ismail WI et al. (n=33)	Akhtaruzzaman M et al. (n=30)	Vardarli MC et al. (n=24)
Triglyceride (mg/dl)	↓ *	↓ *	↑* (N BMI) ↓* (≥25 BMI)	↑* (Female) ↓ (Male)	↓ *	↑	
LDL-Cholesterol (mg/dl)	↑ *	↓	↑ *	↓ *	↓ *	↓ *	↑ *
HDL-Cholesterol (mg/dl)	↓ *	↑ *	↓ *	↑ *	↑ *	↑ *	↓ *
Total Cholesterol (mg/dl)	↑ *			↑ (Female) ↓ (Male)	↑	↓ *	

↑: Increase, ↓: Decrease, *: p <0.05 statistically significant

Few studies have been focused on the effects of Ramadan fasting on the liver function. In a study of 34 patients with NAFLD, ALT levels were reported to increase significantly [5]. In

addition, Ibrahim NSI et al. observed a significant decrease in the AST and ALT levels in 18 healthy young males [14]. Another study performed on 57 healthy individuals indicated a significant increase in the AST and total bilirubin levels, as well as a significant decrease in the ALT levels [14]. In the present study, ALT decreased significantly, which is consistent with the previous studies conducted on healthy participants, while inconsistent with some other findings. Furthermore, AST decreased in the current research although the reduction was not considered significant. On the other hand, DB significantly increased, which is in line with the previous studies in this regard. Although our patients had NAFLD, they had no underlying chronic liver or systemic diseases, which differs with the previous studies [5] in which the ALT levels have been reported to increase. As such, our findings may be in congruence with the studies performed on healthy individuals.

For the first time, we evaluated the effects of short-term fasting on hepatic steatosis and liver volume based on the steatosis level using MRI as the most sensitive and reliable quantitative measurement method. According to the obtained results, the fat signal fraction increased at the upper, middle, and lower levels of the liver, while the liver volume decreased; notably, these changes were not considered significant. Although only a few studies have been conducted in this regard so far, we suggest that the duration of daily fasting short as 29 days is probably not sufficient for a significant morphological change to occur in the liver MRI. We also believe that significant results may be obtained with longer periods of fasting. Although it was not visually detected in the liver MRI of our patients, the quantitative measurements indicated the hepatic steatosis to be larger in the lower levels of the liver in both the BR and AR regions.

The main limitations of the present study were the lack of participants with the BMI of <25 kg/m^2, no sonographic grading of the hepatic steatosis, and the small sample size.

Conclusion

Initially, we hypothesized that prolonged daily fasting in healthy individuals with NAFLD and no underlying diseases may have positive metabolic effects on hepatic steatosis and planned to demonstrate these effects using imaging modalities. However, no significant increase was observed in the liver fat signal fraction, and no significant decrease was denoted in the liver volume as measured by MRI during the one-month period of the study. In contrast, positive, significant changes were observed in several biochemical blood parameters in this period. The excess consumption of food in the evening and at night and a sedentary lifestyle may have affected our findings, while the prolonged avoidance of a predetermined amount of food and drinks regularly may also lead to statistical changes. Therefore, it is recommended that larger longitudinal studies in this regard be conducted on subjects with the BMI of <25 kg/m^2.

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Conflicts of Interest

None declared.

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Validity and Reliability of the Persian Version of the Sports Nutritional Knowledge, Attitudes, Behaviors questionnaire in Adolescent Athletes

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ABSTRACT

Introduction: The present study aimed to evaluate the validity and reliability of the Persian version of the Sports Nutritional Knowledge, Attitudes, Behaviors questionnaire (SNKABQ) in adolescent athletes.

Methods: SNKABQ was designed by Walsh et al. The questionnaire was translated and re-translated using the WHO standard method and in line with the Iranian culture and nutrition habits. The qualitative content validity of the questionnaire was assessed by five experts, time reliability was assessed using the test-retest method, and the correlation coefficient and differential validity were determined by comparing novice and skilled athletes using the Mann-Whitney U test. In addition, the internal consistency of the questionnaire was assessed based on Cronbach's alpha, and the questionnaire was completed by 191 male and female students aged 15-18 years.

Results: The total time reliability of the questionnaire was estimated at 0.86, while it was 0.92 for eating and drinking habits, 0.75 for nutrition attitudes, 0.88 for nutrition knowledge, and 0.89 for nutrition information sources. Moreover, the Mann-Whitney U test indicated that the questionnaire has significant differential validity (P=0.00). The total internal reliability of the questionnaire was determined to be 0.74. The internal reliability of the subscales was also measured and estimated at 0.71 for eating and drinking habits, 0.80 for nutrition attitudes, and 0.70 for nutrition knowledge and nutrition information sources.

Conclusion: SNKABQ had acceptable validity and reliability in the adolescent athletes and could be used as a tool for the assessment of Iranian adolescent athletes.

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Introduction

Poor eating behaviors play a key role in the development of chronic diseases, and changes in eating habits could largely influence the human health throughout life. Recognition of the nutritional status of various communities is paramount, especially in school-age children (1, 2) to resolve the lack of knowledge in this regard through the implementation of training courses.

Extensive research has confirmed the effects of proper nutrition and physical activity on the growth and overall health of children. Furthermore, In Adolescents who are involved in championship sports, nutrition plays a key role in exercise performance and training adaptations. In addition to the ratio of nutrients, meal and snack timing is just as important because it plays a pivotal role in athletes' performance and recovery (3).

Terms such as nutrients, dietary supplements, ergogenic supplements, and performance-enhancing supplements are used frequently in various products claiming to improve athletic performance and health to attract the attention of athletes. Proper sports nutrition strategies and selecting the optimal supplements (if needed) require an adequate knowledge of nutrition (4). Therefore, the knowledge and awareness of athletes should increase in this regard, especially in the case of adolescent (5). Notably, the level of knowledge, attitude, and practice should be initially enhanced by valid tools for the evaluation of these parameters. According to the literature, the general knowledge of nutrition and sports may vary with changes in social conditions and

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geographical climate, and even the gender of athletes is considered to be an influential factor in this regard (6). The healthy diet of athletes requires a high level of nutrition knowledge (7). A study indicated that after the nutrition education of adolescent soccer players, they obtained higher scores of nutrition knowledge, which encouraged them to take healthier meals (8). In another research conducted in 2014, it was reported that adolescent rugby players had an adequate, general knowledge of nutrition and sports, while they were not sufficiently aware of carbohydrates and their dietary role (9).

Given the diversities in dietary habits and cultures and type and name of foods consumed across the world, a valid questionnaire is required for each population based on their specific cultural habits and native language (10). According to research in Iran, no complete and comprehensive studies have investigated the attitudes and nutrition knowledge of adolescent athletes, and data are scarce on the nutrition knowledge of adolescents; therefore, extensive research is required in this regard. Moreover, the validity and reliability of the sports nutrition knowledge questionnaire has not been assessed for young Iranian students.

The present study aimed to evaluate the validity and reliability of the sports nutrition knowledge questionnaire in adolescent athletes.

Materials and Methods

SNKABQ was first used by Walsh et al. (2011) to assess the sports nutrition knowledge, attitudes, and behavior of Irish athletic male students. The original version of the questionnaire was completed by 203 rugby players who were selected from six boys' schools in Ireland to determine their knowledge, attitudes, and behavior toward sports nutrition. In the present study, the questionnaire was translated based on the cultural and nutrition habits of Iranians and re-translated using the World Health Organization (WHO) standard method for the conversion of the original language into the language of the target community (11). After performing the translation-Retranslation process, the researcher examined the validity and reliability of the Persian version of the Sports Nutrition Knowledge Questionnaire in Iranian athletes aged 15 to 18 years (12). The final questionnaire used in this study was translated into Persian and modified by Iranian culture and eating habits and, includes the following five sections: 1. Athlete training program inside and outside the school, 2. Eating habits and Drinking (10 questions), 3. Nutrition attitude (5 questions), 4. Nutrition knowledge (13 questions), 5. Sources of the nutritional information of athletes.

In two-choice questions, each correct answer has 1 point, and the fault answer has 0, also in more than two-choice questions, the correct answer has 2 points, the fault answer has 0, and the “don’t know” response has 1 point. The minimum score that the subjects can receive in the translated version is zero and, the maximum score is ninety-eight points.

A panel of five experts also determined the face validity of the questionnaire, including three professors of sports sciences (Semnan University), one nutritionist, and one psychologist (Qazvin International University) who was familiar with Iranian cultural and dietary habits. The viewpoints of the experts were applied to the questionnaire. All the experts confirmed that the items of the Persian version of the sports nutrition knowledge questionnaire could accurately assess the knowledge and attitude of sports nutrition in athletes. In the Persian version of the questionnaire, the original version was modified to some extent. For instance, we added the choice of 4-6 days to item four (due to the possibility of selecting the choice by those consuming breakfasts four, five, or six days a week) and the milk choice to item eight regarding snack consumption (milk is a usual snack among Iranians). In addition, we changed the term "non-alcoholic beverage" to "drink" in item nine due to the prohibition of using the words describing alcohol and its derivatives in research questionnaires of students, and item 10 was also eliminated since it was also focused on the consumption of alcoholic beverages (non-compliance with the nutritional culture of Iranians).

To determine the time reliability using the test-retest method, the questionnaire was completed by 19 adolescent football players twice at two-week intervals. Following that, the correlation-coefficient between the obtained scores of the two tests was calculated, and the reliability coefficient was determined. The final version of the questionnaire was distributed among 191

athlete students (more than 5 sample per each item of questionnaire).

With regard to the sample population, we selected 174 adolescent athletes from the schools in districts one, four, seven, and 11 of Tehran (Iran) and 10 participants from the Wushu athletes who were involved in the Gold Belt Competitions held in Tehran in December 2019. In addition, seven other subjects from different cities in Iran completed the questionnaire online via a link.

The differential (diagnostic) validity of the questionnaire was determined by comparing two groups of novice and skilled athletes, which were classified based on the longest presence or gaining a sports position in national/provincial competitions or in Tehran. In addition, validity was assessed using Mann-Whitney test in SPSS version 24, and descriptive statistics were also used to categorize the raw data and adjust the tables.

The obtained data were not normally distributed as indicated by the K-S test (Table 1).

Result

The following tables (table 2-4) show the demographic information of the subjects. Table 5 shows the comparison of the novice and skilled athlete groups.

Table 1. Tests of Normality (Kolmogorov-Smirnov)

	Statistics	df	Sig
Habits	0.095	191	0.00
Attitude	0.117	191	0.00
Knowledge	0.098	191	0.00
Total	0.092	191	0.00

Table 2. Demographic Information (School Type)

	Female	Male	Total
All regions	99	92	191
District one of Tehran	1	10	11
District four of Tehran	0	19	19
District seven of Tehran	57	42	99
District ten of Tehran	1	0	1
District eleven of Tehran	28	19	47
District fourteen of Tehran	1	0	1
Cities around Tehran	6	0	6
Ilam	0	1	1
Markazi	2	0	2
Golestan	2	0	2
North khorasan	1	1	2

Table 3. Demographic Information (School Type)

School Type	Governmental	70	55	125
	Non-Governmental	29	37	66

Table 4. Demographic Information (Sports)

Sports	Female	Male	Total
Aerobics	1	0	1
badminton	6	3	9
Bodybuilding	2	7	9
basketball	5	6	11
Shooting	2	0	2
table tennis	5	2	7
Cycling	0	1	1
Track and Fields	1	1	2
Rock climbing	1	0	1
Equestrian	1	0	1
Swimming	7	0	7
Soccer	2	46	48
Futsal	6	2	8
Wrestling	0	3	3
Volleyball	23	7	30
Handball	1	1	2
Martial Arts	21	9	30
No answer to the relevant question	16	4	20

Table 5. Results of Mann-Whitney U Test for Differential Validity

	N	Mean Rank	Sum of Ranks
Elite	97	111.28	10794
Amateur	94	80.23	7542
Total	191		

Table 6. Test Statistics

Mann-Whitney U	3077
Wilcoxon W	7542
Z	-3.88
Asymptotes Sig. (two-tailed)	0.00

A significant difference was observed between the beginners and skilled athletes. With regard to reliability, the Cronbach's alpha coefficient indicated the total internal reliability of the questionnaire was 0.74. The coefficient was also determined for the subscales of eating and drinking habits (0.71), nutrition attitude (0.80), nutrition knowledge (0.70), and nutrition information sources (0.72) (Table 7).

Table 7. Results of Cronbach's Alpha Coefficient

Subscale	Number of Items	Cronbach's Alpha Coefficient
Eating and Drinking Habits	10	0.71
Nutrition Attitude	5	0.80
Nutrition Knowledge	13	0.70
Sources of Nutrition Information	7	0.72
Total	39	0.74

According to the information in Table 8, the intraclass correlation-coefficients were determined for the subscales of eating and drinking habits (0.92), nutrition attitude (0.75), nutrition knowledge (0.88), and nutrition information sources (0.89), as well as the entire questionnaire (0.86).

Table 8. Results of Intragroup Correlation-coefficients

Subscale	Number of Items	ICC
Eating and Drinking Habits	10	0.92
Nutrition Attitude	5	0.75
Nutrition Knowledge	13	0.88
Sources of Nutrition Information	7	0.89
Total	39	0.86

Discussion

Adequate knowledge of nutrition is essential to achieving sports goals and maintaining health and athletic success in childhood and adolescence. In Iran, the lack of a valid measurement tool for this purpose is an obstacle to the research regarding the sports nutrition of adolescents. Therefore, we aimed to prepare a questionnaire in Persian.

Due to the nature of the questionnaire (multiple-choice questions), we measured the internal reliability of the questionnaire based on Cronbach's alpha coefficient, and the internal reliability of the entire questionnaire was estimated at 0.74. In addition, we calculated the internal reliability of the subscales of eating and drinking habits (0.71), nutrition attitude (0.80), nutrition knowledge (0.70), and nutrition information sources (0.72). The obtained values in the present study were considered to be within the optimal range (α>0.7), which is consistent with the previous studies in this regard. Therefore, it was concluded that the questionnaire has acceptable internal reliability.

In a study Aylin Alsaffar et al. (2011) compared the nutrition scores obtained by engineering students and nutrition students to determine the validity of a questionnaire (13). To measure the construct validity of the questionnaire in the present study, the researcher divided the students into two groups of elites (skilled) and novice athletes based on standard definitions. Comparisons were made using different tests, and the results showed that the questionnaire could make fitting distinctions between the amateur and professional athletes. Therefore, the questionnaire was considered an accurate tool to assess the knowledge of sports nutrition in adolescent athletes.

Mai Matsumoto et al. (2017) evaluated the validity and reliability of the nutrition knowledge questionnaire in Japan, reporting the Cronbach's alpha coefficient of the entire questionnaire to be 0.95 (10). Furthermore, the findings of Wardell (1999), Hendrich (2008), and Al-Safar (2011) indicated this value to be 0.97, 0.92, and 0.89 in the United Kingdom, Australia, and Turkey, respectively. The values reported in the aforementioned studies confirm the optimal internal reliability of the general nutrition knowledge questionnaire in the selected samples (13-15), as well as the temporal reliability of the questionnaire. In another study performed in 2017, the reliability of the sports nutrition knowledge questionnaire in Italian adolescents was confirmed at the Cronbach's alpha coefficient of 0.86, indicating the optimal internal reliability of the questionnaire in the Italian adolescent population (16).

According to the previous studies regarding the nutrition knowledge questionnaire, the intergroup correlation-coefficient for temporal reliability in Japan has been reported to be 0.75, while the estimated coefficient in the Turkey, Australia, and United Kingdom is 0.86, 0.87, and 0.98, respectively (13-15). In a study conducted in Italy in 2017, the intragroup correlation-coefficient of the questionnaire was estimated at 0.83 using the test-retest method, which indicated proper time reliability as well (16). Similar to the mentioned study, we also applied the test-retest method to evaluate time reliability, and the intragroup correlation-coefficient of the eating and drinking habits subscale was considered excellent (0.929). In addition, the time reliability coefficient of the subscale of nutrition attitude was considered acceptable (0.75). Acceptable values were also obtained in the subscales of nutrition knowledge (0.88) and nutrition information resources (0.89).

Conclusion

According to the results, the Persian version of SNKABQ is a reliable and valid tool to be used by sports organizations, managers, coaches, and sports teachers to assess nutrition knowledge, attitude and behavior of adolescent athletes in Iran. Getting the right information and making decisions can ultimately lead to optimal sports performance and guaranteed health in children and adolescents.

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Effect of 12 Weeks of Regular Exercise with Vitamin C Supplementation on the Salivary IgA of Male High School Students

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ABSTRACT

Introduction: Immunoglobulins are humoral elements, which indicate the function of the immune system. Studies have shown that exercise and vitamin C supplementation could increase immunoglobulin levels. The present study aimed to evaluate the effect of 12 weeks of regular exercise with vitamin C supplementation on the salivary IgA of male high school students.

Methods: This clinical trial was conducted on 40 male high school students who were randomly assigned to four groups of 10, including exercise with vitamin C supplementation, exercise, vitamin C supplementation, and control group. Groups one and two performed the selected exercises for three sessions, and groups one and three consumed three vitamin C tablets (250 mg) per week. Saliva samples were collected 24 hours before the first training session (pretest) and 48 hours after the last training session (posttest). Data analysis was performed in SPSS version 22 using t-test, one-way ANOVA, and Tukey's post-hoc test at the significance level of P≤0.05.

Results: Salivary IgA was significantly affected by 12 weeks of regular exercise and vitamin C supplementation. The differences between the groups indicated that the IgA levels in the exercise with vitamin C group (P=0.001) and exercise group (P=0.001) were significantly higher compared to the control group. However, no significant difference was observed between the control and vitamin C groups in this regard (P=0.49).

Conclusion: According to the results, regular exercise and vitamin C supplementation could increase the salivary IgA levels of the male high school students.

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Introduction

The immune system is a collection of cells and molecules that respond to foreign substances and infectious microbes. Innate immunity is the primary defense provided by the immune system, followed by acquired immunity as secondary defense mechanisms. Acquired immunity involves lymphocytes and immunoglobulins (1). Immunoglobulins are generated and secreted by B lymphocytes and plasma cells. These substances are classified into five categories of IgG, IgA, IgM, IgD, and IgE. IgA is the predominant immunoglobulin in the mucosal, gastrointestinal, respiratory, tear, and milk fluids, as well as an important defense mechanism factor against the germs that enter the body through mucosal surfaces (2). Antibodies are involved in the identification of antigens and developing the immunological memory of antigens. The function of antibodies is to bind to antigens, so that they could not reach the cells and macrophages and other killer cells would be stimulated for the elimination of microbes (2). Factors such as physical activity, stress, nutrition, vitamin supplementation, and diseases may alter the quantitative and qualitative capacity of the immune system (3), and the role of minerals is considered pivotal in this regard (4).

According to a study, the intake of vitamin A, iron, folic acid, vitamin D, calcium, zinc, and vitamin C and their combined consumption could significantly affect the immune function (5). Vitamin C is the most important water-soluble antioxidant in the body, which reduces fat peroxidation through the inhibition of free radicals (6). According to the literature, vitamin C has remarkable antioxidant properties that may be dose-dependent to help improve the immune function and reduce respiratory infections (7). In addition, longitudinal research confirms the effects of vitamins (especially

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vitamin C) on the function and modulation of immunoglobulins, particularly IgA and IgG, which could prevent and enhance the symptoms of colds (7). The same study also demonstrated that vitamin C has modulatory effects on cytokines, as well as tonic effects on other immune system factors, such as immunoglobulins in acute respiratory distress syndrome (7).

Moderate exercise increases the respiratory capacity and resistance against infections (e.g., colds), while strenuous exercise is associated with the increased risk of upper respiratory tract infections (8). Regular and long-term aerobic exercise in elderly men has been reported to increase the number of $CD25^+$ cells, interleukin-2 (IL-2), and salivary IgA (9). Moreover, moderate exercise has been shown to reduce the incidence of upper respiratory tract infections by 29%, while prolonged, high-intensity physical exercise is associated with a higher risk of these infections (10).

Evidence suggests that 12 weeks of exercise combined with breathing exercises could improve the pulmonary function and maximize oxygen consumption, while also decreasing the risk of depression in women with mild-to-moderate asthma (11). Similarly, eight weeks of modified Pilates exercise has been reported to enhance the pulmonary function and quality of life of chemical warfare victims (12). On the other hand, the results of a study indicated that breathing exercises had no significant effect on the pulmonary function of patients with chronic bronchitis (13).

In another research, the effects of two consecutive weekly training sessions were investigated on the levels of IgA and cortisol 20 in professional male gymnastics. The obtained results indicated no significant change in IgA, while the cortisol levels were observed to increase in the subjects. In the mentioned study, no significant correlation was denoted between IgA and cortisol (14). In a similar study, the effects of a soccer simulation training session were evaluated on salivary IgA, IgG, IgM and cortisol concentrations, and the researchers concluded that the exercises may cause the temporary weakening of IgA in the athletes possibly due to their duration and intensity (15). Given the discrepancy in the proposed findings in sport science studies, it seems that the effects of factors such as exercise, sleep, rest, nutrition, and vitamins on the immune function should be further explored. The research of the World Health Organization (WHO) has highlighted the role of factors such as an active lifestyle and nutrition in this regard (7).

The present study aimed to investigate the effect of 12 weeks of regular exercise with vitamin C supplementation in winter on the rate of salivary IgA in male high school students in District 17 of Tehran, Iran.

Materials and Methods

This clinical trial was conducted with a pretest-posttest design and a control group. Initially, the authors announced the conduction of the research to all the high schools of second period in District 17 of Tehran, and two high schools were selected via cluster sampling. At the next stage, the research objectives, benefits, and limitations were explained to the participants. In total, 40 students aged 15-18 years with the height of 160-175 centimeters, weight of 62-70 kilograms, and no history of cardiovascular diseases, acute pulmonary diseases, and other diseases affecting the research process were randomly selected as the sample population. Written informed consent was obtained from the subjects and their parents prior to enrollment.

The participants were randomly divided into four groups of 10, including exercise with vitamin C supplementation, exercise, vitamin C supplementation, and control. Data were collected using a researcher-made checklist to assess the level of salivary IgA at the pretest and posttest.

Exercise Protocol

The exercise groups performed the selected exercises for 12 weeks three sessions per week (total: 36 sessions, 90 minutes each). The exercise protocol consisted of general aerobic and stretching warm-ups and special combination exercises (10 minutes), skills training or review of the techniques and tactics (20 minutes), handball and futsal exercises (50 minutes), and aerobic and stretching exercises for cool-down (10 minutes) (16). The vitamin C supplementation groups received three vitamin C tablets (250 mg each) weekly during the study period (18).

The study protocol was approved by the Ethics Committee of Tehran Islamic Azad University of

Medical Sciences (code: IR.IAU.TMU.REC.1399.109).

Variable Measurement

Saliva samples of the subjects were collected 24 hours before the first training session (pretest) and 48 hours after the last training session(posttest) by a laboratory specialist and the researcher early in the day using DEXK276 laboratory kit (made in Germany), with the accuracy of microgram per microliter. Initially, each subject rinsed their mouths with water for one minute and rested for one minute. Following that, saliva samples were collected from their mouths within 60 seconds.

The samples were transferred to the laboratory for measurements. Salivary IgA sample levels were determined using the mentioned kits in the ELISA reader device (18). Data were recorded as the pretest and posttest of the study groups. Data analysis was performed in SPSS version 22 using t-test, one-way analysis of variance (ANOVA), and Tukey's post-hoc test at the significance level of P≤0.05. In addition, Shapiro-Wilk test was applied to assess the normal distribution of the data, and t-test was used to evaluate the differences between the pretest and posttest of the study groups. The differences between the groups were also assessed using one-way ANOVA and Tukey's post-hoc test.

Results

Table 1 shows the mean demographic variables of the study groups. Table 2 shows the mean salivary IgA levels of the study groups at the pretest and posttest. Figure 1 depicts the results of Tukey's post-hoc test regarding the differences between the study groups.

Table 1. The mean demographic variables of the study groups

Experimental Groups	Age (year)	Height (cm)	Weight (kg)
Exercise with Vitamin C	1.10±16.01	7.95±168.85	6.80±69.00
Exercise	0.84±16.28	8.81±168.40	8.07±67.80
Vitamin C	0.99±16.16	7.92±169.50	7.45±68.45
Control	0.71±16.00	8.89±168.83	8.11±68.50

Table 2. The mean salivary IgA levels of groups at the pre-test and post-test

Experimental groups	Pretest IgA	Posttest IgA
Exercise with Vitamin C	13.25±69.49	11.10±97.95
Exercise	13.18±69.64	11.10±91.97
Vitamin C	13.10±68.90	11.58±78.52
Control	14.03±69.87	14.03±74.08

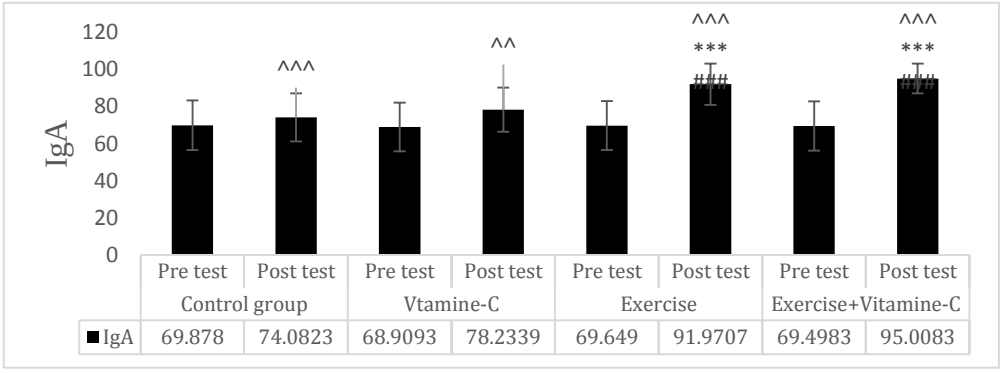

Figure 1. IgA Levels in Study Groups (^^P=0.01 and ^^^P=0.0001 show significant increase compared to pretest; ***P=0.001 shows significant increase compared to control group; ###P=0.001 shows significant increase compared to vitaminC group)

The results of one-way ANOVA indicated significant differences between the groups at the posttest. Moreover, the results of Tukey's post-hoc test showed that the salivary IgA levels of the exercise with vitamin C group (P=0.001) and exercise group (P=0.001) were significantly higher compared to the control group. However, no significant difference was observed in the IgA level between the control and vitamin C groups (P=0.49). The IgA levels in the exercise with vitamin C (P=0.001) and exercise groups (P=0.001) were significantly higher compared to the vitamin C group. Nevertheless, no significant difference was observed between the exercise

group and the vitamin C with exercise group in this regard (P=0.76).

The results of paired t-test regarding the changes at the posttest compared to the pretest of each study group indicated that the posttest IgA levels of the exercise with vitamin C supplementation group (P=0.001; t=-21.56), exercise group (P=0.001; t=-21.58), vitamin C supplementation group (P=0.001; t=2.47), and control group (P=0.001; t=8.35) were significantly higher compared to the pretest levels.

Discussion

According to the results of the present study, salivary IgA levels increased more significantly in the male high school students of the regular exercise with vitamin C supplementation group and regular exercise group compared to the vitamin C supplementation and control groups at the posttest. However, the difference between the vitamin C supplementation and control groups at the posttest was not considered significant in this regard. Notably, salivary IgA levels increased at the posttest in all the study groups compared to the pretest.

In the present study, regular exercise increased the level of salivary IgA at the posttest compared to the pretest. Therefore, it could be inferred that regular exercise could enhance the immune function through mechanisms such as the differentiation of Th1 to Th2 defense cells, increasing the level of anti-inflammatory factors (e.g., IL-10), increasing the secretion of immunoglobulins by lymphocytes, and decreasing cytokines. Furthermore, pro-inflammatory outcomes may be possible in the form of releasing interferon gamma, IL-1 receptors, and tumor necrosis factor receptor alpha, which improve the antigen expression of macrophages (Mϕ or Mp) followed by the development of phagocytic and cytotoxic in the mucosa (16). Previous findings in this regard have demonstrated that low- and moderate-intensity exercise could enhance the immune function (e.g., IgA) (19). Furthermore, another study on children with spastic cerebral palsy (age: 9.5±1.8 years) indicated that 12 weeks of resistance and endurance exercise training had significant effects on salivary immunoglobulin A, salivary alpha-amylase, and total salivary protein (20).

According to a research conducted on 216 subjects with depressive symptoms and IgA nephropathy, regular physical activity was reported to improve the cardiopulmonary function, depressive symptoms, and health-related quality of life in the patients (21). On the other hand, several studies have confirmed that intense exercise, poor nutrition, and insufficient sleep may modulate immunoglobulins and suppress the immune system (1). Furthermore, high-intensity exercise has been shown to decrease the immune function and some immune factors, such as immunoglobulins (16).

In an eight-week study aiming to investigate the effects of continuous aerobic training with a low-carb diet on the serum immunoglobulins in overweight adult men aged 36-50 years, the levels of IgA, IgG, and IgM were reported to decrease (17). Moreover, the results of a six-week study regarding the effects of aerobic exercise (three 60-minute sessions per week) on the body composition, cortisol, and salivary IgA levels of 19-year-old subjects indicated no significant impact on the salivary IgA (22). Evidence suggests that depending on intensity and duration, exercise could improve general health and the immune function (23).

According to the current research, regular exercise with vitamin C supplementation could increase the salivary IgA levels at the posttest compared to the pretest. In another research, five days of vitamin C supplementation (500 mg/day) followed by an exhaustive aerobic exercise session increased serum IgA and IgG levels on the sixth day in 32-year-old women (24). Another study showed the significant effects of vitamin C intake (500 mg per day for five days) on serum IgA and IgG in 24 male Wushu athletes following exhaustive aerobic training (3). Meanwhile, some findings have indicated that high-intensity exercise and vitamin C have no significant effects on immunoglobulin levels (19, 17). For instance, a seven-day study of young women with the mean age of 22 years showed that the effects of exercise (two sessions of exhaustive exercise) and vitamin C (1,000 mg per day) on salivary IgA, cortisol, and total salivary proteins were not significant (25).

Some studies have also denoted that factors such as vitamin dosage, type of exercise, age, and geographical location could affect the cellular and humoral immune function (2). By

participating in the structure of antioxidants, vitamin C could increase antioxidant and anti-inflammatory activity and improve the molecular and humoral immune function (26). In addition to vitamin C, the presence of other structural vitamins (e.g., vitamins E and D) in the production of antioxidants may be effective in the improvement of the cellular and humoral immune function and protection against pulmonary diseases (26, 27).

The results of the present study indicated that vitamin C consumption could increase the level of salivary IgA (immune factor) at the posttest compared to the pretest. According to the literature, consuming the minimum vitamin C dosage of 10 milligrams per day could enhance the immune function, while two grams of this vitamin per day is recommended to adults by the United States Food and Drug Administration (FDA) (27). In addition, consuming 1,000 milligrams of vitamin C per day may be beneficial and uncomplicated for pneumonia patients, while 2-4 grams of vitamin C could decrease the risk of catching a cold more significantly (27). In line with these data, vitamins C, E, and D have been shown to improve the immune function and several immune factors (26, 27).

Diarrhea is the only side-effect of high-dose vitamin C consumption, and no other complications have been attributed to this supplement (8). Notably, the consumption of 10-15 milligrams and 0.3 gram of vitamin C per day has been reported to be insufficient for the improvement of the immune function and the prevention of acute pulmonary diseases in men (28). Therefore, it could be inferred that various doses, combinations, and duration of vitamin C intake could exert different effects on the immune function and immune factors.

According to the results of the present study, the salivary IgA level of the control group increased slightly at the posttest compared to the pretest, which could be due to the fact that our study was performed during winter, in which the probability of antigens entering the body is higher, which in turn increases the immune response and secretion of more antibodies (2).

One of the limitations of current research was the lack of control over the nutrition and medication of the subjects. However, recommendations were made to avoid the unnecessary use of medications during the research. Another limitation was failure to measure other immunoglobulin levels. Therefore, it is recommended that the levels of these physiological variables be evaluated in the further investigations in this regard. Moreover, we were not able to examine various doses of vitamin C and other vitamins, as well as exercises with varied intensity, which should be addressed in similar studies.

Conclusion

According to the results, regular exercise and vitamin C supplementation alone and exercise combined with vitamin C supplementation could increase the salivary IgA levels of the male high school students after 12 weeks. Therefore, it is recommended that further investigations assess the impact of various exercises or doses of different vitamins on immunoglobulin levels.

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Effects of Cinnamon Extract Consumption and Swimming Exercise on the Expression of ATGL and CGI-58 in the Visceral Adipose Tissue of Streptozotocin-induced Diabetic Rats

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ABSTRACT

Introduction: Diabetes is a widespread disease, and various techniques are used for its prevention, control, and treatment, including physical exercises and medicinal herbs. Natural medicines and exercise are comparatively inexpensive and cause fewer complications compared to chemical drugs. The present study aimed to evaluate the effects of a swimming course and cinnamon extract consumption on the expression of adipose triglyceride lipase and CGI-58 in the visceral adipose tissue of streptozotocin-induced diabetic rats.

Methods: This experimental study was conducted on 28 diabetic rats, which were randomly divided into four groups of seven, including 1) control (C),2) cinnamon use (Ci),3) swimming course(S), and 4) cinnamon extract with swimming (S+Ci). The animals received the intended treatment for six weeks in packs of three and four and five classes per week, while the rats in groups two and four received 200mg/kg of cinnamon orally every day for six weeks. Data analysis was performed using the one-way analysis of variance (ANOVA) and Tukey's post-hoc test at the significance level of P≤0.050.

Results: The results of ANOVA indicated that *ATGL* gene expression in the S (P=0.04) and S+Ci groups (P=0.0001) was significantly higher than the control group. In addition, *ATGL* gene expression in the S group (P=0.0006) was higher compared to the Ci group, while it was significantly higher in the S+Ci group (P=0.0001) compared to the Ci and S groups. *CGI-58* gene expression in the S+Ci group (P=0.0001) was also significantly higher than the control, S, and Ci groups, while it was significantly higher in the S group (P=0.036) compared to the Ci group.

Conclusion: According to the results, swimming exercise and cinnamon extract consumption increased the expression of CGI-58 and ATGL protein in the diabetic rats. Therefore, it seems that CGI-58 plays a key role in the activation of lipolysis by ATGL, and higher CGI-58 could increase ATGL, which ultimately accelerates the lipolysis process, reduces fat, and improves insulin resistance in the visceral adipose tissue.

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Introduction

Diabetes is a common metabolic disorder characterized by high blood sugar levels due to insufficient insulin secretion, insulin resistance, or a combination of both. Insulin resistance plays a pivotal role in the pathogenesis of type II diabetes (1). The chronic complications of diabetes are directly correlated with high blood glucose levels (2).Hyperglycemia leads to the non-enzymatic binding of glucose to the proteins inside and outside the cell, and patients with long-term diabetes often experience kidney failure, eye damage, cardiovascular failure, and central nervous system deficiency.

Type II diabetes is often associated with the disorders of lipid metabolism, and plasma fatty acids play a key role in increasing insulin resistance. As such, plasma fatty acids cause dyslipidemia in diabetic patients through increasing the very-low-density lipoprotein center in the liver and cholesterol-transporting proteins, thereby increasing low-density lipoprotein (LDL) and decreasing high-density lipoprotein (HDL).This atherogenic function of lipoproteins (i.e., increased triglycerides, increased LDL, and decreased HDL) causes atherosclerosis and increases the risk of cardiovascular events, which is the most common cause of death in type II diabetic patients (3). Therefore, extensive research is required on the factors that could reduce the pathogens in this patient population.

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Comparative gene identification-58 (CGI-58) is an extremely small protein that controls intracellular triglyceride levels by activating adipose triglyceride lipase (ATGL). Recent studies have emphasized the importance of CGI-58 as a regulator of intracellular energy homeostasis by modeling the hydrolysis of triacylglycerol (TAG) using ATGL. Correspondingly, the rats and humans lacking CGI-58 accumulate TAG in various tissues (4). Not only the presence of CGI-58 will increase ATGL activity, but it also expands its specific substrate, which highlights the significance of this protein in fat metabolism and energy (4). In the absence of ATGL, CGI-58 cannot increase lipolysis activity through any other pathways (5). Therefore, the interaction between ATGL and CGI-58 plays a pivotal role in the lipolysis and hydrolysis of TAG by lipases (6-8). If CGI-58 is combined with ATGL, lipolytic activity increases significantly compared to ATGL alone (9).

Physical exercise could effectively improve obesity through the activation of lipolysis (10). Evidence suggests that the visceral fat mass increases after detraining, and exercise without calorie restriction increases the level of fat-dependent proteins in the adipose tissue of obese rats, thereby reducing the volume of visceral fat and the size of fat droplets. Visceral fat is considered to be a stronger cause of metabolic diseases compared to subcutaneous fat. Moreover, it has been proposed that exercise after 15 weeks of high-fat diet could increase CGI-58 and ATGL by activating the catecholamine pathway and increasing cAMP and perilipin-1 levels, which in turn has a positive effect on increased lipolysis and decreased fat mass, while non-training has an opposite effect (11).

Research indicates that ATGL activity may be regulated by phosphorylation in response to hormonal stimulation during exercise (12). Therefore, high-intensity exercise training increases ATGL levels by increasing beta-adrenergic stimulation, which could increase the lipolysis process. In this regard, Hashimut et al. (2013) investigated obese rats and reported that six weeks of moderate aerobic training causeda significant decrease in epidermal fat and a significant increase in ATGL and CGI-58 (13).Furthermore, the findings of Jo Yang B et al. (2017) indicated that eight weeks of training could increase CGI-58 and ATGL (11).

Given the importance of the intracellular mechanisms of IMTG lipolysis in reducing or increasing the inflammatory processes caused by diabetes and other metabolic disorders, the storage and release of the regulatory factors of IMTG (e.g., exercise) must be investigated. Several studies have shown that exercise could further reduce the fat mass by activating the AMPK, PPARy, and PPARG pathways as the important activators of cAMP, thereby increasing the levels of perilipin-1, ATGL, and CGI-58 (14-16).

Today, the traditional treatment of diabetes by using medicinal plants and herbal extracts is widely practiced across the world (17).Cinnamon (*Cinnamomum zeylanicum*) is a plant belonging to the Lauraceae family, which is has long proven effective in the treatment of diabetes (18).Recent finding son cinnamon have confirmed its ability to lower blood sugar (19). Different parts of the cinnamon plant (including its skin) are reported to have multiple the rapeutic properties, and the consumption of cinnamon is known to strengthen the heart, stomach, and intestines. Moreover, cinnamon could improve kidney function and increase sexual potency (20).High levels of antioxidants in cinnamon cause the plant to act as a cell protector against chemical damage (e.g., environmental toxins), reduce lipid peroxides, and protect the liver against stress. Therefore, cinnamon may play a key role in improving the antioxidant state of obese diabetic patients, as well as patients with cardiovascular diseases and metabolic syndrome (21). Studies have also shown that cinnamon is more effective in the regulation of glucose metabolism compared to other herbal products, such as green tea, olive oil, garlic, and onion (22,23).

According to the literature, daily consumption of one gram of cinnamon for 30 days could reduce the blood glucose and lipid levels of diabetic patients (22). Today, experts believe that diet and medication alone are not sufficient in the treatment and control of the glucose and fat metabolism of diabetic patients, and physical activity and exercise should be incorporated into the daily routine of this patient population.

Given the beneficial effects of cinnamon consumption and sports activities on diabetic patients and considering that no research has been focused on the effects of these factors on these patients, the present study aimed to

evaluate the correlation between exercise training and cinnamon extract consumption on the expression of adipose glyceride lipase and CGI-58 in the visceral adipose tissue of streptozotocin-induced diabetic rats.

Materials and Methods

Subjects

This experimental, applied research was conducted on 28 rats aged 8-10 weeks, which were purchased from the Center for the Breeding and Reproduction of Laboratory Animals affiliated to Islamic Azad University of Marvdasht Branch, Iran. The animals were kept in the Animal Sports Physiology Laboratory of the university for one week to be subjected to the adaptation course. Notably, the animals were kept in standard conditions within a 12-hour light/dark cycle with 55% humidity at the temperature of 22-24° C inside transparent polycarbonate cages, which were equipped with autoclave functionality and free meals. After one week, 28 rats received a peritoneal injection of streptozotocin (Sigma, USA) at the concentration of55 mg/kg of the body weight. After four days, the blood glucose range of the rats was measured using the tail punching method.

To homogenize the study groups, the diabetic rats were divided into four groups of seven based on their fasting blood glucose, including 1) control, 2) cinnamon consumption(Ci), 3) swimming course (S), and 4)swimming course plus cinnamon consumption(S+Ci). The rats in the training groups completed the intended course for six weeks (five sessions per week), and each session was implemented in line with the specific protocol of the current research. The rats in the cinnamon consumption groups were administered with an aqueous extract of cinnamon orally at the concentration of 200 mg/kg of the body weight.

After six weeks of the intervention and 48 hours after the final training session, the rats in all the study groups were anesthetized via the peritoneal injection of ketamine and xylazine (3:1) following 16 hours of fasting. After confirming full anesthesia by ache reflex tests through squeezing the tail and ensuring that analgesia by laboratory specialists, blood samples were collected directly from the target tissues using a 5 cc syringe, and biopsies were extracted by the lab specialists. The blood samples were immediately frozen at the temperature of -80°C.

Swimming Exercise Protocol

To evaluate the ability of the rats to perform the swimming training, they swam in a special rats' swimming pool at a moderate temperature. Afterwards, their activities were meticulously observed for two minutes, and the process was repeated three times a week to familiarize the rats with the training conditions. Subsequently, swimming training was performed for two minutes five days a week in the first week in accordance with the study by Lubkaska et al. (2019). Moreover, 30 seconds was added to the training time in each training session until the training duration reached four minutes. Following that, the rats trained for four minutes until the end of the sixth week. The rats' swimming pool was composed of a special swimming tank for rats with the length of 100 centimeters, width of 50 centimeters, and depth of 50 centimeters (24).

Cinnamon Supplementation

Initially, 200 grams of cinnamon powder was dissolved in 1000 milliliters of pure water and boiled for 10 minutes. After cooling, the solution was filtered through paper filter No. 1. The prepared solution contained 20% cinnamon extract, with each milliliter containing 200 milligrams of cinnamon extract. For the daily consumption of each rat in a cage (equivalent to approximately one kilogram), 0.2 cc of the solution was added to their drinking water (25).

Measurement of the Research Variables

A molecular research was conducted at a gene expression level. For this purpose, RNA was initially extracted from the visceral fat tissues, and the process was performed in accordance with the manufacture's protocol in Iran. A mild absorption at the wavelength of 260 nanometers was used to quantitatively acquire the concentration and purity of the RNA samples. To this end, we used the following formula:

$C\ (\mu g/\mu l) = A260\times \varepsilon\times d/1000$

After extracting extremely high purity and RNA concentration from the samples, the guidelines for cDNA synthesis in the fermentas kit (K1621) were applied, and the synthesized cDNA was processed for a reverse transcription reaction. Initially, the designed primer-related genes were examined, and the expression of the genes was assessed using the quantitative q-RT-PCR

method. In addition, their relative expression was determined using the 2-ΔΔCt formula.

Statistical Analysis

Data analysis was performed in SPSS version 24 using descriptive statistics (mean and standard deviation [SD]) to obtain the data. Moreover, the Shapiro-Wilk test was used to assess the normality of data distribution, and one-way analysis of variance (ANOVA) and Tukey's post-hoc test were employed to compare the performance of the animals. All the statistical analyses were carried out at the significance level of P<0.05.

Results

According to the results of one-way ANOVA, eight weeks of swimming and cinnamon consumption significantly increased the *ATGL* gene expression in the visceral adipose tissue of the diabetic rats(P=0.001). Furthermore, the results of Tukey's post-hoc demonstrated that the *ATGL* gene expression in the S group (P=0.04) and S+Ci group (P=0.0001) was significantly higher compared to the control group. *ATGL* gene expression was also significantly higher in the S group (P=0.0006) compared to the Ci group, while it was significantly higher in the S+Ci group (P=0.0001) compared to the Ci and S groups (Figure 1).

The findings of one-way ANOVA confirmed that eight weeks of a swimming course along with cinnamon consumption could significantly increase*CGI-58* gene expression in the adipose tissue of the diabetic rats(P=0.001). Furthermore, the results of Tukey's post-hoc test indicated that *CGI-58* gene expression in the S+Ci group was significantly higher than the control, S, and Ci groups (P=0.0001). *CGI-58* gene expression in the S group was also significantly higher compared to the Ci group (P=0.036) (Figure 2).

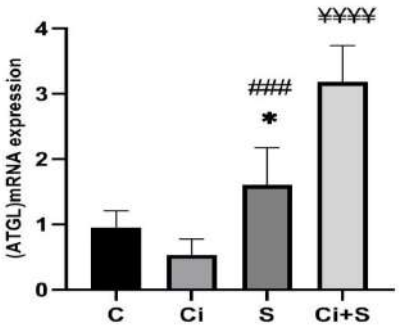

Figure1. ATGL gene expression levels in the visceral adipose tissue of diabetic rats in the four study groups
* (P= 0.01): significantly increase compared to the C groups;
(P = 0.001): significant increase compared to the Ci group;
¥¥¥¥ (P = 0.0001): significant increase compared to the Ci, C, and S groups.

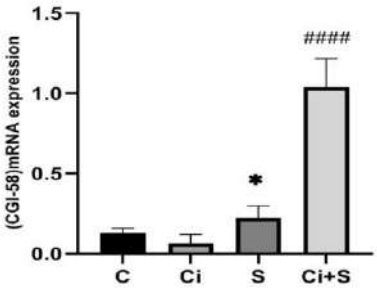

Figure 2: CGI-58 gene expression levels in the visceral adipose tissue of diabetic rats in the four study groups
*(P= 0.01): significant increase compared to the Ci group;
####(P = 0.0001): significant increase compared to the Ci, C, and S groups.

Discussion

The present study aimed to investigate the effects of swimming training with cinnamon extract consumption on the expression of adipose triglyceride lipase (*ATGL* and *CGI-58* genes) in the visceral adipose tissue of streptozotocin-induced diabetic rats. The results of the first hypothesis showed a significant difference in the gene expression of adipose triglyceride lipase (i.e., ATGL) in the visceral adipose tissue after the training intervention with cinnamon supplementation. It seems that a significant increase in the ATGL of the trained diabetic rats could regulate the mobilization and fat consumption of IMTGs through intracellular and hormonal metabolites. Unlike adipose tissue lipolysis, which is stimulated over time by the depletion of the body's energy, lipolysis in the skeletal muscles responds specifically to local muscle demands. The results of research by Tronbol et al. (2016) also indicated that eight weeks of endurance training increased the ATGL protein in all the training skeletal muscles of rats (26), and the increased ATGL expression could probably maintain the low metabolic concentration of muscle fatty acids, thereby improving insulin sensitivity in strenuous training.

In another study, Yao Brongaser et al. reported that the increased rate of IMGT breakdown was associated with increased insulin sensitivity following exercise training. The increased expression or activity of lipolytic enzyme (i.e., ATGL) by endurance training facilitates the breakdown of IMGT, thereby providing the adequate substrate to the muscles. Due to the intensity and duration of training and the level of access to the substrate, the amount of FA used for oxidation metabolism during endurance training reaches its maximum (27), while at higher intensities, plasma free fatty acid levels do not change despite increased adipose tissue lipolysis, and the higher ATGL expression ultimately in diabetic rat's increases lipolysis. As a result, the release of fatty acids will increase, leading to cell requirements in the absence of energy glucose. Other proven factors (e.g., insulin depletion and alpha-adrenergic pathways) have also been reported to increase lipolysis in diabetic patients (28).

According to the findings of Morville et al. (2017), the repetition of long-term training could reduce fat oxidation in elderly men and significantly increase the expression of HKII, GLUT4, and ATGL proteins, which in turn increases the glucose transport capacity and muscle lipolysis capacity, contributing to higher external glucose and intracellular fat during training (29).In the current research, the consumption of cinnamon caused ATGL expression in the diabetic rats compared to the control group. In contrast, Belvin et al. conducted a study in the United States, reporting that consuming one gram of cinnamon per day for three months had no effects on the fat, triglyceride, cholesterol, LDL, and HDL profiles of the subjects, and no significant reduction was denoted in these markers (22).

A meta-analysis performed by Baker et al. indicated that cinnamon could not affect blood lipid levels compared to placebo (30).In another study conducted by Mirfeiz et al. in Karaj (Iran), consuming 500-milligram cinnamon capsules twice a day for 90 days caused no significant difference in blood lipid profiles compared to the control group(31). Furthermore, the results of a clinical trial conducted by Zahmatkesh et al. in Yazd (Iran) demonstrated that receiving two grams of cinnamon per day for eight weeks had no effects on the required indicators compared to the placebo group, and no significant differences were observed between the two groups in this regard (32).Previous findings in this regard are not consistent with the present study, which could be due to differences in the amount of prescribed cinnamon, duration of cinnamon consumption, and the sample populations.

Cinnamon could lower blood glucose and lipids due to its cinnamaldehyde content. Cinnamaldehyde is responsible for increasing insulin release, increasing insulin sensitivity, and regulating the activity of the tyrosine phosphatase enzyme (33). In the current research, the results of the second hypothesis test indicated a significant difference in the gene expression of *CGI-58* in the visceral adipose tissue after the training intervention combined with cinnamon supplementation. Moreover, a significant difference was observed in CGI-58 protein expression between the intervention and the control groups. Therefore, a tendency to increase was observed in CGI-58 with exercise training.

Since CGI-58 is associated with ATGL and the interaction between CGI-58 and ATGL increases AGTL regulatory activity from baseline to active, increasing lipolysis by ATGL could also be attributed to higher CGI-58. According to the

study by Nagy et al. (2014), ATGL is required for the effective mobilization of triglycerides in the adipose and non-adipose tissues, and ATGL is essential to the availability of fatty acids for metabolic reactions. ATGL is regulated by a complex network of lipolytic and anti-lipolytic hormones. In addition, signals by controlling enzymatic expression and ATGL interaction with the regulatory protein CGI-58 in the ATGL regulatory function indicate the key role of this protein particularly in fat metabolism and muscle energy (34). On the other hand, the up-regulation of CGI-58 expression in primary human myotome accelerates TAG depletion, while increasing lipolysis and FA oxidation (35). In contrast, degenerated GCI-58 decreases lipolysis and increases TAG accumulation, indicating thatCGI-58 plays a pivotal role in the regulation of skeletal muscle ATGL activity and TAG dynamics, especially during training(36).

Recent findings have confirmed the key role of CGI-58 in the activation of lipolysis by ATGL, as well as its effects on the adipose tissue metabolism. Therefore, the increase in CGI-58 through ATGL could be justified in the present study. On the other hand, cinnamon consumption caused the expression of CGI-58 in the diabetic rats compared to the diabetic control group. Due to the presence of hydroxyl groups in its molecular structure, the polyphenolic compounds of cinnamon affect the lipid and phospholipid membranes of cells and integrate with lipid layers, thereby increasing intracellular dynamics and biological activity. In addition, they stimulate the sympathetic system and increase basal metabolism, exerting a greater impact on the fat stores through oxidation and heat production in the body, followed by an increase in energy consumption 24 hours a day (21). According to the findings of Brodhurst et al., the compounds in cinnamon could increase the action of insulin three fold and decrease the insulin resistance of the epidermal fat cells in rats (37).

Conclusion

According to the results regarding the effects of combined exercise training and cinnamon extract as an antioxidant supplement, these two factors could increase the expression of ATGL protein and decrease serum insulin and glucose levels in diabetic rats. Moreover, increased levels of ATGL lead to higher insulin sensitivity and improved insulin resistance in diabetic rats. Therefore, it seems that CGI-58 plays a pivotal role in the activation of lipolysis by ATGL, and increased CGI-58 leads to ATGL, which ultimately accelerates the lipolysis process, reduces fat, and improves insulin resistance in the visceral adipose tissue.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Does Ramadan Fasting Alter the Resting Metabolic Rate, Body Composition and Dietary Intake of Overweight and Obese Adults?

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ABSTRACT

Introduction: Ramadan fasting (RF) is a model of calorie restriction similar to intermittent fasting, which is extensively practiced by a large population of Muslims. Limited and controversial studies have investigated the physiological changes induced by 30 days of RF. The present study aimed to evaluate the effects of Ramadan fasting on the resting metabolic rate and body composition of overweight and obese individuals.

Methods: This study was conducted on 21 men and women aged 18-40 years. The subjects avoided eating and drinking for one month during the holy month of Ramadan (at least 20 days) and received a routine diet without an intervention between Iftar and dawn (Sahur). Before and after the study, the energy of the basal metabolic rate was measured via indirect calorimetry, and body composition was measured by bioimpedance (InBody s10). In addition, the physical activity of each participant was evaluated using a pedometer during one week. During the study, each participant received a 24-hour recall once a week.

Results: Compared to the pre-study period and after four weeks of RF, body weight (77.91±11.83 vs. 77.01 ±11.75 kg; P<0.05) decreased, while the body fat percentage (34.84±7.07 vs. 38.48±5.93%; P<0.001) increased. However, the resting metabolic rate had no significant changes (P=0.641), while the total fat-free mass and visceral fat mass significantly deceased after the study (P<0.001). The mean daily energy intake increased after the study compared to before RF (3,290±785.82 vs. 2,458±535.32 kcal; P<0.05). Moreover, the total carbohydrate and sugar intake increased significantly after RF (437.04±101.02 and 194.04±56.87 g per day vs. 310.09±87.12 and 60.42±31.57 g per day).

Conclusion: According to the results, RF may effectively improve metabolic parameters and prevent the decline of the basal metabolic rate if accompanied by nutritional support and healthy dietary recommendations.

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Introduction

Obesity and overweight have recently reached epidemic proportions in the world. The current prevalence of overweight and obesity in Iran has increased to 36.6% and 22.7%, respectively (1). A sedentary lifestyle and the over-consumption of energy-dense foods are considered to be the primary risk factors for obesity and overweight (2). Weight reduction could be optimally achieved by a multimodality approach and adapting to a healthy lifestyle through diet modification and physical activity (3). Several meta-analyses and systematic reviews have been focused on the effects of various dietary restriction patterns on weight loss and metabolic changes. However, conflicting results have been proposed regarding fasting, starvation, and weight gain (4).

Ramadan fasting (RF) is a model of calorie restriction *similar to intermittent fasting, which is extensively practiced by a large population of Muslims (5). Nevertheless, limited and*

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controversial studies have investigated physiological changes induced by 30 consecutive days of RF (6). The inconsistency of the results could be attributed to confounding factors such as ethnicity, fasting duration, climatic conditions, cultural influences, physical activity, and mostly dietary patterns. The duration of RF on a certain day varies in different geographical regions (8-18 hours) depending on the equator and season of the year (7).

Previous studies have denoted that RF causes profound changes in the body composition and metabolic balance. The basic data regarding the changes in dietary intake and body composition in most of these investigations have indicated that the majority of Muslims typically consume two meals per day during Ramadan, one of which is before dawn, and the other is taken after sunset (8). Recently, investigations on energy intake and body composition changes during Ramadan have drawn varied conclusions, which may be due to the differences in nutritional habits and duration of fasting (9). Changes in dietary habits during Ramadan occur in the form of the reduced frequency of food and beverage intake and the increased tendency to the consumption of high-calorie foods and drinks (10, 11). These changes could lead to declined energy intake, weight loss, and dehydration. Dehydration is reflected in the reduced body weight and changes in the biochemical parameters associated with the body water status (12, 13). In a study in this regard, Al-Hourani H. M. reported that body weight and body mass index (BMI) decreased significantly during fasting periods (14). Although reduced body weight has been reported frequently, the association of weight loss and body fat reduction remains to be further investigated (15). Inconsistent findings have also been reported regarding the effects of fasting on the body composition (16, 17), and some investigations have not detected significant changes in the body weight or body composition during Ramadan (16). In addition, the exact effects of RF on metabolic parameters remain debatable.

To the best of our knowledge, no reports have been previously published on the effects of RF on the resting metabolic rate and body composition of obese and overweight individuals. The present pilot study was conducted at *Imam Reza Hospital* in Mashhad, Iran to evaluate the effects of RF on the resting metabolic rate, body composition, and dietary intake of overweight and obese adults.

Materials and Methods

Subjects

This cross-sectional trial was conducted in Mashhad, Iran in 2019. The subjects were randomly selected based on the inclusion and exclusion criteria. In total, 25 subjects were recruited, four of whom withdrew, and 21 subjects (16 females and five males) aged 18-40 years completed the study. The inclusion criteria were as follows: 1) BMI ≥25 and <40 kg/m^2; 2) stable body weight within three months before the study; 3) no history of bariatric surgery; 4) no use of weight loss drugs within the past six months and 5) no medical history of hypertension, diabetes, fatty liver, dyslipidemia, and cardiovascular diseases. The exclusion criteria of the study were pregnancy and lactation.

The patients stated that they had not changed any medications within the past three months and had no acute diseases or infections within two weeks prior to the study. Data were collected on the sociodemographic characteristics, physical activity, food/fluid intake (24-hour recall), anthropometric indices, and indirect calorimetry assessments. Data collection was performed one week before and during the fourth week of Ramadan.

Anthropometric and Body Composition Measurements

Body weight and height were measured by the same person using an electronic balance and a stadiometer (Seca, Germany) and recorded to the nearest 0.1 kilogram and 0.1 centimeter, respectively. Body composition was determined via bioelectric impedance using InBody-S10 (South Korea) to assess the fat mass, skeletal muscle mass (SMM), total body water, fat-free mass (FFM), body fat percentage (BFP), protein, bone mineral content, visceral fat, waist circumference, BMI, and basal metabolic rate (BMR). The analysis was performed in standardized conditions each time, which encompassed two hours of fasting and no intense physical exercise 12 hours prior to the test in the fourth week of Ramadan before midday.

Dietary Intake and Physical Activity

Data on food intake were collected four times 24 hours a day (once a week) using a 24-hour

dietary recall questionnaire, which were completed via phone, and the data were analyzed using the N4 software (First Databank Inc., San Bruno, CA, USA). To evaluate the physical activity of the participants during the study and adjust the effects on the main outcome, all the participants were provided with a step-by-step device (model: HJ303, Omron, made in England) with the measure accuracy of 36.86±28.58 steps, which had to be brought to the study setting every day for one week. Afterwards, the device information was recorded in special forms, and the average of one week of physical activity was calculated for each individual.

Resting Metabolic Rate Assessment

The resting metabolic rate (RMR) value was determined via indirect calorimetry, which was performed using Metalyzer3B-R3 (Cortex Company, Germany). The measurement accuracy of the device covers the O_2 sensor of <0.1 Vol% , Co_2 sensor of <0.1 Vol%, and volume turbine of 50 milliliters (±2%).

Volunteers were advised to fast 6-8 hours before referral, drink sufficient water while fasting, and avoid tobacco, heavy exercise, nutritional supplements, energy drinks, and caffeine and alcoholic beverages within four hours before testing. In addition, it was explained that they should be completely calm and not become excited/stressed, and there would be no need to discontinue possible medications. Before the test, the volunteers were provided with the instructions on using the device, and it was emphasized that no gases should be inhaled by the volunteers through the masks. Before calorimetry, the anthropometric data of the volunteers were made available. The BFP, waist-to-hip ratio, height, and body weight of the volunteers were also recorded in the device. The volunteers rested on a bed for 20 minutes before the test, and the temperature of the site was maintained at 20-25°C. Moreover, it was attempted to test the environment slowly and without commuting in order to prevent the unwanted entry of others.

Statistical Analysis

Data analysis was performed in SPSS version 20 (IBM Co., Armonk, NY, USA). Before performing the statistical tests on the quantitative variables, the distribution normality hypothesis of the quantitative variables was initially evaluated using the Kolmogorov-Smirnov test, and the data were observed to be normal. The obtained data were expressed as the mean values and standard deviations, and the comparison of the group means before and after Ramadan was carried out using paired t-test. In all the statistical analyses, the P-value of less than 0.05 was considered significant.

Results

In total, 21 subjects were enrolled in the study, 76.19% and 23.8% of whom were female and male, respectively. The mean age of the subjects was 27.79±6.09 years, and their mean body weight was 77.91±11.83 kilograms. Table 1 shows the anthropometric and body composition indices of the participants.

Table 1. Anthropometric and Body Composition Indices of Subjects before and after Study

Parameters	Before	After	Mean Changes	SD	P-value
Weight (Kg)	77.91	77.01	0.900	1.27	0.040
BMI (kg/m²)	28.14	28.05	0.0855	0.6174	0.532
Fat (kg)	26.98	29.61	-2.6381	2.73	<0.001
FFM (kg)	50.93	47.39	3.5381	3.05	<0.001
BFP (%)	34.82	38.48	-3.6428	3.30	<0.001
WHR	2.91	2.89	0.0219	0.064	0.135
Visceral Fat (kg)	34.28	33.22	1.0571	0.805	<0.001
SMM (kg)	28.55	26.07	2.48	1.92	<0.001
Protein (kg)	10.12	3.57	0.852	0.65	<0.001
Minerals (kg)	33.54	30.83	2.71	2.11	<0.001
BMR (kcal/kg)	1447.46	1398.67	48.79	107.01	0.012

*BMI: body mass index, FFM: fat-free mass, BFP: body fat percentage, WHR: waist-to-hip ratio, SMM: skeletal muscle mass, BMR: basal metabolic rate; data expressed as mean±SD; *P<0.05*

According to the obtained results, the body weight of the subjects decreased significantly following one month of Ramadan fasting (0.09±1.27 kg; P=0.04). However, the adipose tissue and BFP showed a significant increase (2.6±2.7 kg; P<0.001 and 3.64±3.30%; P<0.001), while the mean visceral fat decreased significantly (-1.05±0.8 kg; P<0.001). Table 2 shows the results of dietary intake assessment during the study.

According to the findings, the mean energy intake significantly increased during Ramadan compared to the pre-intervention phase (3,290.85±785.82 vs. 2,458.23±535.69 kcal/day; P<0.001). Furthermore, the mean fat and carbohydrate intakes of the participants significantly increased during Ramadan compared to the pre-Ramadan phase (127.27±47.70 vs. 92.46±52.31 g/day; P<0.001 and 437.04±101.02 vs. 310.09±87.12 g/day; P<0.001).

Table 2. Dietary Intake of Subjects before and during Study

Parameters	Before Study	SD	During Study	SD	P-value
Energy (kcal)	2,458.23	535.69	3290.85	785.82	<0.001
Starch (g)	198.32	41.89	222.58	56.21	<0.001
Total Sugar (g)	60.42	31.57	194.04	56.87	<0.001
Protein (g)	112.38	21.87	122.46	34.85	<0.001
Fat (g)	92.46	52.31	127.27	47.70	<0.001
Carbohydrate (g)	310.09	87.12	437.04	101.02	<0.001
Iron (mg)	15.23	3.21	16.86	4.75	<0.001
Copper (mg)	2.42	.693	2.55	.829	<0.001
Zinc (mg)	21.96	19.63	23.19	22.24	<0.001
Vitamin D (µg)	2.12	1.02	2.49	1.18	<0.001
Vitamin E (mg)	29.65	21.54	31.14	26.96	<0.001
Vitamin C (mg)	135.25	84.21	140.38	47.52	<0.001

Table 3. Metabolic Parameters of Subjects before and after Study via Indirect Calorimetry

Parameters		N	Before	SD	After	SD	P. Value
RMR (kj/day)		21	1,628.04	396.03	1,664.80	440.46	0.614
RQ		21	0.84	0.05	0.88	0.105	0.009
BMR (kj/day; adjusted for PA)	**Sedentary**	14	1,551.07	237.49	1,386.07	163.29	0.069
	Active	7	1,343.85	107.01	1,411.28	263.34	0.820
RMR (kj/day; adjusted for PA)	**Sedentary**	14	1,620.35	439.43	1,604.42	450.04	0.012
	Active	7	1,643.42	322.48	1,785.57	427.01	0.006

RMR: resting metabolic rate, RQ: respiratory quotient, BMR: basal metabolic rate

Discussion

The present study aimed to investigate the effects of RF on the body composition and basal metabolic rate of overweight and obese individuals. During Ramadan, the type and amount of energy intake, sleep patterns, and daily physical activity may change, and these changes could affect the body composition. Our research indicated that during one month of fasting, body weight and visceral fat decreased significantly, while the body fat percentage increased significantly. In addition, daily energy intake and consumption of simple carbohydrates and free sugars increased significantly during Ramadan by the participants. Previous studies have reported varying changes in the body weight during Ramadan or even other changes were observed in the mealtime when fasting was not involved (18). Our findings are consistent with the studies by Ziaee et al. (19), Khattak et al. (8), and Mansi et al. (9) in terms of weight loss, decreased BMI, and increased energy intake during Ramadan.

In a research in this regard, Al-Hourani et al. reported that weight and BMI decreased during Ramadan (20). In a meta-analysis conducted in 2019, it was observed that one month of RF significantly decreased the body weight and fat-free mass, which is in line with our study (21). Furthermore, the study by Nachvak et al. (2019), which was conducted on 160 healthy men, indicated that the body weight and BMI decreased significantly during Ramadan. In the mentioned research, the analysis of the dietary intakes demonstrated that carbohydrate intake during Ramadan significantly increased compared to the pre-Ramadan phase, which is in congruence with our findings (22). Contrary to the results of the present study, J. Ramadan et al. reported no significant changes in the body weight and body composition during Ramadan,

which may be due to small sample size of the mentioned research (23).

During Ramadan, changes in the mealtime and fluid intake, along with reduced meal frequency, could cause various physiological changes. Evidently, dietary habits and choices vary depending on culture, and the percentage of the energy obtained from carbohydrates, protein, and fat varies in different Islamic countries (10). In the present study, the energy intake and consumption of simple carbohydrates and free sugars increased significantly during Ramadan, possibly indicating the inappropriate dietary pattern during Ramadan and justifying the increased fat percentage in the participants.

Possible weight loss mechanisms could be defined by metabolic responses. For instance, the glucose load is slower in the afternoon compared to the morning. In addition, gastric emptying and blood flow are better during the day than at night, resulting in the faster absorption of substances from the gastrointestinal tract during the day. Therefore, another possible description for weight loss could be the less absorption of the food eaten at night during Ramadan (11). Weight loss is followed by fatigue and constant tiredness, and decreased fluid intake could lead to the reduction of glycogen levels and the extracellular fluid volume, as well as moderate dehydration.

The impact of Ramadan on metabolism is very complex. Many bodily mechanisms are involved in maintaining balance while fasting, and changes in the physical activity level, mealtime, and bedtime alter the metabolism discretely (13). However, it is difficult to determine the exact effects of each factor alone on the metabolism while fasting. RF involves a particular dietary pattern, which differs from long-term and short-term starvation (12). In short-term starvation (less than four days), RMR may increase due to the increased norepinephrine concentration (14). In the present study, RMR did not change significantly before and after Ramadan, which is consistent with the results obtained by El Ati et al. (15), McNail et al. (16), and Harder-Lauridsen N. M. et al. (17).

Limitations of the Study

The special circumstances associated with RF (e.g., changed daily dietary patterns, fasting hours, fewer daily meals, and reduced physical activity) may limit research in this regard. In addition, differences in the dietary habits of diverse communities and cultures could lead to inaccurate results.

Conclusion

According to the results, fasting during Ramadan may be considered a dietary intervention for weight loss and the improvement of metabolic parameters without the reduction of the basal metabolism as opposed to weight loss diets provided that it is accompanied by nutritional support and healthy eating patterns.

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Interactive Effect of the *Linum Usitatissimum* Extracts and Exercise Rehabilitation on Aorta Endothelial and Heart Tissues Apoptosis Biomarkers

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ABSTRACT

Introduction: As one of the most life-threatening illnesses, cardiovascular diseases are often discerned with a high apoptosis rate because of exposure to the high level of oxidative stresses. The present study has investigated the interaction of the *Linum Usitatissimum* (Lu) and aerobic exercise (Ae) on apoptosis of aortic endothelial and heart tissue in rats intoxicated by H_2O_2.

Methods: 56 male Albino Wistar rats were divided into 7 groups, included HC (Healthy Control), TC (Toxic-Control), Toxic-Lu1 (Received Lu, 5 mg/kg), Toxic-Lu2 (Received Lu, 10 mg/kg), Toxic-Ae (Received Aerobic Exercise), Toxic-Ae+Lu1, and Toxic-Ae+Lu2. Finally, the rats were sacrificed ethically, and the apoptotic biomarkers were measured in isolated aortic endothelial and heart tissues.

Results: The interactive comparisons showed that the Ae and Lu had a significant interactive change on pro-apoptosis biomarkers. The BAX in aortic endothelial (P=0.0011) and heart (P=0.0007), caspase-3 in aortic endothelial (P=0.0006) and heart (P=0.0016), and Bcl-2 in aortic endothelial (P=0.0018) and heart (P=0.0016) have significant interactive changes. No significant independent effect was observed. Post hoc test showed that group Toxic-Ae+Lu2 have the most significant improvement compared to the TC group (P≤0.05).

Conclusions: The simultaneous effect of Ae and Lu supplementation most effectively improved the apoptosis biomarkers and displayed potent cardioprotective effects compared to the singular administration of each intervention. Probably, the short rehabilitation period has caused non-significant independent changes. However, the interaction of Ae and Lu has shortened the treatment period.

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Introduction

Apoptosis is one of the main cellular processes that guarantee homeostasis in organisms, leads to the cells (its poor performance) being condemned to death, and new cells are replaced under extensive control (1). Thus, the apoptosis signaling and effector molecules form a complicated network by which the pro-apoptotic and anti-apoptotic factors lead to balanced apoptosis, not more than the level needed to maintain a normal tissue (2). Pathologic apoptosis occurs when the balanced scale is weighted on one side, meaning apoptosis exceeds or falls short of the standard rate. Hence, disorders in modulating cell death or apoptosis can significantly cause cancer, autoimmune lymphoproliferative syndrome, and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease (3, 4). The Bcl-2 protein family, which has consisted of compliant (puma, Noxa, BAD, BAX) and opposing (Bcl-2, Bcl-x_L, Bcl-w) members of apoptosis, partly regulate this phenomenon (5). Puma and Noxa are the BH3-only protein that inactivates the prosurvival Bcl-2 family proteins and subsequently activates the second pro-apoptotic protein Bax or Bak to induce Cytochrome-C release from the mitochondria outer membrane (MOM) (6). Afterward, Cytochrome-C binds to and stimulates Apaf-1 to form an apoptosome complex, leading to caspases activation. In addition to the p53 gene, the mammalian genome encodes two other transcription-related factors,

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p63, and p73 that appear to stimulate the expression of pro-apoptotic genes (7, 8).

Researches have shown that oxidative stresses, especially H_2O_2, are involved in different diseases by leading normal cells to apoptosis (9). The ROS (Reactive oxygen species) molecules such as H_2O_2 are mainly formed in high-energy demanding tissues like the heart, in which a high level of ATP is produced via oxidative phosphorylation (10). These dangerous molecules could seriously damage cell DNA leading to pro-apoptotic factors and cellular death (11). Many cardiomyopathies are associated with mitochondrial DNA damage, leading to defects in the electron transport chain, and an increase in ROS production and disruption of these essential cellular organelle function and mitochondria as the most crucial organelle for apoptosis regulation and initiation could drive the cell to death in this situation (12, 13). It has been indicated that many medicinal herbs are rich sources of compounds with ant apoptotic effects, which made them worth having pharmaceutical agents (14) so that many studies have shown beneficial influences of various herbs in attenuation of apoptosis in patients with cardiac disorders, which resulted in an improved condition of these people (15).

Flax (*Linum Usitatissimum*) or Flaxseed belongs *Linaceae* plants family, and it is a well-known growing plant in tropical and subtropical regions for general medicinal uses from the past till now (16). It is a pharmaceutically accepted plant because of its high level of beneficial omega-3 fatty acids (PUFA) and other compounds such as lignans, fibers, minerals, and vitamins (17). As reported, the lignans, especially Secoisolariciresinol diglucoside (SDG), potentially possess antioxidant, anti-inflammation, anticoagulant, activity, and cytotoxic effects on some human cancers. Interestingly, *Linum Usitatissimum* has paradoxical activity in apoptosis in cancerous and normal cells, so that researchers have shown that flaxseed extract increases and decreases the apoptosis rate in abnormal and healthy cells, respectively (18). As this plant is a rich source of unsaturated omega-3 fatty acid such as α-linoleic acid (ALA) and regarding ant apoptotic traits of flaxseed, the hypothesis comes up that this plant could benefit cardiovascular disease in 2 ways; it supplies a healthier food with less saturated fatty acids, on the one hand. Secondly, it can diminish the apoptosis severity in damaged cardiovascular tissues (19).

Furthermore, studies have shown that exercise reduces apoptosis by modulation of stress-sensitive proteins such as the nuclear factor NF-κB, insulin-like growth factor (IGF-1), and heat shock protein (HSP90 and HSP70) (20). Furthermore, it is known that aerobic exercise declines the Bax/Bcl-2 ratio and activation of caspases, including 3 and 9, and also fewer DNA fragments are observed in trained rats (21). It is suggested that exercise may stimulate cell survival proteins including MnSO3, MnSOD, NF-kB, extracellular kinase receptor (ERK), IGF-1/Akt pathway, and heat shock protein (HSP) to reduce cell death in the heart (22, 23). Moreover, exercise has shown to be an anti-oxidative intervention as it has been found to elevate anti-oxidative enzymes and free radical scavenger molecules. Thus, many researchers have stated that aerobic exercise could efficiently increase cell survival in patients suffering from cardiovascular disease in which oxidative stress-induced cardiomyocytes death is found (23). As most cardiovascular diseases are accompanied by the high rate of apoptosis in endothelial and cardiomyocyte cells (24), aerobic exercise could be considered a dynamic therapeutic approach.

According to the cardioprotective effects of *Linum Usitatissimum* and exercise, the present study investigates the combined effect of these interventions on the apoptosis rate of heart and aortic endothelial of rats poisoned with H_2O_2 who artificially experienced a high level of apoptosis in heart and aortic endothelial tissues.

Materials & Methods

The Ethical Approval

The ethics committee approved the whole experimental protocol in this study of Islamic Azad University, Mahallat Branch (IR.IAU.ARAK.REC.1399.043). It was done under the NIH (National Institutes of Health) guide for the care and use of laboratory animals (No. 80–23), which emphasized minimal animals being sacrificed and minimal pain imposed during the study.

Animals and Groups

The rats for the present study included 56 Wistar Albino male rats (Ages 10-12 weeks and weight 200 ± 20 g). They were purchased from the Institute of Pasteur, Tehran, Iran. After direct proof of all rat's health, they laid into the 6

separate cages with temperature 22-27 °C and alternative exposure to 12 hours of light and 12 hours of darkness to adapt to the conditions before experiments at the university's Animal Care Facility. The animal also had optional access to water and food in all study periods. Then, the rats were categorized into 7 groups (n=8), and all got poisoned but group HC. The groups included as following: HC (Healthy Control), TC (Toxic-Control), Toxic-Lu1 (Received Lu, 5 mg/kg), Toxic-Lu2 (Received Lu, 10 mg/kg), Toxic-Ae (Received Aerobic Exercise), Toxic-Ae+Lu1, and Toxic-Ae+Lu2.

For treating the rats, TC group was only were poisoned by H_2O_2 without any treatment. Toxic-Lu1 and Toxic-Lu2 Group received 5 and 10 mg/kg of herbal extract, respectively, without aerobic exercise. The Toxic-Ae group experienced only an aerobic exercise program without any herbal extract. Also, Toxic-Ae+Lu1 and Ae+Lu2 groups were delivered 5 and 10 mg/kg of herbal extract, respectively. Besides, they underwent an aerobic exercise program. Eventually, HC group received no poisoning and no treatments. To make sure that no other chemical effect on data, no sweetener substrate was used. The herbal extract was orally administered to rats daily for 28 days (4 weeks) so that in groups 5 and 6, it was fed one hour after every aerobic exercise session in the 2nd and 3rd weeks. To make sure that no other chemical effect on data, no sweetener substrate was used. The herbal extract was orally administered to rats daily for 28 days (4 weeks) so that in groups 5 and 6, it was fed one hour after every aerobic exercise session in the 2nd and 3rd weeks.

H_2O_2 Induced Toxicity

The animals were poisoned using peroxide hydrogen prepared from Atusa oxidants 9% product (Grape Oxidant 6 Number 1 Atusa 60 ml) bought from Atusa Company, Tehran, Iran. This product maintained a lot of hydrogen peroxide (H_2O_2) manufactured for hair dyeing processes. To poison rats, they needed to inhale the oxidants so that 60 mg of oxidant tube was poured inbox with a volume of 125.123 mm^2. The grid-form box did not let oral usage of the substrate by rats. Thus, the rats breathed the air of a cage in which the oxidant-contained box was put. In each poisoning phase, 4-5 mice were allowed to inhale the air for 3 hours a day for one week.

Preparation of Linum Usitatissimum

The seeds (Pakan Bazr Co., Isfahan, Iran) were grinded thoroughly, and 10 g of powder was solved in petroleum ether to prepare Linum Usitatissimum supplementation. The solution was poured into a Soxhlet extractor to extract oil, taking along for 10 hours. In the next step, the solution whose isolated oil was thoroughly dried to purify herbal extract in the next step, in which methanol was added to the dried powder. The primary herbal extract was gained from this mixture using Soxhlet extractorduring16 hours. A yellow solution was obtained as the methanolic extract, kept at 50° C for 5 hours to evaporate the containing methanol. Finally, after drying, the remained yellow powder was dissolved in normal saline and stored a 4 ° C in darkness.

Aerobic Exercise Protocol

The low-intensity interval training (LIIT) was done under 2 separate programs, including adaptation and main. The mice did 4 days of exercise (Each day included 4 times of1 min running at a speed of 20-25 meters per min on a rotational bar). The main program consisted of 1 min of running at 20-25 m/min, followed by 2 min of active rest (running at 10-12 m/min). This protocol was repeated 10 times, meaning that the whole main program took 30 minutes per rat. The main programs were executed 5 sessions a week for 4 weeks (25).

Sacrifice and Laboratory Methods

After the treating period, the cardiovascular tissues were analyzed after 24 hours of the last treating session, during which the rats were not fed for 14h. Qua, the ketamine (30-50 mg/kg), and xylazine (3-5 mg/kg) were utilized via intraperitoneal injection to pass out the rats. After complete anesthesia, a cleft at the center of the rat breast was made using the surgical blade, and the aortic cardiovascular tissue was extracted. Then, the tissues were immediately washed by normal saline and frozen using nitrogen (180 ° C) and stored at -80°C. To be able to assess the apoptotic factors, we first needed to homogenize tissue samples. After the defreeze of tissues, they were transferred into 2 ml microtubes, and 310-340 µl of lysis buffer was added for 62-68 mg of tissue samples, as 500µl of lysis buffer (EPX-99999-000) is recommended for 100 mg of tissue. Significantly, antiprotease existing in lysis buffer prohibited protein

denaturation. We used a 5 mm stainless steel bead in microtubes, which were laid into a TissueLyser device. The Homogenization processes were performed by processing at 25 Hz for 2 minutes and final centrifuge at 4 ° C for 10 minutes. Then, the supernatant was disposed of into new microtubes, and the homogenate sample was diluted at a ratio of 10 mg protein/ml using 1X PBS and was kept at -80 ° C. At the final step, we quantitatively analyzed the pro-apoptotic factors, including Bax (pg/ml) and caspase 3 (ng/ml) (Cas 3) and anti-apoptotic factor Bcl2 (ng/ml) in both heart and aortic endothelial cells using ELISA (Mabtech, Sweden), to assess the influence of *Linum Usitatissimum* methanolic extract and aerobic exercise on cellular apoptosis.

Statistical Analysis

The Kolmogorov-Smirnov test was used to determine the normality of the distribution. All results were expressed as mean ± standard deviation. In order to analyze the data and investigate the inconsistencies of the observation amongst different groups, the two-way analysis of variance ANOVA method was used, followed by the LSD posthoc test. The data were analyzed using the Prism 8 software at a statistically significant level ($P \leq 0.05$).

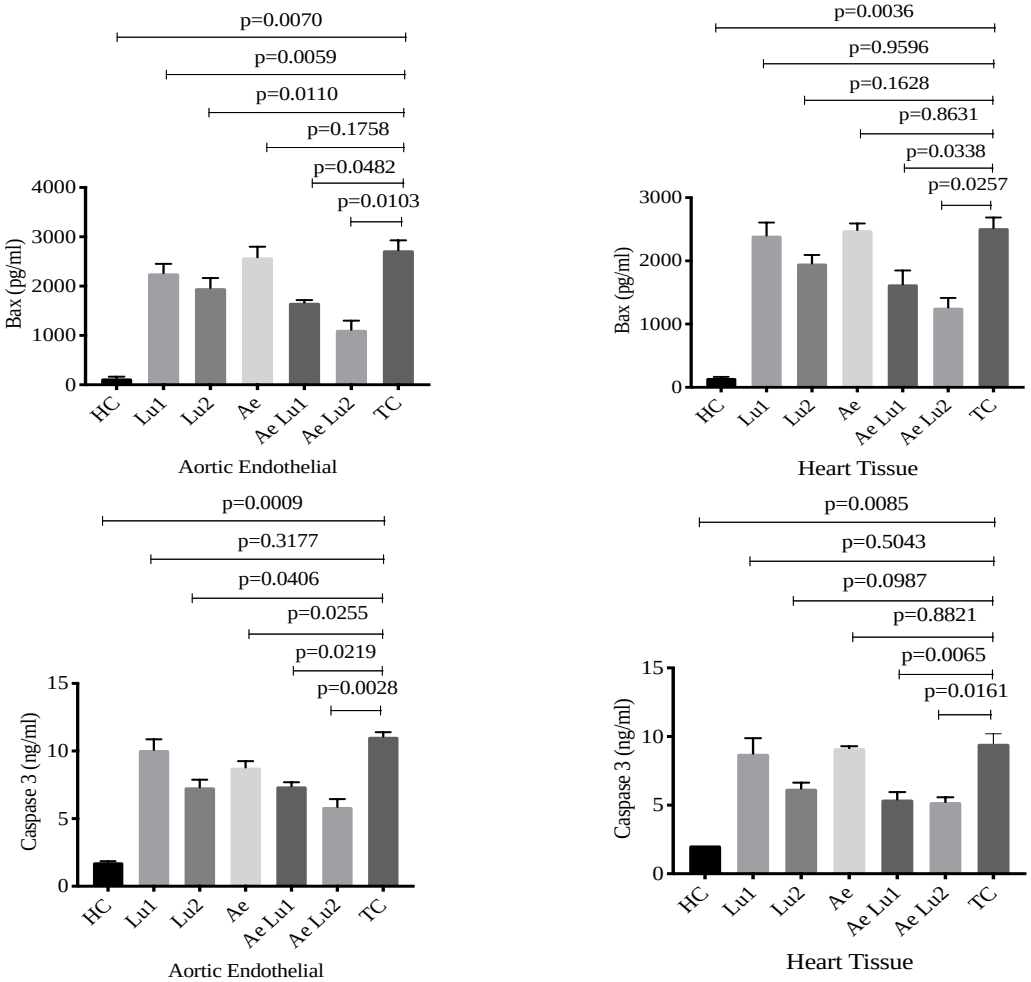

Figure 1. The graphs show the difference between apoptotic biomarkers (Bax and caspase 3) in the studied groups.
*HC (Healthy Control), TC (Toxic-Control), Toxic-Lu1 (Received Lu, 5 mg/kg), Toxic-Lu2 (Received Lu, 10 mg/kg), Toxic-Ae (Received Aerobic Exercise), Toxic-Ae+Lu1, and Toxic-Ae+Lu2.

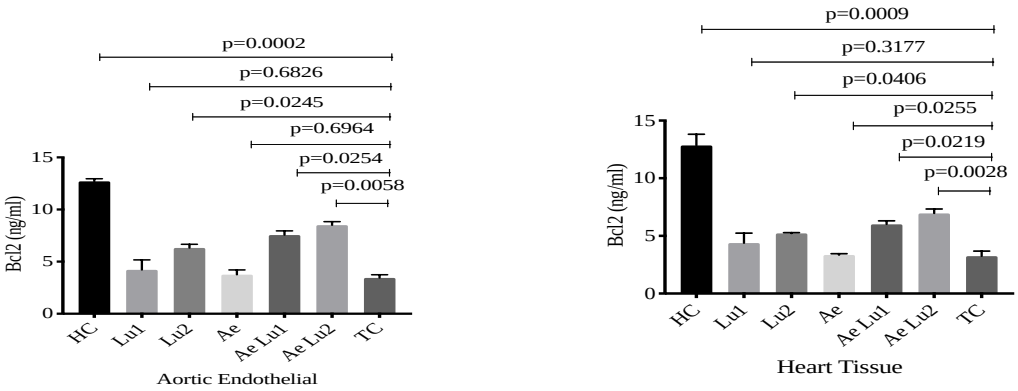

Figure 2. The graphs show the difference of apoptotic biomarkers (Bcl-2) in the studied groups.
*HC (Healthy Control), TC (Toxic-Control), Toxic-Lu1 (Received Lu, 5 mg/kg), Toxic-Lu2 (Received Lu, 10 mg/kg), Toxic-Ae (Received Aerobic Exercise), Toxic-Ae+Lu1, and Toxic-Ae+Lu2.

Results

The Synchronous Administration of Lu and Ae Most Effectively Attenuates Apoptosis Rate in Cardiovascular Tissue

The interactive comparisons showed that the Linum Usitatissimum and aerobic exercise had lessened the pro-apoptosis biomarker BAX in aortic endothelial (F=90.41, p=0.0011, η=0.9784) and heart tissue (F=87.89, p=0.0007, η=0.9777). The same results were found for Cas-3, so that the interventions meaningfully decreased the level of Cas-3 in aortic endothelial (F=114.3, p=0.0006, η=0.9828) and heart tissue (F=71.32, p=0.0016, η=0.9727). No significant independent effect was observed. Post hoc test showed that group Toxic-Ae+Lu2 showed the most significant improvement compared to the TC group for Bax and Caspase-3 (Fig-1). A posthoc test showed a significant difference between the studied groups for Bax in the heart (P=0.0257) and in aortic endothelial (P=0.0103) in the Toxic-Ae+Lu2 group compared to the TC group. A posthoc test showed a significant difference between the studied groups for caspase-3 in the heart (P=0.0161) and aortic endothelial (P=0.0028) in group Toxic-Ae+Lu2 compared to the TC group. Other group comparisons are shown in the figure-1.

Moreover, according to interactive analysis, the Lu and Ae have efficiently decreased the level of antiapoptosis biomarker Bcl2 in aortic endothelial (F=119.3, p=0.0018, η=0.9735) and heart tissue (F=71.32, p=0.0016, η=0. 9805). No significant independent effect was observed. Post hoc test showed that group Toxic-Ae+Lu2 showed the most significant improvement compared to the TC group for Bcl-2 (Fig-2). A posthoc test showed a significant difference between the studied groups for Bcl-2 in the heart (P=0.0028) and aortic endothelial (P=0.0058) in the Toxic-Ae+Lu2 group compared to the TC group. Other group comparisons are shown in the figure-2.

Table 1. The mean level of BAX in studied groups in aortic endothelial and heart tissue cells. The data were analyzed by a two-way ANOVA.

Groups		Mean	F	P.value	η
Aortic endothelial	HC	132.8 ± 31.62	90.41	0.0011	0.9784
	Lu1	2261 ± 191.7			
	Lu2	1962 ± 204.3			
	Ae	2586 ± 212.8			
	Ae Lu1	1667 ± 51.28			
	Ae Lu2	1116 ± 187.3			
	TC	2729 ± 201.1			
Heart Tissue	HC	148.1 ± 17.97	87.89	0.0007	0.9777
	Lu1	2400 ± 206.2			
	Lu2	1958 ± 136.3			
	Ae	2485 ± 105.7			
	Ae Lu1	1630 ± 217.9			
	Ae Lu2	1262 ± 152.6			
	TC	2518 ± 169.6			

Table 2. The mean level of Caspase 3 in studied groups in aortic endothelial and heart tissue cells. The data were analyzed by a two-way ANOVA.

Groups		Mean	F	P.value	η
Aortic endothelial	HC	1.78 ± 0.06	114.3	0.0006	0.9828
	Lu1	10.0 ± 0.77			
	Lu2	7.31 ± 0.56			
	Ae	8.78 ± 0.47			
	Ae Lu1	7.39 ± 0.29			
	Ae Lu2	5.86 ± 0.58			
	TC	11.06 ± 0.33			
Heart Tissue	HC	2.06 ± 0.00	71.32	0.0016	0.9727
	Lu1	8.76 ± 1.12			
	Lu2	6.20 ± 0.44			
	Ae	9.15 ± 0.14			
	Ae Lu1	5.41 ± 0.54			
	Ae Lu2	5.23 ± 0.35			
	TC	9.47 ± 0.72			

Table 3. The mean level of Bcl2in studied groups in aortic endothelial and heart tissue cells. The data were analyzed by a two-way ANOVA.

Groups		Mean	F	P.value	η
Aortic endothelial	HC	3.44 ± 0.29	119.3	0.0018	0.9835
	Lu1	4.21 ± 0.95			
	Lu2	6.32 ± 0.34			
	Ae	3.75 ± 0.44			
	Ae Lu1	7.55 ± 0.41			
	Ae Lu2	8.50 ± 0.23			
	TC	12.7 ± 0.25			
Heart Tissue	HC	3.242 ± 0.2519	71.32	0.0016	0.9805
	Lu1	4.381 ± 0.499			
	Lu2	5.221 ± 0.0342			
	Ae	3.338 ± 0.0714			
	Ae Lu1	6 ± 0.1752			
	Ae Lu2	6.954 ± 0.219			
	TC	12.85 ± 0.5632			

Discussion

The literature strongly supports the increased apoptosis rate in cardiovascular diseases such as ischemia-reperfusion, heart failure, and cardiac hypertrophy (26). The cytochrome C leakage and caspases activation in animals who ischemic heart cells are solid supportive data in this context. Researchers have shown that many cardiovascular disorders are due to oxidative stresses and resulted in ROS molecules, mainly H_2O_2 (27). These hazardous molecules could lead cardiomyocytes to death through various pathways, mainly by macromolecule damage such as DNA and ATP depletion, which put the cells in a stressed condition and initiates apoptosis cascade (28). Thus, many efforts have been made to identify interventions with anti-oxidative effects to attenuate the apoptosis phenomenon in these patients to improve their overall clinical condition and lessen mortality. One of the most studied interventions is a physical exercise that has strongly displayed antioxidant properties. It has been observed that aerobic exercise leads to lessened oxidative-induced apoptosis in animal models with cardiovascular diseases so that the lower level of apoptosis markers such as Bax, Caspase 3, fragmented DNA and Bax to Bcl2 ratio (29).

On the other hand, the studied plant, *Linum Usitatissimum*, has been shown to contain many beneficial chemicals with pharmacologic traits that could benefit patients with cardiovascular diseases partly in 2 ways. *The Lu* is a rich source of many anti-apoptotic compounds include polyunsaturated fatty acids (PUFA), docosahexaenoic acid (DHA), α-linoleic acid (ALA), and eicosapentaenoic acid (EPA) that play cardioprotective roles (30). Moreover, the anti-apoptotic and anti-inflammation features of Lu have been revealed, while that inflammation and oxidative stresses enhance cardiocyte death in various cardiac disorders (31). Due to reports, it has also been indicated that *Linum Usitatissimum* extract declines the DNA fragmentations, pro-

apoptotic factors caspase 3 and 9, and inflammatory factors such as IL-1, 2, 6, and INF-γ by its containing linoleic acid and SDG compounds (32).

The present study data demonstrated that aerobic exercise and herbal extract might trigger significant alterations in specific apoptosis biomarkers when they were lonely administered to rats. However, the most significant upregulation of Bcl-2 and downregulation of Bax and caspase 3were observed while continuously delivered. In this regard, shirvani et al. implied that the combination of training and flaxseed oil upregulated the expression of cardioprotective engaging genes UCP-2, UCP-3, and eNOS genes more than when each of them was used solitarily (33). These findings indicated that the herbal extract interference could benefit patients with cardiovascular disease because of 2 main characteristics: healthy unsaturated fatty acids (omega-3 fatty acid) and, secondly, due to advantageous cellular activities such as anti-oxidative traits. This study showed, though, the Lu supplementation (at a high dose) or Ae individually may have altered apoptosis biomarkers, but these alterations are intensified in the presence of both interventions. In this regard, Ghosh et al. stated that moderate exercise has caused Bax/Bcl-2 ratio and caspase 3 to be diminished in the hippocampus of rats (34). Following our data, derbali et al. also mentioned that linseed oil has a cardioprotective effect in rats with isoproterenol-induced myocardial infarction as it lowered the level of myocardial infarction, including LDH, ALP, AST, and CK-MB (35). Furthermore, Hasan et al. showed that *Linum Usitatissimum* extracted lessened the troponin I, LDL, VLDL and heightened the level of antioxidant enzymes Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) in rabbits who had experienced the isoproterenol-induced myocardial infarction (36). In support of previous studies, the present study states the robust anti-oxidative features of *Linum Usitatissimum* and LIIT, which could be cardioprotective in cardiovascular tissues.

Conclusions

As our data demonstrated, the Linum Usitatissimum and aerobic exercise could attenuate the apoptosis rate in aortic endothelial and heart tissues. The concurrent administration of both results in more antiapoptosis effects compared to when each of them is applied lonely. Probably, the short training period has caused a non-significant independent changes. However, the interaction of Ae and Lu has shortened the treatment period. Thus, regarding chemicals drug's side-effects and other preventive advantages of natural medications, combining herbal-physical therapy could be a suitable approach in future studies as an efficient treatment approach for cardiovascular diseases.

Conflict of Interest

The authors declare that no conflict of interest exists with the present study.

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