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Acute Pancreatitis (AP) and Dietary Habbits

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Inflammation of pancreatic parenchyma is said to be pancreatitis. It can be acute, presenting as emergency with short history or chronic as a continuation of acute with a long history. Autodigestion is responsible for pancreatitis on background of premature activation of pancreatic enzymes within the pancreas. Acute Pancreatitis is acute inflammation of the pancrease which can be mild to moderate requiring hospital admission or severe leading to distressing outcomes such as systemic inflammatory response syndrome or multi organ failure. Acute pancreatitis is a leading cause in emergency admissions to hospital with a rise in its incidence (1). It may present as pain in epigastrium, retching, nausea, shock, relieve of pain on lying forward and increase in enzymes levels. There are several factors contributing to its occurrence. There are multiple etiologies for developing AP including gallstones, hypertriglyceridemia, and certain medications such as angiotensin-converting enzyme (ACE) inhibitors, azathioprine, furosemide, 6-mercaptopurine, pentamidine, sulfa drugs, and valproate. The effects of two modifiable risk factors, alcohol consumption and cigarettes smoking, have been extensively evaluated as contributing to the development and progression of AP (2, 3). But of all causes, there are two most important, they are gall stones and alcohol. However, another potential important modifiable risk factor for AP that has not been studied as well is diet. Diet can affect causing pancreatitis directly and indirectly. Directly by effects produced from substances like fat and indirect by formation of gall stones which lead to pancreatitis.

As proved by the study that diet rich in fat leads to gall stone formation (4). It also led to hypercholesteremia and hyperlipidemia which cause AP. A large cohort study was published in US in the year 2017 which studied association between certain dietary patterns and the risk of acute pancreatitis AP (5). The authors found that the dietary intake of food rich in saturated fat and cholesterol such as red meat and eggs was associated with an increased risk of biliary AP, whereas a study done in US showed fiber intake was inversely associated with both biliary and non-biliary AP (6). Vitamin D intake was inversely associated with biliary AP, whereas coffee intake was protective toward non-biliary AP and recurrent or chronic pancreatitis (6). Another cohort study done on a large multi ethic group concluded that, associations between dietary factors and pancreatitis were observed mainly for gallstone-related AP. Interestingly, dietary fiber protected against AP related and unrelated to gallstones. Coffee drinking about four cups/day protected against AP not associated with gallstones (6).

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However, there is another study, where it was examined whether overall diet quality influences the natural history of non-gallstone-related acute pancreatitis, it turned out that with non-gallstone-related acute pancreatitis, and there was no clear association between overall diet quality and risk of recurrent and progressive pancreatic disease (7). Here comes another study that studied association of dietary habits with severity of Acute Pancreatitis and concluded that a meat-rich diet is independently associated with the development of persistent organ failure (severe disease) in patients with AP (8). Vegetable consumption, but not fruit consumption, may play a role in the prevention of non-gallstone-related acute pancreatitis (9). Coffee consumption was also studied but there was found no association between its consumption and occurrence of AP (10). Diets with high glycemic load are associated with an increased risk of non-gallstone-related acute pancreatitis (11). Consumption of fish was studied too, but consumption of total fish (fatty fish and lean fish combined) may be associated with decreased risk of non-gallstone-related acute pancreatitis (12). Because of limited work present on this subject of acute pancreatitis and dietary habits, and also due to conflicting outcomes among studies, further work need to be done to find the association. So we can conclude that there are several dietary factors which might be associated with acute pancreatitis like diet rich in saturated fat and cholesterol for example alcohol, eggs and red meat while some diet like intakes of vitamin D, milk, and fruits are associated with a reduced risk.

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The Anti-inflammatory and Antioxidative Effects of Selenium Supplementation on Critical Post-surgical Pediatric Patients

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ABSTRACT

Oxidative stress after major surgeries is associated with poor clinical outcomes, such as delayed wound healing and increased length of stay in the pediatric intensive care unit. Due to the growth and development phase in childhood, changes in the levels of oxidative stress and inflammation are of paramount importance in pediatric patients. Acute metabolic stress is correlated with the rate of oxidative stress and is believed to increase after major surgeries in pediatric patients. Therefore, it has been suggested that the presence of selenium in various selenoenzymes and selenoproteins may be largely involved in the antioxidative defense system in surgical inflammation through the regulation of glycolysis, gluconeogenesis, insulin transport pathways, gene expression of inflammatory mediators, and other functions of lymphocytes B and T, natural killer, and lymphokine activated killer cells. In acute metabolic stress, selenium requirement following major surgeries is considered essential in pediatric patients, and selenium supplementation in these patients may be helpful and cost-effective in the long run. Further clinical studies are required to clarify the potential beneficial effects of selenium supplementation, as well as its dose safety and efficacy rate.

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Introduction

Major surgeries may cause acute damages that could stimulate the immune system, thereby increasing the inflammatory mediators and reactive oxygen species (ROS) mainly at the surgical site (1, 2). Surgery-induced metabolic and hormonal alterations, inflammation, and oxidative stress may lead to the intensive care unit (ICU) admission of the children undergoing major surgeries (1). Given the importance of the growth and development phase in childhood, the interventions that might reduce the rates of inflammation and oxidative stress in pediatric patients could effectively decrease the length of ICU stay and mortality rate (3).

Selenium is an essential micronutrient, which modulates the antioxidative defense system of the body. Several studies have reported the beneficial effects of selenium on inflammation (4-6).

This review study aimed to determine the possible beneficial effects of selenium supplementation on the reduction of inflammation and oxidative stress in critical post-surgical pediatric patients.

Systemic Inflammatory Response to Post-surgical Oxidative Stress

Major surgeries are considered to be controlled traumas, which induce acute-phase response and lead to local and systemic inflammatory responses (14). Inflammation is known as a protective process, which is induced due to cell and tissue damage against acute stress (14). Although it is believed that the presence of ROS is essential to the activation of the signaling pathways of the antioxidant defense mechanisms, multiple complications are expected in case of their excessive production; such examples are increased lipid peroxidation, delayed wound healing, and damage to the DNA and protein content of the cells. Furthermore, this process could deteriorate the clinical condition of patients postoperatively (11, 15). Postoperative oxidative stress and inflammatory responses may lead to insulin resistance, cell

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necrosis and apoptosis, immunosuppression, organ failure, and increased length of ICU and hospital stay (16). Figure 1 depicts the pathophysiology of oxidative stress and inflammation after surgical traumas.

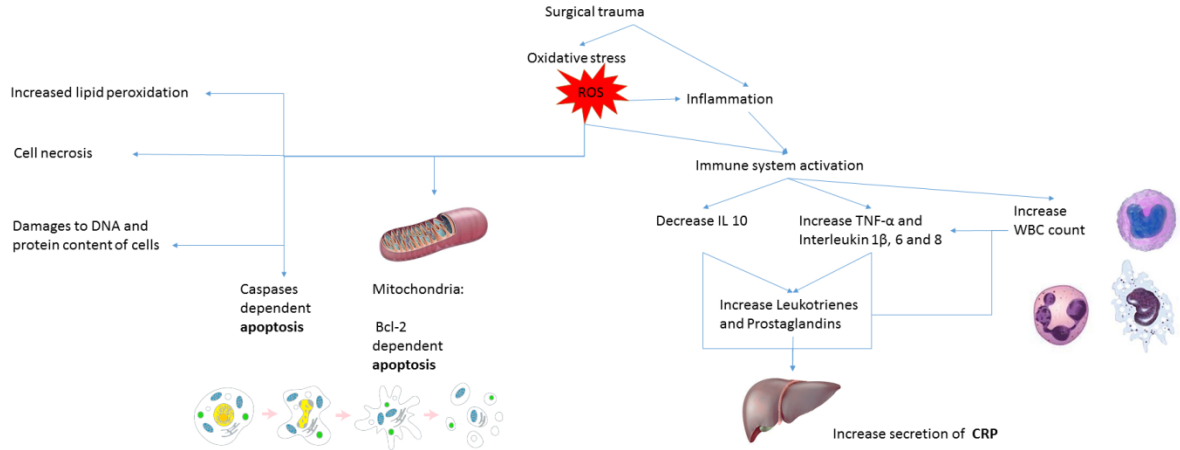


Figure 1. Pathophysiology of Oxidative Stress and Inflammatory Responses after Surgical Trauma

According to the literature, the metabolic responses to surgeries occur through insulin resistance and secretion of catabolic hormones (e.g., glucagon, catecholamines, and corticosteroids). Therefore, these factors may affect the metabolism through reducing cellular glucose uptake and increasing the breakdown of triglycerides to free fatty acids (17). However, the most significant influential factors in the mentioned processes are systemic and local inflammatory cytokines and oxygen free radicals. Additionally, amino acids are effective in the production of acute-phase proteins and wound healing (17). As such, regulation of balance between the inflammatory and oxidative stress status in critical post-surgical pediatric patients may improve their clinical outcomes at the pediatric intensive care unit (PICU).

Properties of Selenium

Selenium is an essential micronutrient, which induces the endogenous antioxidative defense system and is expressed as selenoproteins and selenoenzymes, especially glutathione peroxidase and selenoprotein S (5, 6). The recommended daily intake of selenium varies depending on age in preterm infants, infants, and children; in these age groups, the recommended values have been determine to be 2-3 and 1-3 µg/kg of the body weight (up to 100 µg/day) (7, 8). Selenium is involved in both the thioredoxin and glutathione antioxidative systems (9). Moreover, selenium plays a key role in the regulation of the glycolysis pathways, protection of the body against lipid peroxidation, DNA synthesis, metabolism of thyroid hormones, and activity of natural killer cells and lymphocytes B and T (6, 10). This micronutrient is metabolized by the liver to selenoproteins and selenium metabolites (mainly trimethyselenonium), which are excreted into the urine and slightly through feces (9).

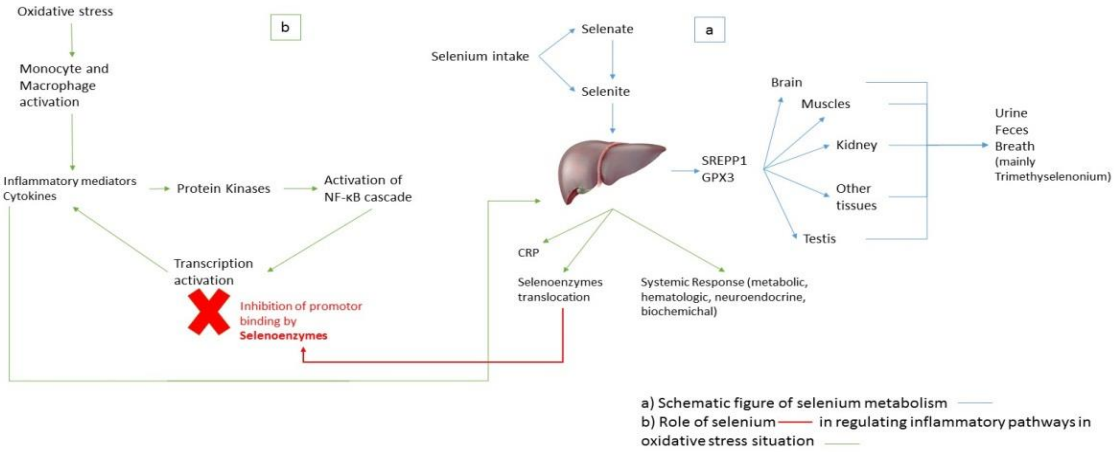
The most informative biomarkers used for the estimation of the selenium status in the body include extracellular glutathione peroxidase (10-25% of the plasma selenium) and selenoprotein P1 (40-60% of the plasma selenium) (9). The plasma concentrations of selenium and selenium-containing proteins are mainly amount-dependent, while factors such as age, smoking habits, malnutrition, obesity, race, and inflammation may also affect the plasma levels of selenium biomarkers (9). These selenoproteins show their plateau expression in conditions with adequate selenium intake (9).

Approximately 90% of the general pediatric population have adequate serum concentrations of selenium. On the other hand, this rate decreases to almost 10% in critically ill children (1, 11, 12). According to a recent study by Safaralizadeh et al., the serum selenium reference range in Iranian children has been estimated at 63-106 µg/l (13).

Selenium and Inflammation

According to the literature, selenium supplementation in the diseases with inflammatory pathophysiology (e.g., rheumatoid

arthritis, asthma, and inflammatory bowel disease) may reduce the levels of inflammatory interleukins, NF-kB and TNF-α (6). Additionally, selenium supplementation may lead to oxidative stress reduction and regulation of glycolysis, gluconeogenesis, and insulin transport pathways (11, 16). Selenium may also play a pivotal role in the improvement of the immune function and inflammation reduction through the regulation of the eicosanoids synthesis pathways and increasing the synthesis of prostaglandins and thromboxanes (6, 18). In addition, selenium supplementation could lower the expression of cytokine genes and adhesive molecules, while increasing the expression of the interleukin-2 receptor. Therefore, the activity of lymphocytes B and T, natural killer cells, and lymphokine-activated killer cells is expected to increase in the body (6). Figure 2 shows selenium metabolism and its role in the regulation of inflammatory pathways in association with oxidative stress.



leukotrienes and prostacyclins as opposed to

Figure 2. Selenium Metabolism and Its Role in Regulation of Inflammatory Pathways

Role of Selenium in Post-surgical Inflammation and Oxidative Stress Levels

According to the previous studies conducted on adult populations, most critically ill surgical patients have low plasma/serum selenium levels (19). In addition, previous interventional studies have indicated that selenium supplementation (especially at high doses) may improve the oxidative stress and inflammation status in critically ill adults (19). Evidence suggests that the redistribution of selenium, endothelial injury, altered metabolic process, and insufficient intake of selenium are the main contributing factors for the low selenium concentrations in critically ill patients, particularly in pediatric cases (1, 11, 20). On the other hand, plasma/serum selenium levels have been reported to be directly correlated with organ failure and severity of oxidative stress, while low levels of serum selenium are associated with the increased duration of mechanical ventilation dependency, ICU length of stay, and 28-day mortality (11).

Previous pediatric observational studies have proposed inconsistent results regarding serum/plasma selenium levels in acute-phase stress response. According to the studies conducted by Browman and Leite, 90.7% and 90.9% of critically ill children had low serum selenium levels during ICU admission (1, 11). Moreover, a recent clinical trial investigating the levels of oxidative stress biomarkers after cardiac surgery demonstrated increased plasma selenium concentrations after surgical trauma, and the elevation was attributed to glutathione peroxidase activity in critical conditions. However, the results of analysis of variance indicated no significant increase in the mentioned variable (21).

According to the literature, acute metabolic stress after major surgeries is highly prevalent, and additional selenium intake is required as the dose administration of physiological selenium may be insufficient (19, 22). According to the Australian guidelines of enteral and parenteral nutrition, short-term increase in selenium requirement after surgeries may be

due to the need for metabolic and antioxidative maintenance, and such patients may substantially benefit from high-dose selenium supplementation (22).

To the best of our knowledge, no clinical trials have investigated the possible benefits of high-dose selenium supplementation for critically ill children postoperatively. Therefore, it is recommended that further clinical trials (especially randomized clinical trials) be performed in this regard on critically ill post-surgical pediatric patients admitted to the PICU.

Conclusion

It is believed that oxidative stress and inflammation after major surgeries are prominent causes of poor clinical outcomes. Few studies have demonstrated the benefits of high-dose selenium supplementation through the reduction of oxidative stress and inflammation levels in critically ill post-surgical children. Therefore, further clinical trials are required in order to confirm the safety and efficacy of selenium doses in critically ill pediatric patients admitted at the PICU.

Authors' Contributions

All the authors equally contributed to conducting this review study.

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Conflicts of interest

None declared.

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The Effects of Endurance Training and Purslane (Portulaca oleracea) Seed Consumption on Cytochrome-C and Malondialdehyde in the Heart Tissues of Rats Poisoned with H2O2

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ABSTRACT

Introduction: Oxygenated water intake could increase cell death markers through increasing the free radicals. However, sport activities and antioxidant substances may prevent some of the symptoms caused by free radical production. The present study aimed to investigate the effects of endurance training (ET) and purslane (Portulaca oleracea; PO) seed consumption on cytochrome-C and malondialdehyde (MDA) in the heart tissues of rats poisoned with H2O2.

Methods: In total, 45 male rats were randomly divided into nine groups of five, including control, 50 mg/kg of PO, 200 mg/kg of PO, 400 mg/kg of PO, ET, ET with 50 mg/kg of PO, ET with 200 mg/kg of PO, ET with 400 mg/kg of PO, and healthy control. During eight weeks, groups 1-8 received H2O2 (1 mmol/kg) intraperitoneally three times per week, and groups 5-8 ran on treadmill three days per week.

Results: ET and PO significantly reduced cytochrome-C and MDA (P=0.001), while the interactive effects of ET and PO on the reduction of cytochrome-C (P=0.52) and MDA (P=0.08) were not considered significant. In addition, the administration of 200 mg/kg (P=0.01) and 400 mg/kg of PO (P=0.001) significantly decreased cytochrome-C, while 400 mg/kg of PO had more significant effects on the reduction of cytochrome-C compared to 200 mg/kg of the substance (P=0.01). Moreover, 400 mg/kg of PO significantly reduced MDA (P=0.001).

Conclusion: According to the results, ET and PO could improve cytochrome-C and MDA in the heart tissues of the rats poisoned with H2O2.

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Introduction

Cardiovascular diseases are significant health concerns across the world, which are predicted to account for over 40% of deaths by 2020 (1). Apoptosis is considered to be a major cause of cardiovascular disease, especially heart failure (2). This process plays a key role in the regulation of the balance between cell generation and cell death in various tissues, especially somatic tissues such as the heart muscle. Apoptosis initiates with the fragmentation of chromatins and densification of the cell cytoplasm and terminated by the crimping of the nucleus and cell membranes and production of vacuoles containing apoptotic particles (3).

The imbalance between the production of free radicals and antioxidant defense system is referred to as oxidative stress (4). Free radicals are found in several environmental sources, including photochemical air pollution, electromagnetic/particle radiation, tobacco smoke, and drugs (5). Oxidative stress occurs when the balance between peroxidants and antioxidants is shifted in favor of peroxidants (6). Despite the beneficial health effects of physical activity, oxidative stress is caused by the increased production of reactive oxygen species (ROS) during physical exercise due to increased oxygen consumption and metabolism (7).

Under normal circumstances, free radicals are the byproducts of the metabolism of oxygen in

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the body, which may cause damage to cell membranes and react with the genetic materials that may give rise to numerous diseases (8). Reactive free radicals affect several important cellular components (e.g., DNA, proteins, and membrane lipids), thereby leading to tissue damage. The damage caused by physical exercise is gradual and largely depends on the intensity, timing, and duration of the activity (9). The production of free radicals during physical activity is involved in the development of muscle injury and spread of inflammation after exercise, which in turn increase cellular damage. Endurance training (ET) prevents the manifestation of some of the symptoms induced by free radical production, while the damage caused by free radicals could improve the tissue antioxidant defense through enhancing the activity of antioxidant substances (10). The antioxidant system comprises of a set of biological antioxidants and antioxidant enzymes, which neutralize ROS *in-vivo*, including endogenous enzymatic antioxidants (e.g., superoxide dismutase [SOD] and catalase [CAT]). Among ROS, the hydroxyl radical group could cause fat peroxidation, some of the products of which may be variable concentrations of cytochrome-C and malondialdehyde (MDA), which are considered to be the indicators of oxidative stress.

Dietary factors are believed to play a critical role in the development of various human diseases, such as cardiac and metabolic disorders, atherosclerosis, hyperlipidemia, thrombosis, diabetes, and hypertension (11). In this regard, special attention has been paid to *Portulaca oleracea* (PO) (12), also known as the purslane plant, which belongs to the Portulacaceae family. PO is an annual plant with a succulent stem, reciprocating leaves, and small yellow flowers. This plant grows extensively in Iran and is used as an oral and traditional medicine (12).

PO has notable antiseptic, antispasmodic, diuretic, antioxidant, blood purifying, analgesic, and anti-inflammatory properties, while exhibiting protective functions against heart attack and strengthening the immune system (13). Furthermore, PO is an abundant source of various antioxidants, such as vitamins A, B1, C, and E, beta-carotene, and other essential amino acids (14). To date, several studies have been focused on the protective effects of PO. For instance, Ahangarpour et al. (2016) reported that administrating 200 mg/kg of PO for three weeks could attenuate the aging alternations induced by D-gal, as well as aging in the female reproductive system (15). In addition, Khodadadi et al. (2018) have claimed that 100, 200, and 400 mg/kg of PO could exert protective effects on cardiac dysfunction caused by subclinical hyperthyroidism induced by levothyroxine sodium in rats (16). Hozayen et al. (2011) have also concluded that aqueous PO extract could improve the adverse changes in renal function through increasing antioxidant activities and reducing peroxidation (17).

Considering the lack of observational studies regarding the simultaneous effects of ET and PO seed consumption on cytochrome-C and MDA in H_2O_2 poisoning, the present study aimed to investigate the effects of ET and PO consumption on cytochrome-C and MDA in the heart tissues of rats poisoned with H_2O_2.

Materials and Methods

This experimental study was conducted on 45 male Wistar rats aged eight weeks, which were purchased and transferred to the laboratory. The sample size was determined based on a previous study in this regard (8, 9). The rats were preserved in standard conditions for one week to adapt to the new environment. In terms of ethical considerations, the **r**esearchers received the required introduction letters from the Islamic Azad University, Central Tehran Branch in Tehran, Iran (ethics code: 10121404981020).

Grouping

On the eighth day, the rats were randomly divided into nine groups of five, including control, 50 mg/kg of PO, 200 mg/kg of PO, 400 mg/kg of PO, ET, ET with 50 mg/kg of PO, ET with 200 mg/kg of PO, ET with 400 mg/kg of PO, and healthy control. For eight weeks, groups 1-8 were administered with H_2O_2 (1 mmol/kg) intraperitoneally three times per week (18), groups two, three, four, and 6–8 received the PO daily at fixed doses via gastric intubation (19), and groups 5-8 ran on a treadmill three days per week (20).

ET Protocol

To preform ET, the rats ran on a treadmill in the first week at the speed of 8 m/min and slope of 10 degrees for 30 minutes. In the second week, the speed was 12 m/min with the same slope and time, and in the third week, the speed was

set at 16 m/min at the same slope for 45 minutes. In the fourth week, the running speed was 20 m/min at the same slope for 45 minutes, and in weeks 5-8, the rats were trained at the speed of 20 m/min with the slope of 10 degrees for 60 minutes per day (20).

PO Extract Preparation

The aqueous extract of PO was prepared in this study. After the preparation and grinding of PO, and 10 milliliters of saline per each gram of the powder was added to the beaker and boiled for 20 minutes. After cooling, the extract was filtered through a clean cloth, followed by Buchner's filter paper and funnel. At the next stage, the extract was heated again to concentrate until high viscosity was achieved. The final extract was transferred to several plates and incubated at the temperature of 70°C until completely dried. Afterwards, 500 milligrams of the dry substance was dissolved in 50 milliliters of saline, and 50, 200, and 400 mg/kg of the solution was injected to the rats (19).

Tissue Sampling

Forty eight hours after the last ET session and PO consumption, the rats were anesthetized with 10% ketamine (50 mg/kg) and 2% xylazine (10 mg/kg) after approximately five minutes. Following that, the heart tissues of the animals were extracted by specialists, and after setting in a cryotube, they were placed in liquid nitrogen and stored at the temperature of -70ºC for further investigation. Cytochrome-C and MDA were measured using the ELISA assay and CUSABIO laboratory kits (catalogue No. CSB-E14281r and CSB-E08558r), respectively.

Statistical Analysis

Data analysis was performed using independent-samples t-test, two-way analysis of variance (ANOVA), and Bonferroni post-hoc test (P≤0.05).

Results

Figures 1 and 2 depict the levels of cytochrome-C and MDA in the nine research groups. The results of independent-samples t-test indicated that the levels of cytochrome-C (P=0.001) and MDA (P=0.001) significantly increased in the control group compared to the healthy control group. In addition, the results of two-way ANOVA (Table 1) showed that ET (P=0.001) and PO consumption (P=0.001) significantly reduced cytochrome-C. However, the interactive effects of ET and PO consumption on the reduction of cytochrome-C were not considered significant (P=0.52).

According to the results of Bonferroni post-hoc test (Table 2), 50 mg/kg of PO had no significant effect on the reduction of cytochrome-C (P=0.99), while 200 mg/kg (P=0.01) and 400 mg/kg of PO (P=0.001) significantly reduced cytochrome-C. Moreover, 400 mg/kg of PO had a more significant effect on the reduction of cytochrome-C compared to 200 mg/kg of PO (P=0.01).

The results of two-way ANOVA (Table 1) indicated that ET (P=0.001) and PO (P=0.001) could significantly reduce MDA, while the interactive effects of ET and PO on the reduction of MDA were not considered significant (P=0.39). Furthermore, the results of Bonferroni post-hoc test (Table 2) showed that 50 mg/kg (P=0.99) and 200 mg/kg of PO (P=0.42) had no significant effects on MDA, while 400 mg/kg of PO could significantly reduce MDA (P=0.001).

Table 1. Results of Two-way ANOVA on Effects of ET and PO on Cytochrome-C and MDA Levels

Variable	Source	Sum of Squares	Mean Square	F	P-value	Partial Eta Squared
Cytochrome- C	ET	17.85	17.85	369.63	0.001	0.92
	PO	2.29	0.76	15.86	0.001	0.59
	Interaction of ET and PO	0.11	0.03	0.76	0.52	0.06
MDA	ET	891007.27	891007.27	318.98	0.001	0.90
	PO	169913.56	56637.85	20.27	0.001	0.65
	Interaction of ET and PO	8528.85	2842.95	1.01	0.39	0.08

ET: endurance training; PO: Portulaca oleracea

Table 2. Results of Bonferroni Post-hoc Test on Effects of PO Seeds (50, 200, and 400 mg/kg) on Cytochrome-C and MDA

Variable	Factor	200 mg/kg	400 mg/kg	No Consumption
Cytochrome- C	50 mg/kg	M=0.19	M=0.50	M=-0.13
		P=0.34	P=0.001	P=0.99
	200 mg/kg	-------	M=0.31	M=-0.32
			P=0.01	P=0.01
	400 mg/kg	-------	-------	M=-0.63
				P=0.001

MDA	50 mg/kg	M=21.59 P=0.99	M=145.80 P=0.001	M=-22.58 P=0.99
	200 mg/kg	-------	M=124.20 P=0.001	M=-44.17 P=0.42
	400 mg/kg	-------	-------	M=-168.38 P=0.001

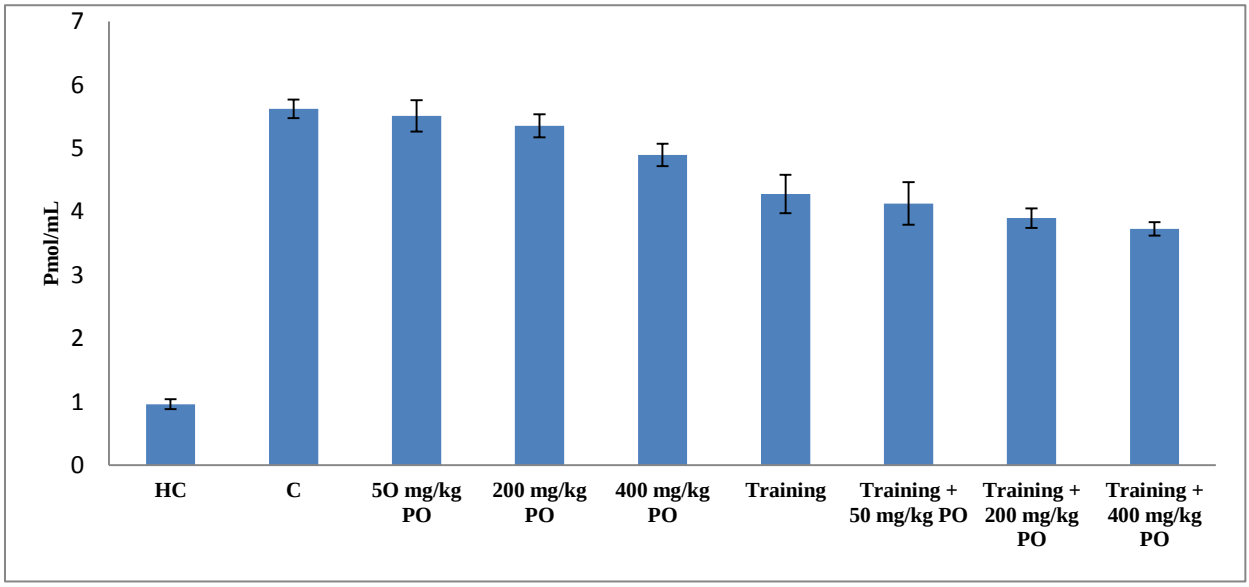

Figure 1. Cytochrome-C Level in Heart Tissues of Rats in nine Study Groups
(HC: healthy control, C: control, PO: *Portulaca oleracea*)

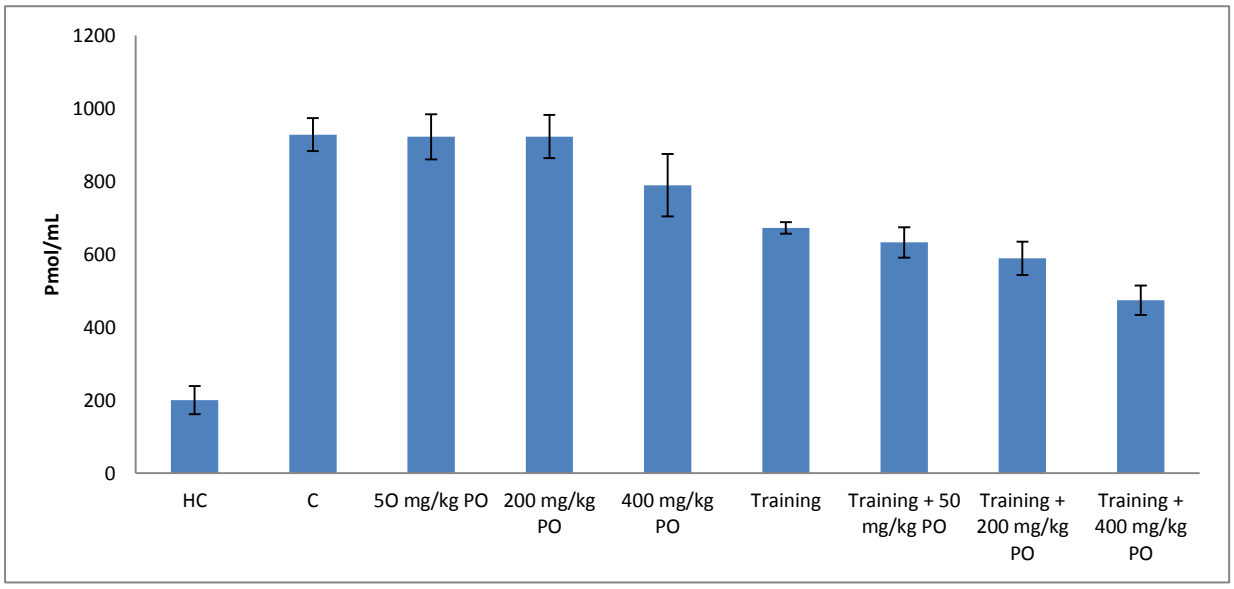

Figure 2. MDA Level in Heart Tissues of Rats in nine Study Groups

Discussion

According to the results of the present study, H_2O_2 poisoning significantly increased cytochrome-C and MDA in the heart tissue of the rats, while eight weeks of ET significantly reduced cytochrome-C and MDA in the heart tissue of the H_2O_2-poisoned rats. In this regard, Balcı et al. (2012) examined the effects of gender, ET, and acute exhaustion exercise on oxidative stress in the cardiac and skeletal muscles of rats, reporting that gender was a determinant of the changes in the MDA, nitric oxide (NO), and glutathione levels in the heart

tissues and skeletal muscles following exhaustive exercise or ET (8).

In another research, Ashrafi et al. examined the antioxidant protective effects of ET on the heart tissues of Wistar rats, claiming that ET significantly increased the levels of apelin, NO, and SOD, while slightly decreasing MDA (9). Moreover, Fakoory et al. investigated the effects of eight weeks of aerobic training on peroxidant and antioxidant indices in women with type II diabetes, reporting that after eight weeks of aerobic training, MDA levels decreased, while SOD and CAT levels significantly increased (4).

Most of the studies in this regard have confirmed the positive effects of sports activities on the improvement of cytochrome-C and MDA. Furthermore, the intensity of these activities is considered to be a significant influential factor in the changes in antioxidant enzyme, and most findings have demonstrated the anti-oxidative effects of physical exercise (21). With respect to the mechanism of the antioxidant effects of physical exercise, it has been suggested that exercise may influence oxidative stress processes through several mechanisms, including oxygen sitting at the electron transfer chain, prostanoid metabolism, xanthine oxidase and macrophage activity, and increased catecholamine activity (21).

According to the findings of the current research, eight weeks of PO consumption significantly reduced cytochrome-C and MDA in the heart tissues of the rats poisoned with H_2O_2. Consistent with our findings, eight weeks of PO consumption was reported to significantly decrease MDA and increase SOD and CAT in women with type II diabetes (4). In addition, the results obtained by Wang et al. (2010) indicated that PO had a significant effect on the reduction of MDA (22).

PO contains high levels of alpha-linoleic acid, beta-carotene, flavonoids, coumarin monoterpene and alkaloid glycosides, antioxidants, and omega-3. Plants containing omega-3 and omega-6 fats could inhibit lipid peroxidation by breaking down the existing oxidizing structure using cytochrome P450 and neutralizing free radicals, the effects of which are associated with these compounds in plants, especially omega-3 and linoleic acid (23).

According to the present study, 400 mg/kg of PO could significantly reduce MDA, while the concentrations of 200 and 400 mg/kg significantly reduced cytochrome-C. In addition, 400 mg/kg of PO had more significant effects on the reduction of cytochrome-C compared to the concentration of 200 mg/kg. Therefore, it could be concluded that the effects of PO on cytochrome-C and MDA were dose-dependent, and the effects significantly increased at higher doses of PO. Previous findings have also denoted that the phenolic compounds in the PO seed could inhibit hydrogen peroxide peroxidation activities in fatty acids, thereby decreasing MDA. Beta-cyanine is an effective compound in PO, which reduces oxidative stress. Several reports have confirmed the antioxidant effects of this plant and its inhibiting properties against lipid peroxidation and membrane vulnerability to free radicals (24).

Regarding the effects of PO on cytochrome- C, Zheng et al. suggested that Portulacerebroside A (PCA), which is a novel cerebroside isolated from PO, could reduce the viability of human liver cancer HCCLM3 cells. Additionally, PCA has been reported to markedly elevate the percentage of apoptotic cells, phosphorylation of *p38* mitogen-activated protein kinase and c-Jun N-terminal kinases, release of mitochondrial cytochrome-C and apoptosis inducing factor to the cytosol, and activation of caspase-9 and caspase-3. Furthermore, previous findings have emphasized that PO could be a proper candidate for cancer treatment (25).

With respect to interactive effects, our findings indicated that eight weeks of ET combined with PO seed consumption had no interactive effects on the reduction of cytochrome-C and MDA in the heart tissues of the H_2O_2-poisoned rats. Therefore, it seems that ET and PO seed differently affect cytochrome-C and MDA and have no synergistic effects.

Some of the limitations of the present study were the lack of control of the calorie content received by the rats and enormity of the oxidative stress pathway factors. Therefore, it is suggested that further investigations in this regard consider these factors in relation to oxidative stress and cell death and assess the effects of ET on various tissues in order to achieve more accurate results.

Conclusion

According to the results, ET and PO seed consumption could improve cytochrome-C and MDA in the heart tissues of the H_2O_2-poisoned rats. Moreover, the effects of PO seed on cytochrome-C and MDA were dose-dependent.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Antioxidant Effects of Continuous Training with Crocin Consumption on Doxorubicin-induced Hepatotoxicity in Rats

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ABSTRACT

Introduction: Doxorubicin has been reported to cause liver damage, while physical exercise and crocin consumption could improve antioxidant defense. The present study aimed to investigate the antioxidant effects of continuous training with crocin consumption on the liver tissues of doxorubicin-poisoned rats.

Methods: This experimental study was conducted on 40 rats, which were divided into five groups of eight, including unhealthy control (Dox), crocin consumption, continuous training, continuous training with crocin consumption, and healthy control (saline). For eight weeks, groups 1-4 received 2 mg/kg of doxorubicin peritoneally seven times every Friday throughout the study period. Groups 1-4 received 10 mg/kg of crocin peritoneally every day, groups three and four performed five sessions of continuous training per week, and group five were only injected with 0.9% normal saline.

Results: Doxorubicin induction could significantly decrease superoxide dismutase (SOD) and catalase (CAT), while increasing *malondialdehyde* (MDA). Continuous training and crocin consumption could significantly increase SOD and CAT in the doxorubicin-poisoned rats ($P<0.05$). However, continuous training with crocin consumption had no interactive effects on the increasing of SOD and CAT in the doxorubicin-poisoned rats ($P>0.05$), while continuous training and crocin consumption could significantly reduce MDA in the doxorubicin-poisoned rats. Moreover, continuous training with crocin consumption had interactive effects on the reduction of MDA in the liver tissues of the doxorubicin-poisoned rats ($P<0.05$).

Conclusion: According to the results, continuous training with crocin consumption had interactive effects on the reduction of MDA in the liver tissues of doxorubicin-poisoned rats, while it had no interactive effects on the increasing of SOD and CAT.

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Introduction

The liver occupies about 3-5% of the body mass, and some of its most important activities include metabolizing and detoxifying chemical drugs, such as anti-inflammatory drugs, painkillers, chemotherapeutic and antidepressant agents, and contaminants that may cause cellular oxidative stress (1, 2). Doxorubicin is a drug that is used in chemotherapy and is considered to be a highly effective anthracycline antibiotic, which is known with the trademark of adriamycin. Doxorubicin is prescribed alone or in combination with other drugs for the treatment of various neoplasms (3). However, the production of various reactive oxygen species (ROSs) and induction of apoptosis in healthy organs (especially the liver) have been reported to occur during the course of treatment with this drug, limiting its use and increasing its challenges (4).

Oxidative stress and inflammation are considered to be the major causes of liver diseases, which ultimately lead to various types of cell death, such as apoptosis, necrosis, necroptosis, and autophagy, as well as vascular injury in the liver. Liver toxicity occurs in response to toxic reactions and chemotherapeutic drugs not only in the hepatocytes, but also in the endothelial cells, Kupffer cells, and satellite cells (5). Therefore, use of chemotherapeutic drugs such as doxorubicin may cause liver damage due to the induced toxicity, with the patterns of damage including necrosis, steatosis, fibrosis, cholestasis,

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and liver vascular injury (6). In this regard, some findings have indicated that regular physical exercise a few days per week at moderate intensity could enhance the antioxidant defense of the body and its physiological adaptation (7). However, other studies have suggested that physical exercise may increase the production of free radicals and their balance with antioxidants (8).

Another approach involves the use of herbal antioxidants in non-pharmacological treatments. Crocin is a water-soluble carotenoid, which constitutes approximately 3.5% of the dried stigma of saffron (9). Numerous studies have shown that the crocin found in saffron is a potential antioxidant owing to its carotenoid structure and is used in clinical therapies (10). The main therapeutic properties of this compound include anti-inflammatory, antioxidant, anticancer, and antitumor effects, which have received special attention from medical researchers (11).

Considering the inadequate studies regarding the interactive effects of antioxidants on physical exercise and crocin antioxidant supplementation with the liver tissues of doxorubicin-poisoned rats, the present study aimed to investigate the antioxidant effects of continuous training along with crocin consumption on the liver tissues of doxorubicin- poisoned rats.

Materials and Methods

This experimental study was conducted on 40 Wistar rats with the mean age of eight weeks and mean weight of 200-220 grams, which were obtained from Ahvaz Jondi Shapur Laboratory of Experimental and Proliferation Animals, Iran and maintained in clean and transparent cages in standard conditions at an ambient temperature (23+3°C) within a 12-hour light-dark cycle at 35±5% humidity. The animals had adequate ventilation and unlimited access to water and food for two weeks prior to the tests in order to adapt to the laboratory conditions.

The adaptation training program was performed on a rodent treadmill at the speed of 8-10 m/min and slope of 0°C for 5-10 minutes during 10 days. After two weeks of adaptation to the environment and training, the rats were randomly divided into five groups of eight, including unhealthy control (Dox), crocin consumption, continuous training, continuous training with crocin consumption, and healthy control (saline).

There were some limitations in the study of the incompatibility of the trained rats and mortality induced by doxorubicin injection, and these animals were excluded from the study and replaced by other rats. This study was performed in accordance with the ethical guidelines of the Ministry of Science and Research as adopted from Marvdasht Islamic Azad University (code: IR.IAU.M.REC.1398.013).

In this experimental study, 40 Wistar rats weighing approximately 220±20 grams were purchased and transferred to the laboratory in standard conditions. The animals went through an adaptation phase for seven days. In total, 40 rats were divided into five groups of eight, including unhealthy (Dox), crocin consumption, continuous training, continuous training with crocin consumption, and healthy (saline).

During the study, all the rats (n=32) were peritoneally administered with 2 mg/kg of doxorubicin (Belgian Abve Company), which was dissolved in normal saline. The drug was administered seven times every Fridays (48 hours after the last training session and 24 hours before the next session). The rats in groups 2-4 received 10 mg/kg of crocin (Sigma-Aldrich Co., St. Louis, MO, USA) via oral gavage daily, and the healthy and doxorubicin groups received the same amount of normal saline via gavage (12, 13). In order to similarize the conditions of the subjects and neutralize the effects of injection on the animals in group five, equal amounts of saline (0.9% sodium chloride) was administered. The animals in groups three and four performed continuous training five sessions per week. In order to initiate and perform the continuous training protocol in the present study, the rats ran 10 minutes on an animal treadmill at the speed of 5 m/min and slope of 0° for one week.

The main part of the continuous training was conducted using an animal treadmill in a one-hour session per day five days a week for eight weeks. The first week of the main training began with 40% of the maximum running speed. From the second to the fourth week, the speed reached 50-55% of the maximum running speed, and from the fifth to the eighth day, it reached 60% of the maximum running speed (14). The maximum running speed was measured using the incremental exercise test protocol. Initially, the rats started running at the speed of 10 meters per minute, and to feel fatigue, the running speed increased 1.7 meters per minute every two minutes until the rats were exhausted.

Exhaustion was considered when the rats touched the bottom of the canal five times in one minute.

The duration of each exercise training session was one hour, with the warm-up program performed at the beginning of each training session, consisting of five minutes of running at 7 m/min. Moreover, cooling down was performed at the end of the exercise through the stepwise reduction of the speed to 7 m/min at the end of each training session (15). It is also notable that in order to investigate the effects of doxorubicin on the study variables, the remaining eight rats were assigned to the healthy control group, and 24 hours after the last training session at the end of the eighth week, the rats underwent surgery in order to measure the studied parameters. To this end, the rats were anesthetized by 10% ketamine and 2% xylazine after approximately five minutes. Following that, their liver tissues were extracted by specialists. A cryotube was inserted into liquid nitrogen, and preserved at the temperature of -70˚C for further examination. Finally, catalase (CAD), superoxide dismutase (SOD), and malondialdehyde (MDA) levels were measured using ELISA, ZellBio GmbH (ZB-CAT-96A at 0.5 kU/l sensitivity), ZB-SOD-96A at 1KU / L sensitivity), and ZB-MDA-96A kits (0.1 µM sensitivity), respectively.

To investigate the normality of the data distribution, the Shapiro-Wilk test was used. To analyze the findings, independent sample t-test and two-way analysis of variance (ANOVA) were applied (P≥0.05).

Results

The levels of SOD, CAT, and MDA are presented in Figures 1-3, respectively.

The results of independent sample t-test (Table 1) indicated that SOD (P=0.001) and CAT levels (P=0.001) significantly decreased in the control group compared to the healthy control group. However, the MDA levels significantly increased (P=0.001).

According to the results of two-way ANOVA (Table 1), eight weeks of continuous training and crocin consumption could significantly increase SOD in the liver tissues of the doxorubicin-poisoned rats. On the other hand, continuous training concurrent with crocin consumption had no interactive effects on the increased SOD in the liver tissues of the doxorubicin-poisoned rats.

According to our findings, eight weeks of continuous training and crocin consumption could significantly increase CAT in the liver tissues of the doxorubicin-poisoned rats, while continuous training concurrent with crocin consumption had no interactive effects on the increasing of CAT in the liver tissues of the doxorubicin-poisoned rats. Furthermore, eight weeks of continuous training and crocin consumption could significantly decrease MDA in the doxorubicin-poisoned rats, while continuous training along with crocin consumption had interactive effects on the reduction of MDA in the liver tissues of the doxorubicin-poisoned rats.

Table 1. Results of Two-way ANOVA and Independent Sample T-test on Effects of Doxorubicin, Continuous Training, and Crocin Consumption on SOD, CAT, and MDA

Parameter	Independent Sample T-test		Two-way ANOVA								
			Training			Crocin Consumption			Interactive Effects		
	t	P-value	F	P-value	Effect Size	F	P-value	Effect Size	F	P-value	Effect Size
SOD	25.24	0.001*	105.91	0.001€	0.79	32.93	0.001¥	0.54	0.21	0.65	0.007
CAT	26.75	0.001*	251.07	0.001€	0.90	91.45	0.001¥	0.76	3.25	0.08	0.10
MDA	35.16	0.001*	933.15	0.001¥	0.97	597.99	0.001¥	0.95	269.82	0.001£	0.90

*Significant difference between healthy control group and control group; €: significant effect of continuous training on increased CAT and SOD and decreased MDA; ¥: significant effect of crocin on increased CAT and SOD and decreased MDA; £: significant interaction of continuous training and crocin on decreased MDA

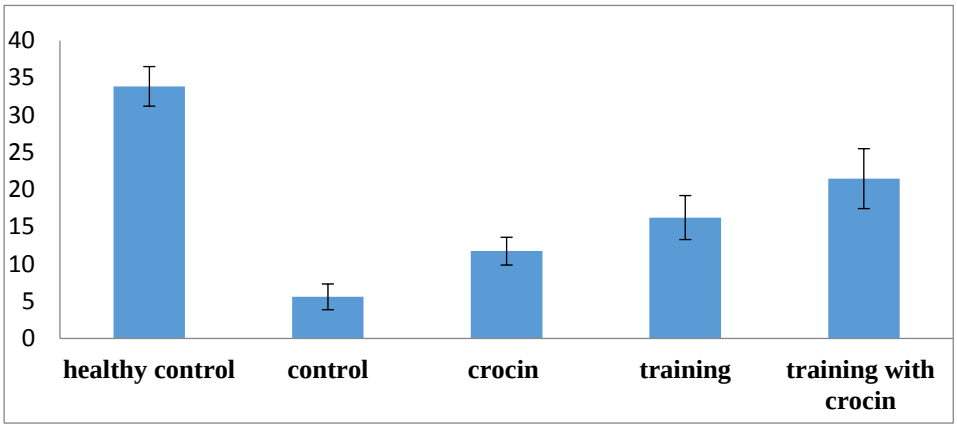

Figure 1. SOD (mg of protein) Levels in Study Groups

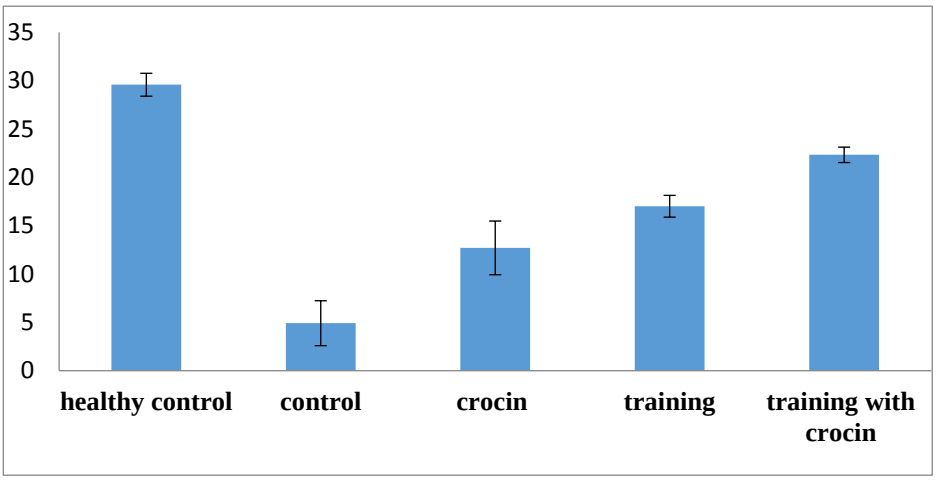

Figure 2. MDA (nmol/mg protein) Levels in Study Groups

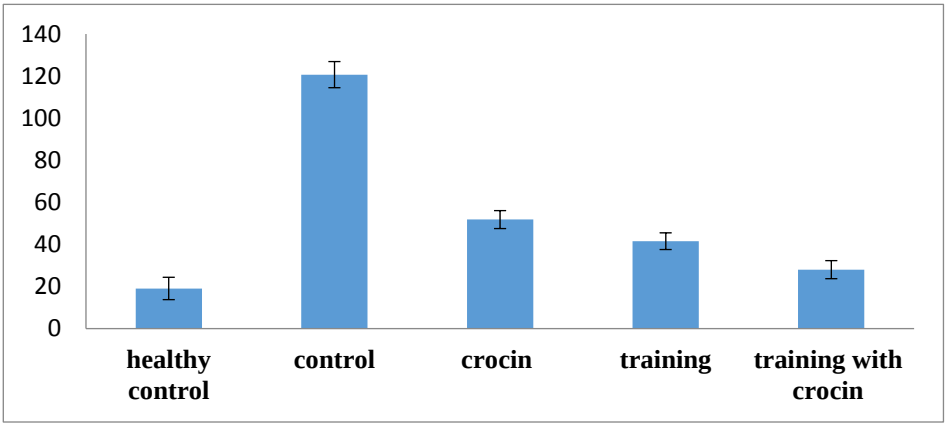

Figure 3. CAT (mg of protein) Levels in Study Groups

Discussion

The present study aimed to investigate the antioxidant effects of continuous training and crocin consumption on the liver tissues of doxorubicin-poisoned rats. According to the findings, doxorubicin induction significantly

increased MDA and significantly decreased SOD and CAD in the liver tissues of the rats. Consistent with our findings, doxorubicin has been reported to significantly increase MDA and significantly reduce SOD, which is an influential factor in oxidative stress and liver toxicity in rats (16). Furthermore, previous studies have denoted that the acute induction of various doses of doxorubicin could cause a marked decrease in CAT (17).

According to the results of the present study, eight weeks of continuous training significantly decreased MDA concentrations and significantly increased SOD and CAT concentrations in the liver tissues of the doxorubicin-poisoned rats. Moderate-intensity exercise is beneficial to health and disease prevention through reducing the production of oxidative stress (18). In addition, physical activity enhances the antioxidant defense and reduces lipid peroxidation in middle-aged and elderly individuals. Moderate-intensity exercise also has beneficial effects on the reduction of oxidative stress (19).

Several factors contribute to the induction of oxidative stress during exercise, including the type, intensity, and duration of exercise, individual characteristics, gender, nutrition, and genetics (20). Following three weeks of aerobic training, we observed a significant increase in the levels of glutathione peroxidase (GPX), as well as a significant decrease in the MDA and carbonyl protein levels in the liver of the doxorubicin-induced rats. Aerobic exercise has been shown to regulate hepatotoxicity indices and could be considered as a non-pharmacological approach for disease treatment (21).

In another study in this regard, the effects of three and six weeks of aerobic training on the liver tissues of doxorubicin-induced rats were compared, and no significant differences were reported between the training sessions and CAT. However, a significant increase was observed in CAT, indicating the reduction of oxidative stress in the liver tissues (22). Moreover, six weeks of aerobic training with doxorubicin induction significantly increased nitric oxide and SOD, while significantly decreasing the MDA, indicating the adaptation and protection of exercise effects on cytotoxicity (23).

Concerning the consistency of our findings with the results of the previous studies in this regard, it could be concluded that continuous exercise reversed the imbalance between the pre-oxidant and oxidant reactions due to doxorubicin induction in the liver tissues of the rats. One possible mechanism is the beneficial effects of exercise and training on the ROS and activation of the NF-KB and MAPK pathways in cells, leading to the increased production of antioxidant enzymes, such as SOD, glutathione reductase (GR), and CAT (24).

The activation of these pathways activates antioxidant enzymes such as manganese superoxide dismutase in the mitochondria (MnSOD or SOD2), as well as the copper-zinc superoxide dismutase in the cytosol and nucleus (Cu Zn SOD or SOD1), which convert peroxidase anion into hydrogen peroxide (H_2O_2), GR, and CAT, transferring hydrogen to water (25). On the other hand, researchers have recommended the strategy of using herbal medicines and supplements along with chemotherapy. Evidence suggests that crocin consumption could reduce the adverse effects of chemotherapy with doxorubicin.

In the present study, eight weeks of using 10 mg/kg of daily crocin significantly decreased the MDA concentration and significantly increased the SOD and CAT concentrations in the liver tissues of the doxorubicin-induced rats. Consistent with our findings, a similar study suggested that crocin supplementation significantly reduced the MDA and significantly increased SOD and CAT antioxidant enzymes in the cardiac tissues of doxorubicin-poisoned rats. In addition, crocin was reported to have protective effects against the damage induced by this drug (26).

According to the literature, the consumption of crocin in the rats exposed to hepatic ischemia-reperfusion could increase cardiac tissue antioxidant activity through the improvement of THE SOD, CAT, and GPX enzymes and protecting the heart against ischemia-reperfusion injury (27). This is in line with the findings of the current research, which indicated that crocin traps free radicals with its specific carotenoid structure, thereby acting as a potential antioxidant.

According to the results of the present study, continuous training along with crocin supplementation in the doxorubicin-induced rats decreased MDA, while no significant changes were observed in the SOD and CAT antioxidant enzymes. Regarding the antioxidant effects of

physical exercise and crocin consumption on intoxication with doxorubicin, our findings could be compared to no studies. Therefore, further investigations are required to obtain more accurate data on the mechanism of the interactive effects of exercise and crocin consumption.

Conclusion

According to the results, performing regular exercise for eight weeks could improve the antioxidant/oxidant balance and decrease doxorubicin-induced toxicity through increasing the SOD and CAT levels and decreasing the MDA oxidant index. In addition, crocin supplementation as an antioxidant alone improved the antioxidant/oxidant balance in favor of reducing the oxidative stress induced by doxorubicin, while concurrent regular exercise and crocin supplementation had no effects on the antioxidant system and only reduced MDA due to doxorubicin induction in the liver tissues of the rats, which requires further investigation.

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The Effects of Alginate Coatings Containing *Zataria multiflora Boiss* Essential Oil in the Forms of Coarse Emulsion and Nano-emulsion on Inoculated *Escherichia coli* O157: H7 in Beef Fillets

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ABSTRACT

The present study aimed to compare the inhibitory effects of alginate coatings containing *Zataria multiflora Boiss* essential oil (ZMEO) in the forms of coarse emulsion and nano-emulsion on the growth of inoculated *Escherichia coli*O157: H7in beef fillet during 16 days of refrigeration at the temperature of 4°C. Alginate solutions (3%) with various concentrations of ZMEO (0.25%, 0.5%, and 1%) were prepared, and the coarse emulsion and nano-emulsion forms were also prepared. The beef fillets were inoculated with *E. coli*O157: H7(1.5×10^5 log CFU/g) and immersed in various alginate treatments, and the bacterial count was performed during refrigeration on days zero, four, eight, 12, and 16.The obtained results indicated that the alginate coating containing ZMEO in both forms (coarse/nano-emulsion) was a proper candidate to control *E. coli*O157: H7at the temperature of 4°C. However, the antibacterial effects were more significant on the samples treated by the nano-emulsion form compared to the coarse emulsion form and controls. In addition, the lowest bacterial growth was observed in the samples coated with the alginate nano-emulsion containing 1% ZMEO (5.3±0.24 log CFU/g) at the end of storage. Therefore, it could be concluded that the use of alginate coatings containing ZMEO (particularly in the nano-emulsion form) could effectively decrease the growth of *E. coli*O157: H7 during storage, and this natural additive could be applied in the food industry, especially the meat industry.

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Introduction

Food-borne diseases are associated with high mortality and economic losses every year and are primarily caused due to the consumption of the products that are contaminated with bacterial pathogens (1).Considering the growing rate of various diseases and their severity across the world, the safety of meat and its products have become increasingly important.

Antibiotics have played a pivotal role in the treatment of infectious diseases over the past decades, while the complications associated with microbial resistance to antibiotic treatment have urged researchers to opt for the use of natural antimicrobial compounds in foods (1).

Enterohemorrhagic *E. coli* is an important foodborne pathogen, which is an *E. coli* subgroup producing Shiga-toxin and could cause hemolytic uremic syndrome and hemorrhagic colitis in humans. The most common serotype is of this bacterium is O157: H7, which has become epidemic, leading to high mortality in Europe, North America, and Canada. Such epidemics are associated with the consumption of half cooked meat and water and food contaminated with cattle manure. This serotype has been isolated from 3.7% (164.6%) of fresh beef samples and 1-2% of other fresh meat products, such as pork, poultry, and lamb (2).Food contamination by *E. coli* O157: H7 has been reported worldwide (3).

An effective approach to enhancing the safety of food such as fresh meat and its products is to use edible coatings. Edible coatings are thin layers of the materials that cover foods, preventing moisture, oxygen, and nutrient-soluble materials from transmission to food products (4). Among the other advantages of edible coatings are eco-friendliness, non-toxicity, non-polluting, and cost-effectiveness. Furthermore, coatings and films could be used as carriers of additives, flavors, antioxidants, and antimicrobials (5).

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Alginate is an important edible coating, which is an alginic acid salt and a polymer of D-β-mannuronic acid (M) and L-α glucuronic (G) units. Alginate is a proper coating candidate owing to its unique colloidal properties, including stability, consistency, suspension, thin-layer coating formation, gel production, and emulsion stabilization (4). In addition, this coating is water-soluble and able to preserve the taste, color, and nutritional value of food products (e.g., vitamin and essential amino acid preservation) (6). The most prominent feature of alginates is their ability to react with multi-capacity metal cations (particularly calcium ions) to produce potent gels or insoluble polymers (7). Essential oils are natural compounds that could be used as additives in coatings and films in order to increase the shelf life and safety of food products. Essential oils are volatile compounds with potent antimicrobial and antioxidant activity and could be applied in various food products to reduce or eliminate pathogens as natural antimicrobial additives (2). *Zataria multiflora Boiss* essential oil (ZMEO) is a well-recognized essential oil containing phenol monoterpene compounds, such as carvacrol, thymol, and P-cymene in various parts of plants (8).

Nanotechnology has become a priority field in today's world trade. The production of nano-emulsions for plasticization and controlling the release of bioactive compounds (e.g., drugs, colors, essential oils, and vitamins) has attracted the attention of researchers in the food industry. Nano-emulsions are submicron emulsions with the droplets size of 50-1,000 nanometers. Nano-emulsions are thermodynamically and synthetically stable, and owing to their special size, they may be transparent or semi-transparent through the naked eye. Moreover, they are stable and resistant against sedimentation and becoming creamy. These properties have rendered nano-emulsions proper options for fundamental and applied studies in chemical, health, pharmaceutical, food sciences, and several other fields (9).

Since beef is a major ingredient in the diet of individuals, its contamination during processing may lead to the survival and growth of *E. coli* O157: H7, which could be easily transmitted to humans, thereby causing severe human epidemics, especially in children, the elderly, and pregnant women. Therefore, the control and prevention of food contamination by this bacterium is of utmost importance.

The present study aimed to enhance food safety against *E. coli* O157: H7 using natural antimicrobial agents as functional coatings in the form of coarse emulsion and nano-emulsion.

Materials and Methods

Experimental Material

Sodium alginate was obtained from Sigma-Aldrich (USA), and sorbitol-MacConkey agar, glycerol, and calcium chloride were provided by Merck (Darmstadt, Germany). The bacterial strain of *E. coli* O157: H7 (NCTC: 12900) was obtained from the microbial collection of the Laboratory of Food and Aquaculture at the School of Veterinary Medicine at Ferdowsi University of Mashhad in Mashhad, Iran.

Preparation of the Bacteria

The bacterial strain was cultured on sorbitol-MacConkey agarcontaining potassium tellurite and cefixime (CT-SMAC) and incubated at the temperature of 37°C for 24 hours. In addition, typical colonies were cultured in 10 milliliters of sterilized brain heart infusion (BHI) broth and incubated at the temperature of 37°C for 24 hours. Afterwards, one milliliter of the sample was collected, re-cultured in 10 milliliters of sterile BHI broth, and incubated at the temperature of 37°C for 18 hours in order to achieve the logarithmic phase of bacterial growth. The bacterial suspension was adjusted to 0.5 McFarland standard turbidity using a spectrophotometer (optical density: 0.85-0.1) at the wavelength of 600 nanometers in order to attain 1.5×10^8 CFU/ml and diluted (1:10) to $1/5\times 10^7$ CFU/ml as the desired bacterial density.

Preparation of the Beef Fillets and Bacterial Inoculation

Fresh beef was purchased from a local market and transferred to the laboratory in an ice box, and 10-gram fillets were prepared. In order to remove the normal microbial flora, the samples were immersed in 70% ethanol solution for 3-5 minutes and sterilized until ethanol was evaporated and the samples were dried. Following the sterilization and drying of the samples, 100 microliters of *E. coli* O157: H7 was inoculated with 1.5×10^7 of the bacteria per milliliter (final dose: 1.5×10^5) on each 10-gram beef fillet, which was finally spread onto the samples using a sterilized L-shaped rod.

Preparation of the Alginate Coating Containing ZMEO in the Form of Simple Emulsion and Nano-emulsion

At this stage, three grams of sodium alginate was dissolved in 100 milliliters of distilled water in order to provide 3% alginate coating. In addition, two grams of calcium chloride was dissolved in 100 milliliters of distilled water in order to prepare 2% calcium chloride solution. Afterwards, 2% glycerol (v/v) was added to the coating solution as the plasticizer and mixed using a heater stirrer. The essential oils were added to the solution at the concentrations of 0.25%, 0.5%, and 1% (w/v). In order to distribute the essential oil in the coating solution, 0.5% of Tween 80was used as the emulsifier.The alginate nano-emulsion containing the essential oil was prepared using the high-energy ultra-sonication method. To this end, the 3% alginate solution was initially prepared, and the nano-emulsion was prepared afterwards using DIAX 900 (Heidolph, Germany) for six minutes and an ultrasound device (200 W HF-power, Bandelin, Germany). At the next stage, the size of the particles was evaluated using DLS (Nano S, Malvern, UK).

Preparation of the Experimental Treatments

All the samples (eight groups) were placed in 3% alginate solution for one minute. After coating, the samples were drained for 30 minutes and re-suspended in 2% calcium chloride solution for 30 seconds (Table 1). Following that, each sample was refrigerated in sterile zipper packs at the temperature of 1±4°C for further analysis on days zero, four, eight, 12, and 16.

Table 1. This study applied treatments

	Treatments	Description
1	Control	Sample without alginate coating
2	Alginate control	Sample with alginate coating
3	0.25% emulsion	Sample with alginate coating containing 0.25% Thymus essential oil as simple emulsion
4	0.5% emulsion	Sample with alginate coating containing 0.5% Thymus essential oil as simple emulsion
5	1% emulsion	Sample with alginate coating containing 1% Thymus essential oil as simple emulsion
6	0.25% Nano-emulsion	Sample with alginate coating containing 0.25% Thymus essential oil as Nano-emulsion
7	0.5% Nano-emulsion	Sample with alginate coating containing 0.5% Thymus essential oil as Nano-emulsion
8	1% Nano-emulsion	Sample with alginate coating containing 1% Thymus essential oil as Nano-emulsion

Counting of E. coli O157: H7

Initially, 10-gram samples were mixed in zipper packs with 90 milliliters of sterilized peptone water and placed in a bag mixer for three minutes in order to obtain a homogeneous suspension (dilution: 10^{-1}). Afterwards, one milliliter of the supernatant was collected by a sampler and poured into a tube containing nine milliliters of sterilized peptone water in order to obtain 10^{-2} dilution. After the preparation of the serial dilutions, they were drop-cultured on the sorbitol-MacConkey agar containing potassium tellurite and cefixime and incubated at the temperature of 37°C for 24 hours of storage.

Statistical Analysis

Data analysis was performed in SPSS version 21, and the data of each group was expressed in the mean, standard deviation, minimum, and maximum of the total bacterial counts during the storage period. Moreover, the bacterial growth of the study groups was analyzed for 16 days using repeated measures ANOVA, and Bonferroni's post-hoc test was also used to compare the treatments. The P-value of less than 0.05 was considered statistically significant.

Results and Discussion

Table 1 shows the growth patterns of the inoculated *E. coli* O157: H7 in various treatments during storage. Accordingly, the number of the bacteria decreased in all the groups during this period as the studied strains were mesophile bacteria. The highest and lowest microbial load were observed in the alginate group (4.46 log CFU/g) and nano-emulsion group with the ZMEO concentration of 1% (2.11 log CFU/g), respectively after the storage period, which confirmed the significant antimicrobial properties of the nano-emulsion of the alginate coating containing the essential oil. In another study, Moghimi et al. (2016) evaluated the effects of the pure form and nano-emulsion of *Thymus daenensis* on *E. coliin-vitro*, reporting that the antibacterial properties of the nano-emulsion form were more significant compared to the pure form due to its increased ability in destroying the bacterium membrane; this is consistent with the results of the present study (10). In addition, Sorino et al. (2015) investigated the antibacterial activity of chitosan-based modified coatings containing nano-treated essential oils, gamma

rays, and modified atmosphere packaging independently and in combination with green beans inoculated with *E. coli* O157: H7 during 13 days at the temperature of 4°C. According to the findings, the nano-treated carvacrol had the highest antibacterial activity against *E. coli* O157: H7. Since the antibacterial activity of *Zataria multiflora Boiss* depends on carvacrol as its major component, the mentioned finding is in line with the current research (11).

According to the results of the present study, the alginate coating containing the essential oil (0.25%, 0.5%, and 1%) was more effective in the reduction of bacterial growth compared to the alginate group alone. In another research, Heydari et al. (2015) assessed the impact of sodium alginate coating containing 0.5% and 1% of oregano essential oil on the quality of carp fillets maintained at the temperature of 4°C (12). According to the obtained results, the samples treated with the sodium alginate containing the essential oil could reduce bacterial growth more effectively compared to the control and alginate groups, which is consistent with our findings (13).

Table 2. Average logarithmic reduction of *E. coli* O_{157}: H_7 in different study groups

Mean Difference I-J / Group (I)	Group (J) Alginate	AL+EO 0.25%	AL+EO 0.5%	AL+EO 1%	AL+EO 0.25% nano	AL+EO 0.5% nano	AL+EO 1% nano
Control	-0/0052	0/51***	0/63***	0/83***	0/82***	1/06***	**1/41*** **
Alginate		0/52***	0/64***	0/87***	0/83***	1/07***	**1/42*** **
AL+EO 0.25%			0/12	0/35***	0/31***	0/55***	**0/90*** **
AL+EO 0.5%				0/22*	0/18***	0/42***	**0/77*** **
AL+EO 1%					-0/04	0/19***	**0/54*** **
AL+EO 0.25% nano						0/24*	**0/58*** **
AL+EO 0.5%nano							**0/34*** **

Description: The values being entered at the intersection of the two groups show logarithmic mean difference (I-J) during the study period. Duplicate values were not written here. *** : 0/001< .p and* :0/05<p،<

Table 2 shows the reduction of *E. coli* O157: H7 counts and their comparison during 16 days of refrigeration. Accordingly, the most significant difference was observed between the alginate group and nano-emulsion at 1% concentration (1.42 log CFU/g). Furthermore, the comparison between the alginate coating treatments containing the essential oil in the form of simple emulsion and nano-emulsion revealed that the nano-emulsion treatments were more effective compared to the simple emulsion treatments at similar concentrations, indicating the enhanced antimicrobial properties of the essential oil in the nano-emulsion treatments, which increased significantly at the higher concentrations of the essential oil.

In a similar research, Hashemi et al. (2017) investigated the antimicrobial properties of the nano-emulsion of *Zataria multiflora Boiss* as an additive to polymer-based packaging materials, and the obtained results indicated that the increased concentration of the nano-treated essential oil was associated with more significant antimicrobial effects, which is consistent with the results of the present study (12).

According to the findings of the current research, the bacterial changes in *E. coli* O157: H7 decreased from 5.36% to 4.46% in the alginate treatment during 16 days of storage, while no significant difference was observed with the control group. On the same note, Fatih et al. (2009) reported that the alginate group with no antimicrobial agents had no impact on the reduction of the *E. coli, Listeria Ivanova*, and *Pseudomonas fluorescens*, which is in line with our findings (15).

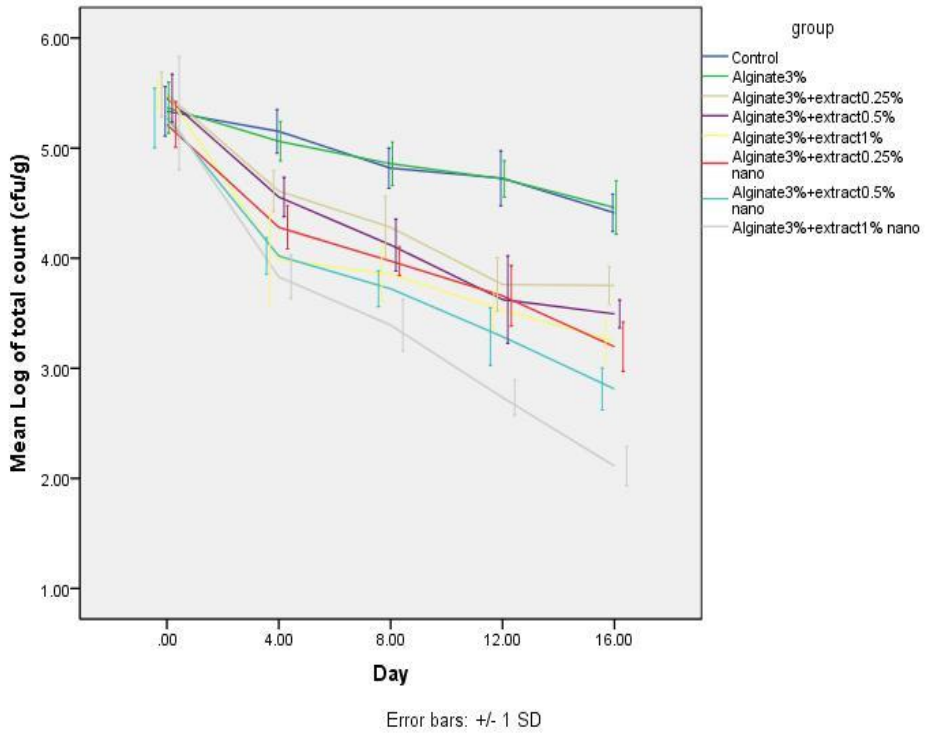

Figure 1. Logarithmic variation of the number of E. coli O_{157}: H_7 of different groups during the study period

Conclusion

The growth inhibition of *E. coli* O157: H7 was investigated in alginate treatments containing the ZMEO in the form of simple emulsion and nano-emulsion at various concentrations (0.25%, 0.5%, and 1%). According to the results, the use of alginate coating containing ZMEO in the nano-emulsion form could reduce the *E. coli* population on the refrigerated beef fillets more effectively compared to the simple emulsion for at identical concentrations. Moreover, significant results were observed regarding the effects of the treatment on the growth of *E. coli* O157: H7during storage.Finally, the obtained results demonstrated that the nano-emulsion group at 1% concentration had the optimal outcomes regarding the growth inhibition of *E. coli* O157: H7 in the beef fillets. Considering the priority of using natural additives in food products by manufacturers and consumers, it is recommended that alginate coating solution containing *Zataria multiflora Boiss* be applied in beef fillets in order to increase its safety against pathogenic bacteria. It is also notable that such treatments alone cannot entirely eliminate food contamination, and hurdle technology and other storage techniques (e.g., heating) should also be applied to effectively remove various contaminants.

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Conflict of Interests

None declared.

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The Effect of Short-term Periodic Fasting on Acetaminophen-induced Liver Injury in Mice

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ABSTRACT

Introduction: In many cultures, fasting is recommended for health protection and promotion. However, few studies have been focused on the effects of fasting on organ function and resistance to toxic agents (e.g., drugs). The present study aimed to investigate the effects of short-term periodic fasting on the hepatotoxic effects induced by acetaminophen in mice.

Methods: This experimental study was conducted on female BALB/c mice to assess the effects of short-term periodic fasting (three consecutive days every two weeks for 10weeks) on the serum levels of aspartate transaminase (AST) and alanine amino transferase (ALT)and hepatotoxic effects induced by acetaminophen. After 10weeksof periodic fasting, the mice were administered with 500 mg/kg of acetaminophen via intra peritoneal injection. After 24 hours, the AST and ALT levels were measured, and the mice were sacrificed to evaluate their liver injury severity using the pathological method as the gold standard.

Results: The AST and ALT enzymes increased in the control group (P=0.0098 and P=0.0004, respectively; Mann-Whitney U test), which was associated with high-grade liver injury (P=0.001; Fisher's exact test). In contrast, the fasting mice had slight changes in the levels of AST and ALT enzymes associated with low-grade liver injury.

Conclusion: Acetaminophen is a common cause of drug-induced liver injury. According to the results of the study, fasting could protect important organs (e.g., liver) against the toxic effects of drugs. Further investigations in this regard could provide insight into human states.

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Introduction

Fasting is an intense mode of dietary restriction, which involves the avoidance of all foods (except water) with intervening periods of normal food consumption. Depending on the duration, fasting could be classified as intermittent fasting (≥16 hours of fasting/day or 48 hours of fasting/week) and periodic fasting (minimum of three days of fasting/two or more weeks)(1).Fasting not only decreases blood glucose levels and growth factor signaling, but it also activates the stress resistance pathways, thereby leading to the modulation of cell growth, energy metabolism, and protection against oxidative stress, inflammation, and cell death(1, 2).

According to Longo and Mattson, intermittent fasting in rodents could exert protective effects against cancer, cardiovascular diseases, diabetes, and neurodegeneration(2). In another study, Brandhorst et al. demonstrated that prolonged fasting (two or more days of dietary restriction followed by at least seven days of normal diet)could promote cognitive performance, decrease inflammation and cancer incidence, and extend the health span and multisystem regeneration of mice (3).

Recent data suggest that prolonged fasting plays a key role in the protection of healthy cells and

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organs against toxicity (4, 5).According to the previous studies on mice, caloric restriction could attenuate the lethal effects of hepatotoxins such as thioacetamide(6) and bleomycin(7).Acetaminophen (APAP) is used as an analgesic and antipyretic drug and has extensive application in medicine (8, 9).APAP is considered safe at therapeutic doses, while its overdose has been reported to be hepatotoxic(10).

The present study aimed to investigate the effect of short-term periodic fasting on liver injury following high-dose APAP administration.

Materials and Methods

Mice and Fasting Regimen

This experimental study was conducted on female BALB/c mice with short-term periodic fasting to investigate the toxic effects of high-dose APAP on the liver. At six weeks of age, the mice were housed in boxes (three animals per box) and randomly assigned to two groups of fasting and control. Both groups were fed *ad libitum*, each consisting of 20mice.

Specific pathogen-free conditions were maintained throughout the study. The animals were kept in a room within a 12-hour light/dark cycle at constant temperature and humidity. The fasting periods encompassed three consecutive days every two weeks. During these periods, the fasting mice only had access to water. Except for these three days, the animals had free access to adequate food and water. The fasting protocol continued for 10weeks.

Four days after the last fasting period, the animals in the fasting and control groups were administered with 500 mg/kg of APAP via intraperitoneal injection. After 24 hours, eight mice were randomly selected from each group to measure the serum levels of aspartate transaminase (AST) and alanine aminotransferase (ALT; U/L) using the commercial enzymatic kits of Biorex Fars Company and an automated analyzer (model: HITACHI 911). Following that, the animals were sacrificed to determine the severity of liver injury using the pathological method as the gold standard. For this purpose, macroscopic and microscopic changes were assessed. Also, liver samples were fixed in 10% formalin, and stained using the H&E method. The induced liver injury was examined based on special criteria, such as the parenchymal distortion of the liver and necrosis (Table 2-A).

Statistical Analysis

Data analysis was performed in SPSS version 17 using Mann-Whitney U test and Fisher's exact test to investigate the statistical differences between the control and fasting groups. In all the statistical analyses, the P-value of less than 0.05 was considered significant.

Results

In normal mice, the serum levels of ALT and AST in plasma is ALT: 25-60 U/L; AST: 50-100 U/L; whereas the increased levels of these enzymes indicate liver injury. After APAP treatment, the AST levels increased in the control group(117.36±48.57U/L), while they remained within the normal range(61.13±21.75U/L) in the fasting group (P=0.0098). More significant results were obtained for the ALT levels., increased ALT (124.55±34.63) observed in the control group, and slightly increased levels (66.06±10.29) observed in the fasting group of mice (P=0.0004) (Table 1).

Table1. Measurement of Serum Aminotransferases (AST and ALT) in the Normal, Control and Fasting Groups of Mice (AST and ALT increased in control group compared to fasting group [P=0.0098 and P=0.0004, respectively]; Since variables had non-normal distribution, they were evaluated using Mann-Whitney U test.)

Serum aminotransferases	Normal mice	Control group	Fasting group	Mann-Whitney Test
AST (U/L)	50-100	117.36 ± 48.57	61.13 ± 21.75	P= 0.0098 df = 14 z = 2.99
ALT (U/L)	25-60	124.55 ± 34.63	66.06 ± 10.29	P = 0.0004 df = 14 z = 4.58

Significant differences were observed in the mean levels of ALT and AST in the control and fasting groups (P<0.05).The liver injury grading showed that all the mice in the control group (n=8) had high-grade injury(grades II, III, and IV), while the animals in the fasting group had low-grade injury (grades zero and I), and high-grade injury was only observed in one fasting

mouse(P=0.001)(Figure 1; Table 2-B).It is notable that the distribution of liver injury was not homogeneous in the study groups.

Table2. A) Histopathological Grading Based on Histopathological Studies; B) Liver Injury Grading in Control and Fasting Groups of Mice (High-grade liver injury in control group compared to fasting group [P=0.001]; Fisher's exact test used for qualitative variables.)

A	
Grading	**microscopic findings**
0	Normal hepatic architecture
I	Mild distortion of liver parenchymal architecture with minimal focal necrosis
II	Mild distortion of liver parenchymal architecture with several foci of liver necrosis
III	Moderate distortion of liver parenchymal architecture with multiple foci of liver necrosis
IV	Severe distortion of liver parenchymal architecture with multiple foci of liver necrosis

B			
Liver injury grade	**Control group**	**Fasting group**	**Fisher's Exact Test**
Low - grade (0, I)	0	7	P = 0.001
High - grade (II, III, IV)	8	1	

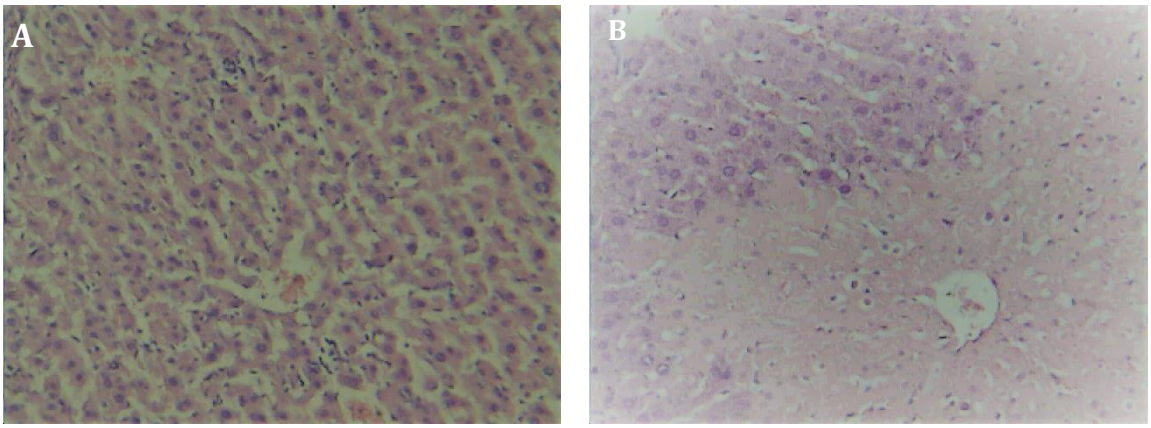

Figure 1. Photomicrograph of Histopathological Examination of Liver Samples (400x magnification; A: hepatocytes necrosis in control liver sample, B: The lack of any serious injury in periodic fasting liver sample.)

Discussion

APAP is a well-known hepatotoxin, which causes drug-induced liver injury in a dose-dependent manner and is also considered to be the most common cause of drug-induced liver injury (11). In addition, the side effects of APAP are regarded as a major public health concern (10). The liver is the main organ that metabolizes APAP extensively and rapidly, while the gut and kidneys are also involved in this process(12).The liver converts APAP into glucuronide and sulfate conjugates although the toxic metabolite of N-acetyl-p-benzoquinoneimine (NAPQI)is also produced in small amounts (13).NAPQI is conjugated with reduced glutathione in the liver and is exerted in the bile and urine. Since the liver has limited glucuronidation and sulfation capacity, the excessive consumption of APAP leads to the excessive production of NAPQI. Surplus NAPQI production is detoxified partially with glutathione, and the functional alteration of cell protein thiol via covalent binds with the remaining NAPQ eventually causes acute hepatic necrosis(13).The cellular events leading to liver injury following high-dose APAP consumption in mice model are associated with mitochondrial damage, oxidative stress, c-jun N-terminal kinase activation, and nuclear DNA fragmentation(14, 15).

Multiple factors potentially influence APAP-induced liver cell damage, including the hepatocyte responses to APAP metabolites(16),

pro-inflammatory cytokine cascades(17), heat shock proteins(18), peroxisome proliferator-activated receptors (19), and glutathione levels(20). Autophagy is a natural cellular mechanism, through which autophagosomes (double-membrane vesicles) engulf cytoplasmic cargos, subsequently fusing with lysosomes to form autolysosomes, where the unnecessary/dysfunctional components are degraded or recycled(21, 22). Stressful stimuli such as fasting (i.e., nutritional restriction), hypoxia, DNA damage, and cytotoxic agents may promote the autophagy phenomenon(23, 24). Furthermore, autophagy is an effective mechanism to adjust stressful conditions in most cells and organs, such as the liver and muscles (24, 25).

In the current research, we evaluated the impact of short-term periodic fasting (three consecutive days every two weeks for 10weeks) on the serum ALT and AST and liver injury following high-dose APAP administration (500 mg/kg) in female BALB/c mice. According to the obtained results, the intraperitoneal injection of APAP caused the AST and ALT serum levels to increase in the control group, while only slight changes were observed in the fasting group in this regard (P=0.0098 and P=0.0004, respectively).

In the present study pathological method was applied to determine the severity of the liver injury. According to the findings, high-dose APAP led to high-grade liver injury in the control group, while the fasting group of mice showed low-grade liver injury (P=0.001). This is in line with the results of the previous studies conducted on mice, which indicated that high-dose APAP may increase serum aminotransferases and leads to extensive liver injury (26-28). Furthermore, the comparison of the fasting group to the control group of mice indicated protection against APAP-induced liver injury which is consistent with the previous findings in this regard (16).

In congruence with our findings, Verweij et al. reported that fasting protects the liver against ischemia-reperfusion injury. Accordingly, fasting may lead to the up-regulation of antioxidant enzymes, such as superoxide dismutase 2, glutathione peroxidase 1, and glutathione reductase, as well as the stress response gene heme oxygenase 1(5). Interestingly, factors such as the inflammatory cascades, peroxisome proliferator-activated receptors, and glutathione levels have been shown to be remarkably influenced by calorie restriction (e.g., fasting)(19, 29-31), which attests to the protective role of fasting against drugs such as APAP(16).

Conclusion

According to the results, short-term periodic fasting could positively influence liver drug detoxification as the fasting mice had low-degree liver injury against high-dose APAP. Undoubtedly, further investigations are required on larger sample population in order to prove such beneficial effects.

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Conflicts of interest

The authors declare no conflict of interest.

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The Effects of Eight Weeks Aerobic Interval Exercise with Variable Volume on the Cardiovascular Risk Factors and Liver Enzymes of Women with Dyslipidemia

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ABSTRACT

Introduction: Metabolic dyslipidemia could lead to non-alcoholic fatty liver disease, and its secondary consequence is the development of metabolic syndrome and diabetes. The present study aimed to investigate the effects of eight weeks of aerobic interval exercise with variable volumes on the cardiovascular risk factors and liver enzymes of the women with dyslipidemia.

Methods: This quasi-experimental study was conducted on 30 middle-aged women with high blood lipids. The patients were selected and divided into three groups of low-volume training (three sessions per week; n=10; LVT), high-volume training (four sessions per week; n=10; HVT), and control (n=10; C).The exercise program was implemented in eight weeks 3-4 sessions per week for 45-60 minutes with the intensity of 65-75% of the maximal heart rate. The inter-group and intra-group comparison were performed using student's t-test, and one-way analysis of variance (ANOVA) was used to assess the differences between the groups.

Results: In the training groups, a significant reduction was observed in weight (LVT: 72.01 vs. 67.26, HVT: 72.80 vs. 68.06), body mass index (LVT: 28.19 vs. 26.31, HVT:27.85 vs. 26.04), body fat (LVT: 26.86 vs. 25.69, HVT:27.21 vs. 25.91), waist-to-hip ratio (LVT: 1.05 vs. 1.03, HVT:1.07 vs. 1.05), alanine transaminase(LVT: 46.60 vs. 39.60, HVT: 43.80 vs. 38.50), aspartate transaminase(LVT: 36.50 vs. 31.00, HVT: 33.50 vs. 29.40), and triglyceride (LVT: 171.80 vs. 163.60, HVT:176.90 vs. 161.40). However, the maximum oxygen uptake increased significantly after the intervention in both the training groups (LVT: 32.17 vs. 35.93, HVT:30.93 vs. 35.98). The levels of total cholesterol (211.20 vs. 204.90) and low-density lipoprotein cholesterol (134.13 vs. 126.68) significantly decreased only in the LVT group, while no such changes were observed in the HVT group. In addition, the systolic blood pressure (LVT: 135.40 vs. 128.60, HVT: 137.00 vs. 129.60) decreased significantly in both groups, while no significant change was observed in the diastolic blood pressure.

Conclusion: According to the results, eight weeks of aerobic interval exercise could improve the cardiovascular risk factors, liver enzymes, and body composition of the women with dyslipidemia. Therefore, it is recommended that some cardiovascular risk factors and liver enzymes of women with dyslipidemia be used for the improvement of these patients.

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Introduction

Dyslipidemia is a disorder associated with metabolic syndrome, which is characterized by disorders at the levels of three lipids, including the increased levels of very-low-density lipoprotein (VLDL) and low-density lipoprotein and decreased high-density lipoprotein [1]. High blood lipids are considered to be the atherogenic factors associated with metabolic syndrome, such as obesity, diabetes [2], and cardiovascular diseases[3]. Most of the studies in this regard have indicated that dyslipidemia is more prevalent in women compared to men[4, 5].

Blood lipids are an important risk factor associated with significant complications, such as coronary artery disease and fatty liver disease [6]. Cardiovascular diseases are known as the leading cause of mortality in most countries (especially the United States), and more than half of the patients with cardiovascular diseases are diagnosed with blood lipid disorders [2, 7],

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obesity [8], and dyslipidemia [9], which in turn contribute to fatty liver diseases.

According to the literature, 35% of the individuals with morbid obesity progress toward non-alcoholic fatty liver disease (NAFLD) [10]. NAFLD is the liver inflammation caused by the excessive accumulation of fats in the liver tissues, which occasionally interferes with the normal function of the liver tissues and maybe progressive, causing liver damage or cirrhosis [11]. Furthermore, the disease has been reported to alter liver enzyme levels, such as aminotransferases, which are among the most sensitive diagnostic liver enzymes (e.g., aspartate aminotransferase and alanine transferase)[12].

On the other hand, previous findings have indicated that hyperlipidemia control is essential to the prevention of cardiovascular events [7], which highlights the utmost importance of preventive and therapeutic strategies for this disease, especially in the female population. Energy consumption, exercise, and physical activity have been reported to be correlated. Exercise is a beneficial and cost-efficient approach to the prevention and treatment of hepatic issues. Moreover, previous studies have indicated that lifestyle changes, physical activity, and exercise could reduce weight and improve liver enzymes [13, 14].

According to the literature, acute exercise activity leads to elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [15]. On the other hand, participation in long-term aerobic activities decreases these enzymes [16-18]. In a study in this regard, Zeinvand et al. (2018)investigated the impact of eight weeks of aerobic and resistance training on the blood lipid profile of elderly women, claiming that aerobic training was more effective than resistance training in improving the blood lipid profile in the elderly with NAFLD [19]. In contrast, Park et al. (2019) reported that eight weeks of training (five times per week) with 50-60% of the heart rate reserved for 1-2 weeks and at 60-80% of the heart rate reserved for 3-8 weeks in eight women caused no significant changes, and only alkaline phosphatase increased significantly [20].

The number of the training sessions per week is an exercise variable that could affect metabolic changes[21, 22]. However, limited and contradictory findings have been reported regarding the effects of exercise training on cardiovascular risk factors and liver enzymes [22, 23]. The sample populations of these studies have mainly been diabetic patients and children with intellectual disability, and the exercise protocols have encompassed aerobic exercises. Nevertheless, evidence is scarce regarding the effects of the weekly volume of aerobic interval exercises on cardiovascular risk factors and liver enzymes in women with hyperlipidemia.

The present study aimed to investigate the effects of eight weeks of aerobic interval exercise with variable volumes on the cardiovascular risk factors and liver enzymes of women with dyslipidemia.

Materials and Methods

Subjects

This quasi-experimental study was conducted with three experimental groups, which were compared with each other. In total, 30 middle-aged women with high blood lipids (age: 35-50 years) were enrolled in the study. The patients had the total cholesterol level of 200-240 mg/dl and body mass index (BMI) of 25-30 kg/m^{2}and were selected voluntarily and objectively. At the first stage of the research, the patients were introduced to the concept and approaches of cooperation. Important notes were also provided to the subjects regarding nutrition, diseases, drug consumption, supplements, drug abuse, no smoking habits, and lack of participation in other studies for a minimum of six months prior to the research schedule.

Participation in the study was voluntary, and written informed consent was obtained from the patients. Afterwards, the subjects were randomly divided into three groups of low-volume training (LVT; three sessions per week; n=10), high-volume training (HVT; four sessions per week; n=10), and control (n=10).

The study protocol was approved by the Ethics Committee of Hakim Sabzevari University, Iran (code: IR-MEDSAB.REC.1396.941). The following equation was used to determine the sample size:

$$n = \frac{2\sigma^2(Z_{1-\alpha/2} + Z_{1-\beta})^2}{d^2} = \frac{2(2.5)^2(2+1.28)^2}{3.5^2} = 10.97 \cong 11$$

Body Composition

At the second stage of the research, the height of the patients was measured in centimeters using stadiometer (Seca, Germany) with the sensitivity of five millimeters, and their weight was calculated using a digital scale (model: PS07-PS06, Beurer, Germany). Following that, the

waist-to-hip ratio (WHR) was determined, and BMI was calculated by dividing the body weight by the squared height in meters (kg/m^2). In order to measure the waist of the participants, a tape measure was used (MABIS, Japan) at the midway between the lowest rib and top of hips (above the navel). The hips were measured at the widest point around the buttocks; to do so, the tape was held snugly without pulling and leveled around, and the value was divided for the WHR.

At the next step, the body fat percentage was measured based on two points (triceps, superficial and abdominal sections) using Lafite type calipers. These measurements were obtained from the right side of the patients, so that the thickness of the subcutaneous fat would be recorded in the three arms of the middle skinfold in the posterior section in the thigh, as well as the thickest section of the tibia. The measured values were applied to the Luhmann-Slater formula, and the body fat percentage was obtained using Equation 1. In this equation, the body density of the subjects is represented by *BdF*, the sum of the three points of the skinfold is shown by *Σ3*, and the age of the patients is indicated by *age*. Based on the satiation equation, the body fat percentage was obtained (Equation 2).

Equation 1: BdF = 1.099421- (0.0009929 × Σ3sits) + (0.0000023 × (Σ3sits)2) - (0.0001392× age)

Equation 2: % F (siri, 1956) = [(4.95÷ BdF) – 4.5] × 100

The blood pressure of each patient was measured before physical activity using the Maximed Exipres TD-3018 machine and converted into the mean blood pressure using the formula of the mean arterial blood pressure, as shown in Equation 3.

Equation3: Mean Blood Pressure – (2× Diastolic Blood Pressure + Systolic Blood Pressure) /3

In order to homogenize the nutritional status of the patients due to its impact on the study parameters, the patients were required to write down a checklist report on a three-day diet. After collecting all the nutrition information, the amount of the received calorie and supplements was determined. In addition, the patients were asked to consume common foods with the same calorie content prior to the two periods of blood testing.

Blood Sample Collection

Blood samples were collected 48 hours before the training and 48 hours after the training sessions. Sampling was performed between 8:0-10:00 AM. After 10-12 hours of fasting in the laboratory, blood sample were collected from the left vein of each subject in the sitting position and at rest. Serum liver enzymes were also determined using the photometric method and a bionic acid kit with the sensitivity of 1 unit per liter. Serum biochemical concentrations were determined using an autoanalyzer spectrophotometer and various kits at different wavelengths. Moreover, serum triglyceride concentration was determined (mg/dl) using the Man kits and GPO-PAP enzymatic method at the wavelength of 546 nanometers. High-density lipoprotein (HDL) and low-density lipoprotein (LDL) were also measured using the enzymatic method (PishtazTeb kit, Tehran, Iran).

Maximum Oxygen Uptake (VO_{2MAX})

The maximum oxygen uptake was estimated based on the Rockport walking protocol, which functions when walking at the maximum speed of one mile (1609 m), recording the heart rate immediately for 15 seconds, and multiplying the obtained number by four. The maximum oxygen consumption in the Rockport walking protocol was calculated using Equation 4, in which the body weight is measured in pounds, and age is determined in years, and other parameters such as the sex factor (men=1, women=0), time to complete one mile per minute, and heart rate after performing the test (beats per minute) are estimated [24].

Equation 4: $VO2_{MAX}$=132.853-(Weight×0.0769)-(0.3877×age)+(6.315×sex)-(3.2649×time)-0.1565×Heart Rate

Exercise Protocol

The training protocol consisted of eight weeks of aerobic interval training with two different volumes (3 and 4 days per week), and the duration of each session was 45-60 minutes with the intensity of 65-75% of the maximal heart rate reserve. The training duration progressively increased from 40 minutes at baseline to 45 minutes at the end of the study period. The training protocol encompassed a general warm-up for 10 minutes (walking, moderate running, stretching, and mobility), and the training intensity was controlled using a pulse meter (model: POLAR, Finland).At the end of each

session, the exercise protocol was performed for 10 minutes to return to baseline and cool down (slow running, walking, and stretching). After the implementation of the protocol (6 weeks), all the measurements were repeated in order to obtain the posttest data. In addition, the control group had no activity during the study period, and the patients inactive similar to their lifestyle before the study.

Statistical Analysis

Data analysis was performed in SPSS version 16. The normality of the theoretical distribution of the data was confirmed using the Shapiro-Wilk test, and variance homogeneity was confirmed using the Levene's test. Moreover, paired sample t-test and repeated measures ANOVA were applied for the inter-group and intra-group comparison of the variance changes. In all the statistical analyses, the significance level was considered to be less than 0.05.

Results

Table 1 shows the characteristics of the patients. According to the information in Table 2, a significant reduction was observed in the body weight (LVT: 72.01 vs. 67.26; HVT: 72.80 vs. 68.06; P=0.001), BMI (LVT: 28.19 vs. 26.31; HVT:27.85 vs. 26.04; P=0.001), body fat(LVT: 26.86 vs. 25.69; HVT:27.21 vs. 25.91; P=0.001), and WHR (LVT: 1.05 vs. 1.03; HVT:1.07 vs. 1.05; P=0.001), in the training groups. On the other hand, a significant increase was denoted in the maximal oxygen consumption after the intervention in both the training groups(LVT: 32.17 vs. 35.93; HVT:30.93 vs. 35.98; P=0.001).In addition, significant differences were observed in intergroup mean changes between the training groups in terms of the body weight, BMI, and maximal oxygen consumption (P<0.05).

Table 1. Characteristic of Patients

Groups	Variations (Mean±SD)			
	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m²)
Low-volume Training (LVT)	40.30±3.74	159.85±7.48	72.01±6.14	28.19±1.76
High-volume Training (HVT)	41.20±3.88	162.04±6.66	72.80±4.72	27.85±2.80
Control	42.90±4.20	160.70±6.11	73.28±5.13	28.36±0.93
	P=0.65	P=0.84	P=0.41	P=0.25

Table 2. Changes in Body Composition in Study Groups

Variables	Groups	Stages	Variations			
		Posttest Mean±SD*	Pretest Mean±SD*	P-value**	P-value***	
				P-value	F	P-value
Weight (kg)	LVT	72.01±6.14	67.26±5.72	0.001†	5.50	0.001†
	HVT	72.80±4.72	68.06±4.29	0.001†		
	Control	73.28±5.13	74.30±5.46	0.206		
BMI (kg/m²)	LVT	28.19±1.76	26.31±0.92	0.001†	6.56	0.005†
	HVT	27.85±2.80	26.04±2.72	0.001†		
	Control	28.36±0.93	28.77±1.46	0.231		
Body Fat Percentage (%)	LVT	26.86±1.28	25.69±1.40	0.002†	1.46	0.25
	HVT	27.21±1.26	25.91±1.06	0.001†		
	Control	26.45±1.17	26.57±1.11	0.633		
WHR (cm)	LVT	1.05±0.07	1.03±0.07	0.009†	1.58	0.224
	HVT	1.07±0.06	1.05±0.05	0.003†		
	Control	1.08±0.07	1.08±0.07	0.558		
$VO2_{max}$ (ml/kg/min)	LVT	32.17±5.48	35.93±5.13	0.001†	9.35	0.001†
	HVT	30.93±3.30	35.98±2.57	0.001†		
	Control	29.76±2.92	29.75±2.87	0.633		

†Significance level P<0.05 ***P-value within groups*
**Data presented as mean±standard deviation* ****P-value between groups*

According to the information in Table 3, the levels of ALT (LVT: 46.60 vs. 39.60; HVT: 43.80 vs. 38.50; P=0.001) and serum AST decreased in both the training groups (LVT: 36.50 vs. 31.00; HVT: 33.50 vs. 29.40; P=0.001).Moreover, serum triglyceride levels decreased significantly in both

groups (LVT: 171.80 vs. 163.60; HVT:176.90 vs. 161.40; P=0.001), while the total cholesterol levels decreased only in the LVT group(211.20 vs. 204.90), and no significant change was observed in the HVT group in this regard. On the other hand, HDL significantly increased in both the training groups (LVT: 42.70 vs. 45.50; HVT: 42.80 vs. 46.10; P=0.001), while a significant reduction was observed in the control group.

According to the findings, LDL significantly reduced only in the LVT group (134.13 vs. 126.68; P=0.002), while no significant changes were observed in the HVT and control groups in this regard. Moreover, the systolic blood pressure decreased significantly in both groups after the intervention (LVT: 135.40 vs. 128.60; HVT: 137.00 vs. 129.60; P=0.001), while no significant changes were observed in the diastolic blood pressure. A significant difference was denoted in the intergroup mean changes in both the training groups in terms of LDL and the systolic blood pressure.

Table 3. Changes in Cardiovascular Risk Factors and Liver Enzymes in Study Groups

Variables	Groups	Stages	Variations			
		Posttest Mean±SD*	Pretest Mean±SD*	P-value**	P-value***	
				P-value	F	P-value
ALT (IU/l)	LVT	46.60±4.95	39.60±5.13	0.001†	2.06	0.146
	HVT	43.80±5.87	38.50±3.92	0.004†		
	Control	43.60±4.88	42.80±5.55	0.619		
AST (IU/l)	LVT	36.50±4.60	31.00±4.85	0.001†	0.67	0.520
	HVT	33.50±6.65	29.40±9.06	0.037†		
	Control	32.80±4.66	33.00±6.33	0.897		
TG (mg/dl)	LVT	171.80±12.81	163.60±8.78	0.029†	0.492	0.057
	HVT	176.90±9.72	161.40±9.88	0.001†		
	Control	168.80±7.45	170.80±7.24	0.427		
TC (mg/dl)	LVT	211.20±7.22	204.90±8.89	0.004†	0.483	0.622
	HVT	206.10±12.28	201.90±11.17	0.166		
	Control	204.50±11.02	200.90±8.08	0.229		
HDL-C (mg/dl)	LVT	42.70±3.19	45.50±2.63	0.004†	17.79	0.001†
	HVT	42.80±3.39	46.10±2.84	0.006†		
	Control	42.40±3.40	39.60±2.59	0.016†		
LDL-C (mg/dl)	LVT	134.14±8.36	126.68±9.11	0.002†	0.386	0.684
	HVT	127.92±13.24	123.52±12.60	0.116		
	Control	128.34±9.34	127.14±7.75	0.635		
Systolic Blood Pressure (mm/mg)	LVT	135.40±5.25	128.60±3.95	0.004†	6.30	0.006†
	HVT	137.00±2.75	129.60±2.72	0.001†		
	Control	132.50±8.36	136.00±7.33	0.265		
Diastolic Blood Pressure (mm/mg)	LVT	85.50±4.74	83.60±4.24	0.25	0.559	0.578
	HVT	85.10±3.14	84.50±3.70	0.61		
	Control	86.60±3.13	85.80±3.36	0.49		

†Significance level P<0.05 ***P-value within groups*
**Data presented as mean±standard deviation* ****P-value between groups*

Discussion

According to the results of the present study, body weight, BMI, body fat, and WHR significantly decreased in the LVT and HVT groups. Furthermore, the exercise protocol increased the energy consumption, and if energy intake was low, it could lead to weight loss in the exercise groups. Hormonal changes such as decreased insulin and increased catecholamines have been reported to stimulate beta-adrenergic receptors and activate hormone-sensitive lipase, which activate adipocyte lipolysis [25]. Through lipolysis, the fatty acids in the body (triglycerides) are broken down and released into the bloodstream as free fatty acids and a substrate for energy metabolism[26]. In the current research, the body composition of the patients improved, which was represented by the decreased weight, fat percentage, and WHR. On the other hand, decreased body fat percentage and increased lean body mass cause the basal metabolism to increase[27], which in turn could increase insulin sensitivity and reduce inflammation [28, 29]. This is considered to be a major clinical goal in the treatment of high blood lipids.

According to the results of the present study, the maximal oxygen consumption increased significantly in both the training groups. The increased $VO2_{max}$ compared to the pre-exercise

activity could be attributed to cardiovascular and metabolic adaptation to the exercises, such as the increased muscle oxidative capacity, total hemoglobin, and fat, decreased glycolysis, increased end-diastolic volume (cardiac load), decreased end-systolic volume, and increased volume impact, arterial-venous blood oxygen difference, activity of Krebs cycle enzymes, number and size of mitochondria, and muscle tissues and their efficiency [30].Therefore, great emphasis has been placed on physical activity and cardiorespiratory fitness in order to prevent cardiovascular diseases[30, 31].

According to the findings of the current research, the ALT and AST levels decreased in both the training groups, which might be due to the improved body composition or positive changes in the lipid profile following aerobic interval exercise. In the present study, a significant decrease was observed in anthropometric indices such as weight, body fat percentage, and lipid profile improvement after the exercise protocol with two volumes of three and four training sessions per week. Considering the associations between these variables (lipid profile and body composition) with the changes in the liver enzyme, better weight control is required, which could be attained through proper diets and exercise periods.

Insulin resistance is another cause of fatty liver [32, 33].Exercise increases insulin sensitivity by increasing the glucose carriers in the muscle cell membrane and improving insulin messaging [32, 33]. A significant association has also been reported between insulin resistance and fatty liver [34].Therefore, it could be stated that aerobic interval exercise could reduce insulin sensitivity and insulin resistance, which in turn reduced the transfer of free fatty acids to the liver and fat accumulation in the liver [35].It is also expected that the increased lipid metabolism in the cells involved in the activity (muscle cells) may contribute to the decreased liver fat content. Considering the association between obesity and inflammation [36, 37] and role of inflammation in the incidence of fatty liver[38], it is possible that aerobic interval exercise decrease the levels of inflammatory cytokines by improving the body composition and reducing plasma lipid oxidation, thereby decreasing ALT and AST in the exercise groups after aerobic training for three and four sessions per week. However, these changes made no significant difference between the training groups and the control group.

In the current research, triglyceride decreased significantly in both the training groups, while the total cholesterol levels decreased only in the LVT group, and no significant changes were observed in the HVT group in this regard. HDL significantly increased in both the training groups, while it significantly decreased in the control group. On the other hand, LDL significantly reduced only in the LVT group, while no significant changes were observed in this variable in the HVT and control groups.

One of the most effective adaptations following aerobic activities is the increased mitochondrial volume and the subsequent activity of lipolysis enzymes, which increase the catabolic ability of fats during exercise [39].Exercise may also exert beneficial effects on plasma HDL levels through affecting the muscle and liver lipoprotein lipase (LPL) activity[40].Changes in the LPL activity are associated with increased VLDL entry from the liver to the bloodstream and its clearance from the bloodstream [41].On the other hand, lecithin cholesterol acyltransferase (LCAT) converts cholesterol into HDL particles, as well as LDL. The increment of this enzyme may be responsible for the increased HDL induced by physical exercise. Furthermore, previous findings have demonstrated that LCAT increases significantly in some sports activities. In this context, the other mechanisms (e.g., decreased insulin sensitivity) that may alter the levels of lipids and blood lipoproteins may be effective as well [42].Other possible mechanisms (e.g., increased cholesterol transmission) may also be important in this regard.

Some of the effects of exercise on fats may be indirect and associated with abdominal fat reduction. The movement of free fatty acids from the abdominal fat to the liver also decreases, and VLDL production decreases as well [43].Continuous and prolonged physical activity leads to a decrease in liver lipase[44].Overall, these changes could improve metabolic adaptation and lipid metabolism (e.g., lipid profile).In the present study, no significant difference was observed in this regard between the exercise groups with different volumes, which could be due to the lack of a significant difference between three and four sessions of calorie intake or other confounding factors, such as nutritional quality, excess calories, and level of daily activity. Increasing the number of the training sessions or length of the intervention

period may cause a significant difference in this regard.

Conclusion

According to the results, exercise training in with the two volumes of three and four sessions per week could reduce cardiovascular risk factors, liver enzymes, and body composition, while increasing the maximal oxygen consumption. However, no significant difference was observed between these two weekly training volumes in these measured variables. There seems to be no significant difference between three and four training sessions regarding cardiovascular variables and the measured liver enzymes. For further changes, coaches are advised to modify other exercise variables, such as increasing the number of the training sessions per week, adjusting other variables (e.g., exercise intensity, training time per training session) or other interventions (e.g., calorie restriction). The main limitation of the present study was the lack of diet control in the women with dyslipidemia. It is hoped that the research scope and observations would be expanded in the future.

Conflicts of interest

None declared.

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The Effects of Aerobic Training and Caffeine Consumption on the Quality of Life and Life Expectancy of Overweight Men

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ABSTRACT

Introduction: Inappropriate diet and inactivity are the leading causes of overweightness and obesity, which adversely affect physical and mental health. The present study aimed to investigate the effects of eight weeks of caffeine supplementation and aerobic training on the quality of life and life expectancy of overweight men.

Methods: This quasi-experimental study was conducted on 60 overweight men aged 40-60 years with the body mass index of ≥30 kg/m^2, who were randomly divided into four groups, including control, caffeine supplementation (Ca), aerobic training (AT), and Ca+AT. Aerobic training was performed three times per week for eight weeks, and the duration of each session was 25-40 minutes at 60-80% of the maximal heart rate. The caffeine supplementation groups consumed 5 mg/kg of caffeine daily in two servings. The research variables were evaluated using the quality of life and life expectancy questionnaires. Data analysis was performed in SPSS using univariate analysis of covariance at P≤0.05.

Results: Aerobic training increased the total score of quality of life (P≤0.05), mental health (P≤0.05), and life expectancy (P≤0.05) in the overweight men. Caffeine supplementation also increased the total score of quality of life (P≤0.05). In addition, the scores of quality of life and the subscales and life expectancy score in the Ca+AT group were significantly higher compared to the control (P≤0.05), training (P≤0.05), and caffeine supplementation groups (P≤0.05).

Conclusion: According to the results, aerobic training was more effective than caffeine consumption in improving the quality of life and life expectancy. However, the interaction between aerobic training and caffeine consumption was considered more effective than aerobic training and caffeine consumption alone in improving the quality of life and life expectancy.

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Introduction

Obesity and overweightness are defined as the abnormal or excessive accumulation of fat in different body parts and are considered to be a public health concern, as well as major risk factors for cardiovascular diseases, type II diabetes, cancer, and the general decline of life expectancy [1]. Inappropriate diet and inactive lifestyle are the leading causes of overweightness and obesity, which not only have adverse effects on the quality of life, but they also increase the economic costs of obesity treatment [2, 3].

Several studies have suggested that in addition to decreased quality of life following obesity and overweightness, there is a correlation between these issues and psychological disorders [1, 4]. Since life expectancy is associated with the attitudes and structures toward life, individuals must be sensitive to lifestyle and the improvement of the quality of life [5].

According to the literature, obesity and overweightness, along with physiological disorders, are linked to increased anxiety and depression and decreased health-related quality of life [6]. Furthermore, researchers have emphasized on the role of physical activity in the improvement of physical and mental health, quality of life, and life expectancy [7]. Physical activity and regular exercise enhance metabolism, prevent excessive fat mass, and improve the quality of life and life expectancy, thereby delaying old age [8, 9].

In a study in this regard, Schop et al. reported that in the quality of life questionnaire, the scores of physical, mental, and overall quality of life were significantly lower in obese children compared to normal children. In addition, exercise training has been shown to improve the quality of life of these children [10]. The quality of life in physically active individuals has also

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been reported to be significantly higher than inactive individuals [7]. According to another research, the quality of life was positively and significantly correlated with health-related quality of life in patients with type II diabetes [11].

High-intensity aerobic training [12] and moderate-intensity aerobic training [13] have been reported to improve the quality of life, increase aerobic fitness, and enhance the mental and psychological health of patients, especially regarding life expectancy and self-reliance. On the other hand, due to the growing issues caused by obesity, researchers believe that a proper diet along with sports activities is the most effective approach to weight loss and achieving various health dimensions. Some researchers have claimed that physical exercise combined with the consumption fat-burning supplements (e.g., caffeine and caffeine compounds) could result in physical and mental health [14].

Caffeine consumption along with moderate-intensity biking training has been reported to increase the fat share of the total energy expenditure. Even when lipolysis and lipid oxidation spike during moderate-intensity training, caffeine could increase fat metabolism [14, 15]. However, the role of caffeine and its ergogenic effects during physical exercise is that caffeine affects the central nervous system (CNS) and adipose tissue through binding to the adenosine receptors and increasing the intracellular concentrations of cyclic adenosine monophosphate [16]. Previous findings have also shown that caffeine consumption could increase energy production for the CNS through increasing fat metabolism, thereby leading to the modification of the CNS function, which confirms the positive effects of low and reliable doses of caffeine on the reduction of anxiety and depression and improvement of mental health [17].

Although limited studies have been focused on the effects of caffeine combined with exercise training on the quality of life and life expectancy in overweight and obese individuals, some studies have indicated that physical exercise with caloric restriction relative to exercise training alone has more favorable effects on the quality of life, cardiovascular risk factors, and physical function in obese women [18]. The previous studies in this regard have mainly been focused on the interactive physiological effects of training and caffeine consumption. Despite numerous investigations, no studies have examined the simultaneous effects of exercise training and caffeine consumption on the psychological characteristics of the elderly, as well as the need to provide nutritional strategies along with exercise activities to expedite conclusion and motivate physical activity.

The present study aimed to investigate the effects of aerobic training with caffeine supplementation on the quality of life and life expectancy of overweight men.

Materials and Methods

This single-blind clinical trial was conducted with a pretest-posttest design and control group. After obtaining the required permit, a notification was issued in the health centers in Gachsaran, Iran. On a preplanned day, the overweight individuals aged 40-60 years with the body mass index (BMI) of ≥30 kg/m^2 who volunteered to participate in the study were invited to the Islamic Azad University of Gachsaran branch. In a briefing session, the objectives, possible problems, and advantages of the research were explained to the volunteers. Afterwards, 60 participants who met the inclusion criteria were selected for the study.

The inclusion criteria were the lack of regular physical activity for a minimum of two years, no history of supplementation and specific medication use, and absence of cardiovascular/respiratory diseases, diabetes, and hypertension. After obtaining written consent from the subjects and completion of the health and physical activity questionnaires, the sample population was selected. The participants were randomly divided into four groups of 15, including control, caffeine supplementation (Ca), aerobic training (AT), and aerobic training with caffeine supplementation (AT+Ca). The participants were asked not to change their diet during the study, avoid the consumption of alcohol, caffeine, and antioxidants, and refrain from physical activity outside of their training program. The supplementation and lack of dietary changes were assured through daily personal visits during the study period.

The quality of life and life expectancy questionnaires were completed by the subjects at the pretest and posttest. Aerobic training was performed for eight weeks three sessions per week; the duration of each session was 25-40 minutes at 60-80% of the maximal heart rate

[19]. The subjects in the caffeine supplementation groups received two caffeine capsules daily, which were manufactured and licensed by Alhavi Co., Iran [20].

Quality of Life Questionnaire

The World Health Organization (WHO) short quality of life scale was used to measure the quality of life of the participants. The questionnaire consists of 26 items to assess the four dimensions of quality of life, including physical health, psychological health, social relations, and quality of the living environment. The tool has seven items to measure physical health (items 3, 4, 10, and 15-18), six items to measure mental health (items 5-7, 11, 19, and 26), three items to measure social relations (items 20-22), and five items to measure the quality of the living environment (items 8, 9, 12-14, and 23-25). In addition, two items evaluate the apparent quality of life and general health of individuals (1-2). Each item is scored within the range of 1-5; physical health has the score range of 7-35, mental health has the score range of 6-30, social relations has the score range of 2-10, and the quality of living environment has the score range of 8-40. The reliability of this scale has been assessed by the developers at 10 WHO International Centers and confirmed at the alpha coefficient of 0.73-0.89 for the subscales and overall scales [21].

Life Expectancy Questionnaire

In this study, life expectancy was measured using Schneider (2005) life expectancy questionnaire. The questionnaire consists of 12 items, eight of are used, and the other four are the falsifiers that are not included in the scoring. Among the eight items, four are focused on factor thinking (agent component; items 2, 9, 10, and 12), and four evaluate strategic thinking (passage component; items 1, 4, 6, and 8), the purpose of which is to assess the life expectancy of the respondents. The items in this questionnaire are scored based on a five-point Likert scale (Strongly Disagree=1, Disagree=2, Do Not Know=3, Agree=4, Strongly Agree=5). Notably, the scoring of items three, seven, and 11 is reversed (Strongly Disagree=5, Disagree=4, Do Not Know=3, Agree=2, Strongly Agree=1). To obtain the overall score of the questionnaire, the scores of each item are summed up, with the higher scores indicating higher life expectancy and vice versa (Schneider, 2005). The validity and reliability of this questionnaire have been confirmed by the faculty members of Mashhad University and the Teacher Training Universities of Management and Experimental Studies. According to Bryant and Cvengros (2001), the internal consistency of the entire test is 0.791-0.711 [21].

Aerobic Training Protocol and Caffeine Supplementation

The training protocol was performed three sessions per week, and the duration of each session was 25-40 minutes. In the training program, the subjects had 60-65% of the maximal heart rate for 25 minutes (first two weeks), 65-70% of the maximal heart rate for 30 minutes (second two weeks), 70-75% of the maximal heart rate for 35 minutes (third two weeks), 75-80% of the maximal heart rate for 40 minutes (fourth two weeks). Before the training, the subjects warmed-up for 15 minutes by jogging, stretching, and elasticity movements, and after the training, they cooled-down for 10 minutes by stretching and flexing [19]. In addition to aerobic training, the participants consumed two caffeine capsules daily, which were licensed by the Ministry of Health in proportion to body weight (5 mg/kg caffeine per body weight) [20].

Statistical Analysis

Data analysis was performed in SPSS version 22 using descriptive statistics (mean and standard deviation) to describe the demographic data and data collected by the questionnaires. In addition, the analysis of covariance (ANCOVA) was applied to assess the research hypotheses.

Results

According to the results of the Kolmogorov-Smirnov test, the distribution of the research data was normal in all the study groups at the pretest and posttest. Considering the heterogeneity of the variances, the nonlinearity and non-homogeneity of the regression slope of the research data, ANCOVA was used to analyze the research findings. The results of ANCOVA indicated significant differences at the posttest in the total scores of the quality of life (P=0.001), mental health (P=0.001), social relations (P=0.001), quality of living environment (P=0.007), and life expectancy (P=0.001). However, no significant difference was observed in the physical health level of the study groups (P=0.05) (Figure 1).

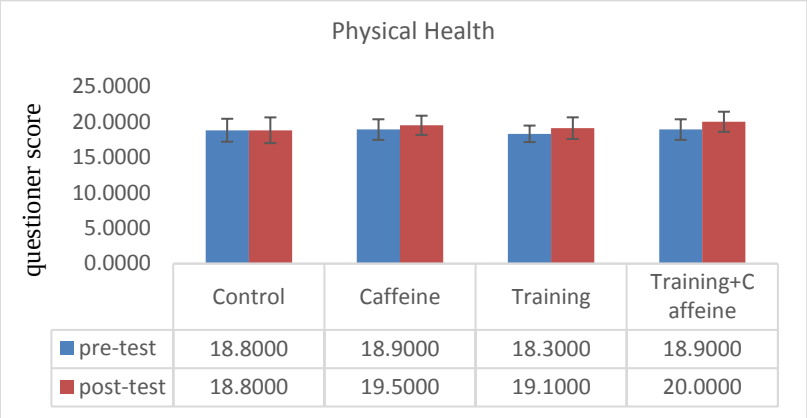

Figure 1. Physical Health Scores at Pretest and Posttest in Study Groups

According to the results of Bonferroni's post-hoc test, the score of mental health was significantly higher in the AT (P=0.001) and AT+Ca groups (P=0.001) compared to the control group. Furthermore, the score of mental health was significantly higher in the AT (P=0.001) and AT+Ca groups (P=0.001) compared to the Ca group, while significantly higher in the AT+Ca group compared to the AT group (P=0.01) (Figure 2).

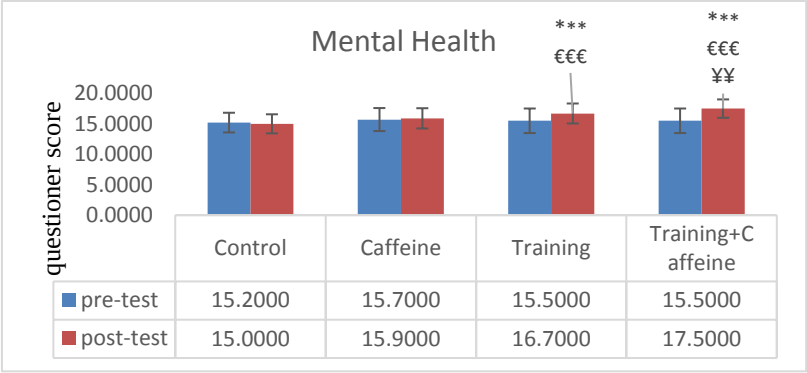

Figure 2. Mental Health Scores at Pretest and Posttest in Study Groups (***Increase compared to control [P=0.001]; €€€increase compared to caffeine consumption [P=0.001]; ¥increase compared to aerobic training [P=0.01])

According to the findings, the score of social relations was significantly higher in the AT+Ca group compared to the control (P=0.001), Ca (P=0.001), and AT groups (P=0.02) (Figure 3).

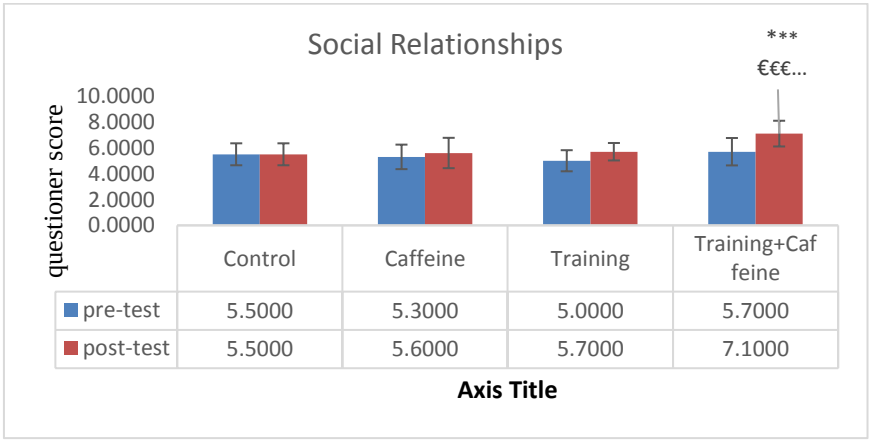

Figure 3. Social Relations Scores at Pretest and Posttest in Study Groups (***Increase compared to control [P=0.001];€€€ Increase compared to caffeine consumption [P=0.001]; ¥increase compared to aerobic training [P=0.02])

Similarly, the score of the quality of the living environment was significantly higher in the AT+Ca group compared to the control (P=0.001), Ca (P=0.015), and AT groups (P=0.015) (Figure 4).

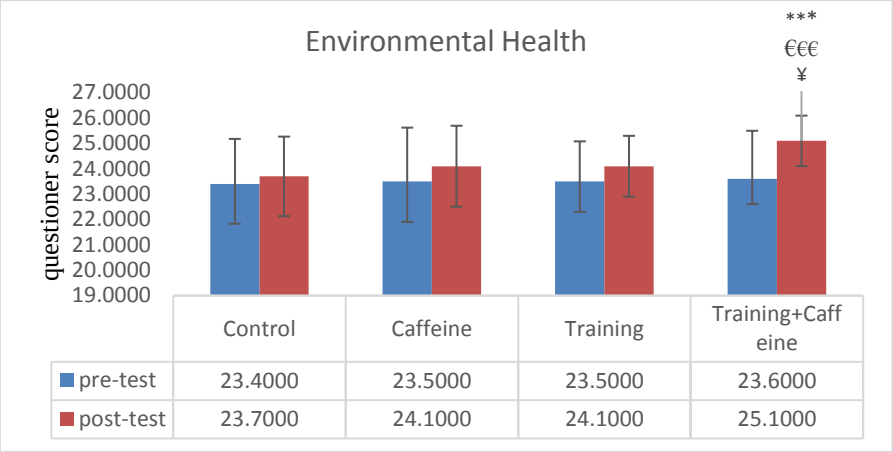

Figure 4. Scores of Quality of Living Environment at Pretest and Posttest in Study Groups (***Increase compared to control [P=0.001]; €€€increase compared to caffeine consumption [P=0.001]; ¥increase compared to aerobic training group [P=0.05])

The obtained results demonstrated that the score of quality of life was significantly higher in the Ca (P=0.02), AT (P=0.001), and AT+Ca groups (P=0.001) compared to the control group. Moreover, the score of quality of life was significantly higher in the AT+Ca group compared to the Ca (P=0.0001) and AT groups (P=0.001). However, no significant difference was observed in the score of quality of life between the Ca and AT groups (P=0.24) (Figure 5).

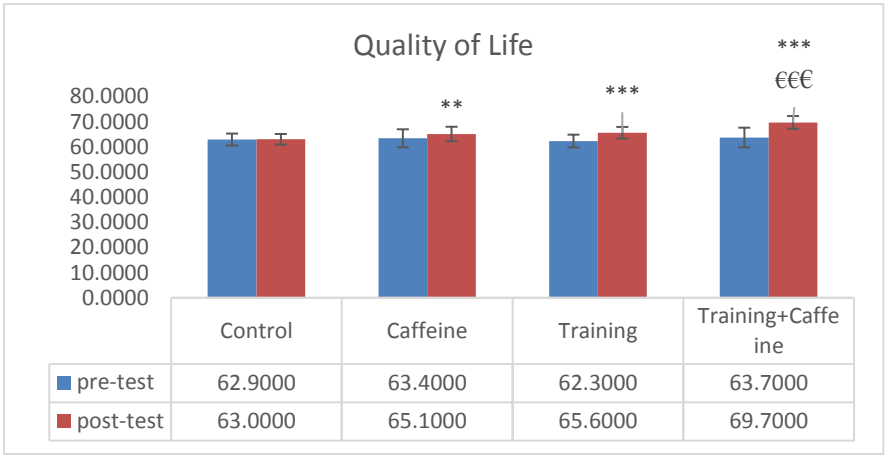

Figure 5. Total Score of Quality of Life at Pretest and Posttest in Study Groups (**P=0.01, ***P=0.001, increase compared to control; €€€increase compared to caffeine consumption and aerobic training [P=0.001])

The score of life expectancy was significantly higher in the AT (P=0.01) and AT+Ca groups (P=0.001) compared to the control group. In addition, the score of life expectancy was significantly higher in the AT+Ca group compared to the control group (P=0.002). However, no significant difference was observed in the score of life expectancy between the AT, Ca (P=0.35), and AT+Ca groups (P=0.25) (Figure 6).

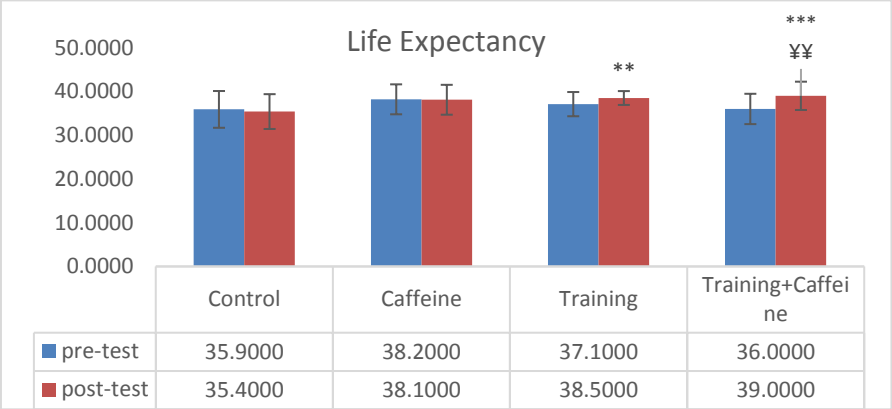

Figure 6. Life Expectancy Scores at Pretest and Posttest in Study Groups (**P=0.01, ***P=0.001, increase compared to control [P=0.001]; increase compared to caffeine consumption [P=0.01])

Discussion

According to the results of the present study, aerobic training could improve the total scores of the quality of life, mental health, and life expectancy in the overweight men. Today, quality of life mainly refers to hopefulness to live. Hopefulness is defined as liberation from suffering and difficulties, which may be real and adaptable to the external environment and the world or remain only as a predictor and bring about happiness and hopefulness to the individual [22]. Obesity and overweightness could be considered the chronic conditions that are associated with hormonal and metabolic changes, thereby leading to physical and psychological consequences and adversely affect physical activity and quality of life [23]. The previous studies in this regard have shown significant associations between increased BMI, decreased quality of life, and cognitive impairment [23].

On the other hand, aerobic activity is associated with numerous adaptations, lowers the blood pressure, and heightens happiness and vitality through increasing the release of hormones such as serotonin and endorphins, modulating the nervous system, and enhancing physical health, quality of life, and life expectancy [9]. Therefore, a highly successful treatment method in this regard involves physical activity, especially aerobic exercises [24].

Although quality of life and life expectancy are complex and multifaceted concepts, studies have shown that physical exercise has numerous beneficial effects on the quality of life [8]. According to a research in this regard, 12 weeks of aerobic training and water exercises could improve the quality of life and happiness of middle-aged female athletes [25]. Furthermore, 12 weeks of a combined training program has been reported to enhance the scores of general health, physical performance, mental health, and pain relief in elderly female patients with knee pain [26]. Researchers have also claimed that physical exercise positively influences the quality of life in physically inactive elderly [27, 28].

According to the findings of another research, weight loss through physical activity and exercise interventions is associated with higher quality of life, while it also improves various aspects of quality of life and life expectancy [29, 30]. The mentioned findings are in line with the results of the present study, and the consistency could be attributed to the positive impact of physical activity on quality of life, which could justify by the fact that physical activity reduces activity limitations and increases independence, thereby making life happier and more successful life. This results in increased quality of life and a sense of general wellbeing. Since quality of life is an intrinsic part of participation in physical activity, it could be stated that physical activity affects the quality of life, which in turn affects life expectancy.

According to the results of the present study, caffeine consumption could increase the total score of quality of life, while it had no significant effects on improving the total scores of physical health, mental health, social relations, environmental health, and life expectancy in the overweight men. Researchers believe that caffeine consumption could have several positive psychological effects through several

mechanisms, such as antioxidant and anti-inflammatory effects. Similar to benzodiazepine anxiolytic drugs and through the agonist function of benzodiazepine receptors and increased GABA receptor function, caffeine could induce chloride ion entry and cell hyperpolarization, thereby inhibiting cellular excitability and exerting its anxiolytic effects [31].

Limited studies have been focused on the effects of caffeine supplementation on quality of life and life expectancy, while previous findings have indicated that consuming 250 milligrams of green coffee daily for six weeks could reduce anxiety and depression in overweight women [32]. In another study, the consumption of caffeine-containing tea was reported to have antidepressant effects on elderly men and women [32]. However, studies have also denoted that caffeine consumption may variably affect the consumers depending on the dose, and immediate effects have been observed on the cardiovascular system. Furthermore, the increased levels of catecholamines following caffeine consumption could activate the beta adrenergic system and lead to hypertension [14]. Interestingly, previous studies have demonstrated the sedative and antidepressant effects of caffeine to be dose-dependent, so that the conventional doses of caffeine could have favorable effects on depression. Nonetheless, the mentioned study showed that coffee consumption in women drinking 1-4 cups of coffee per day could not significantly reduce sleep disorders and depression [33]. The discrepancy in this regard could be due to the differences in the sample populations, caffeine intake, and the duration of the supplementation period.

In another study, coffee consumption was significantly associated with reduced depression following the consumption of 1-4 cups of coffee per week, while no association was observed between caffeine consumption and depression in the women consuming five cups of coffee [34]. Therefore, non-significant changes have been reported in some parameters of quality of life and life expectancy following caffeine consumption, which could be attributed to the dose and duration of the consumption.

According to the results of the present study, the scores of mental health, social relations, environmental health, overall quality of life, and life expectancy were significantly higher in the aerobic training group with caffeine consumption compared to the control group. Although aerobic training had a more favorable effect on the score of mental health, aerobic training along with caffeine consumption had more favorable effects on the variables of quality of life and life expectancy compared to training and supplementation alone in the overweight men.

In the current research, eight weeks of caffeine supplementation along with aerobic training could increase the components of quality of life in the experimental group compared to the control group. According to the literature, exercise training could improve the metabolism of sugar and lipid substrates and weight loss through the reduction of the resting levels of cortisol, endorphin release, and release of euphoric hormones (e.g., serotonin), while also modulating the hypothalamic-pituitary-adrenal axis activity [29]. This in turn enhances self-confidence, happiness, and quality of life [26-29]. On the other hand, caffeine consumption could reduce anxiety via the antioxidant, anti-inflammatory, and sedative pathways [31], while increasing fat metabolism and reducing fat levels, thereby leading to weight loss and weight reduction. This helps with the proper body image of individuals, enhancing their quality of life [16]. In this regard, the results of a study indicated that six weeks of combined training (aerobic and resistance) along with consuming 250 milligrams of green coffee per day had significantly more favorable effects on the reduction of anxiety and depression in overweight women [31].

Despite extensive research in this regard, no studies have investigated the interactive effects of exercise training and caffeine supplementation on the quality of life and life expectancy. According to a study, the interaction of moderate-intensity aerobic training and caffeine consumption could significantly increase fat metabolism compared to each alone [14, 15]. Furthermore, performing physical exercise along with lifestyle changes and calories intake has been reported to exert more favorable effects on the quality of life, cardiovascular risk factors, and physical performance in obese women with grade I, II, and III [18].

With respect to the role of anxiety reduction in improving quality of life, it seems that one of the limitations of the present study was the lack of investigating anxiety and depression in the subjects. Therefore, it is suggested that further

investigations measure the anxiety and depression markers that are associated with quality of life and life expectancy at physiological and psychological levels. Since the effects of caffeine on psychological factors are dose-dependent, the history and duration of use was another limitation of the present study as we did not assess the history of caffeine consumption in the participants. Therefore, it is recommended that the consumption of caffeine at different doses with longer consumptions periods and history of caffeine consumption be considered in similar studies.

Conclusion

According to the results, aerobic training along with caffeine consumption had more significant effects on improving the quality of life and life expectancy compared to training and supplementation alone. Therefore, aerobic training seems to be more effective than caffeine consumption in improving the quality of life and life expectancy. However, the interaction between aerobic training and caffeine consumption has more significant effects of the quality of life and life expectancy compared to training and supplementation alone.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Prevalence of Underweight and Overweight and the Influential Factors in the Adolescent Students in Finote Selam Town in Amhara Region, Ethiopia

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ABSTRACT

Introduction: Adolescence is the transition from childhood to adulthood, which occurs within the age range of 10-19 years. The present study aimed to assess the nutritional status and influential factors in the adolescent students of Finote Selam town in Ethiopia in 2018.

Methods: This school-based, cross-sectional study was conducted on 437 adolescent students, who were selected via stratified simple random sampling. Data analysis was performed in the EPI Info version 7 and SPSS version 20 using binary logistic regression to identify the influential factors in the underweight students. In addition, crude and adjusted odds ratios with 95% significance level were used to measure the strength of the associations, and statistical significance was considered at the P-value of less than 0.05.

Results: The total prevalence of underweight, normal weight, and overweight in the adolescent students in Finote Selam town was 46.2%, 51.0%, and 2.7%, respectively. Significant associations were observed between underweight and the male gender, living in rural areas, having illiterate fathers and uneducated mothers, and the family size of larger than or equal to five (P<0.05).

Conclusion: According to the results, underweight was the most prevalent issue in the study area. The most influential factors in this regard were gender, place of residence, parental education level, family size, and occupation status of the father. Therefore, the impact of these factors should be further investigated to develop strategies for the reduction of malnutrition in Finote Selam town.

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Introduction

Adolescence is a decisive period of development as it represents the transition from childhood to adulthood, occurring within the age range of 10-19 years (1). During this crucial period, dietary patterns have a vital impact on lifetime nutritional status and health (2). Increased nutritional needs at this life juncture are due to the fact that adolescents gain up to 50% of their adult weight, more than 20% of their adult height, and 50% of their adult skeletal mass during this period. However, adolescents are faced with a series of severe nutritional challenges, which may affect the rapid growth spurt and their health as adults (3). Adolescents are considered to be the best human resources, yet for many years, their health has been compromised as they have been considered to be less susceptible to diseases compared to younger children or the elderly. Adolescent health attracted global attention only in the past decade. The assessment of the nutritional status of adolescent girls has been the latest explored area of research in the world (4-7).

Poor nutritional status during adolescence is an important determinant of health outcomes. Adolescents have various needs and diverse problems. Chronic energy deficiency in adolescents leads to short stature and lean body mass and is also associated with deficiencies in the muscle strength and working capacities (8). In females, short stature persisting into adulthood increases the risk of adverse reproductive outcomes. In many Western countries, children and adolescents seem to increasingly adopt lifestyles that adversely affect

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their nutritional and health status, substantially increasing the risk of the premature development of chronic diseases, such as cardiovascular diseases, metabolic syndrome, osteoporosis, and some cancers (9). Poor dietary habits, sedentary leisure time, and lack of physical activity are among the lifestyle patterns that once instilled, have a strong tendency to track from childhood into adulthood and become extremely resistant to modification (10).

Several studies have indicated that the educational achievement of parents is associated with the nutritional status of the children. The educational attainment of parents results in the higher income of the family, implying the higher availability of various foods and household resources (11). On the other hand, it might be positively associated with higher nutritional awareness, as well as better child care. Furthermore, maternal education level has been positively associated with the body mass index (BMI) of adolescents, which could be explained through the assumption that maternal education is also an income determinant in families. Mothers are also responsible for shopping and cooking, and their education level affects the purchased food items and methods of cooking (12).

Data is scarce regarding the nutritional status of adolescents in Ethiopia. A study conducted in Addis Ababa (the capital of Ethiopia) on the elementary school children to investigate child and adolescent obesity indicated that the total prevalence of underweight, overweight, and obesity was 13.0%, 7.6%, and 0.9%, respectively (13). In the mentioned study, the prevalence of underweight, overweight, and obesity in the female students was reported to be 9.0%, 9.4%, and 0.8%, respectively, while it was estimated at 18.1%, 5.4%, and 1.1% in the males, respectively. In addition, the prevalence of adolescent obesity was 0.9% (95% CI: 0.027-1.53%), and the gender-specific prevalence was slightly higher in the proportion of obesity in the boys (1.1%) compared to the girls (0.8%) (14).

Another study conducted in the elementary and secondary schools of Ambo town in Ethiopia showed the prevalence of obesity, overweight, normal weight, and underweight to be 1.7%, 2.6%, 68.2%, and 27.5%, respectively (15). In the mentioned research, the prevalence of obesity, overweight, normal weight, and underweight in the females was reported to be 1.1%, 3.8%, 70.5%, and 24.6%, respectively (16).

To date, no studies have been focused on the nutritional status of the adolescent students in Finote Selam town. The present study aimed to assess the nutritional status and the influential factors in the adolescent students in Finote Selam town, Ethiopia.

Materials and Methods

Study Design and Setting

This school-based, cross-sectional study was conducted on the adolescent students aged 10-19 years in public primary and secondary schools during February 5-March 27, 2018 in Finote Selam town, Ethiopia. Finote Selam town has six primary schools, one high school, one preparatory school, and five colleges, and the total number of the students in grades 5-12 is 12,289 (17).

Source and Sample Population

All the adolescent students (10-19) of the schools in Finote Selam town were considered as the source population, and the randomly selected adolescent students of the schools (10-19) were considered as the sample population.

Sample Size and Sampling Procedure

The minimum sample size required for the study was calculated using a single proportion formula. The proportion of the female underweight adolescent students in Adama city in central Ethiopia has been reported to be 21.3% (18) at 95% confidence interval (CI) and 4% margin of error, with an added 10% as a contingency for the non-response rate.

$$n = \left(\frac{z}{d}\right)^2 xP(1-p) \quad (19)$$

In the formula above, *n* shows the sample size, *Z* represents the Z score at 95% CI of 1.96 and P-value of 21.3%, and *d* is the marginal error (0.04).

$$n = \frac{(1.96)^2 * 0.213(1-0.213)}{(0.04)^2} = \frac{3.8416 * 0.213*0.787}{(0.04)^2} = 403$$

By adding 10% of the non-response rate, the minimum sample size required to estimate the prevalence of stunting and the influential factors among the adolescent students was calculated to be 403+10% (403+41)=444.

To obtain the sample size, the stratified random sampling technique was used. To this end, the schools were stratified into primary schools, junior high schools, high schools, and preparatory schools. Three primary schools (Bata, Bakel, and Efrata) and three junior high schools (Edgetber, Bata, and Bakel) were selected via simple random sampling from six primary schools and six junior high schools, respectively. On the other hand, one high school (Finote Selam Secondary School) and one preparatory school (Damot Preparatory School) were selected purposively since Finote Selam town has one high school and one preparatory school. The total sample size was distributed proportionally to the schools. The sampling frame was the identification number of the students as recorded in the respective schools, and the number of the students to be enrolled in the study was determined via simple random sampling (Figure 1).

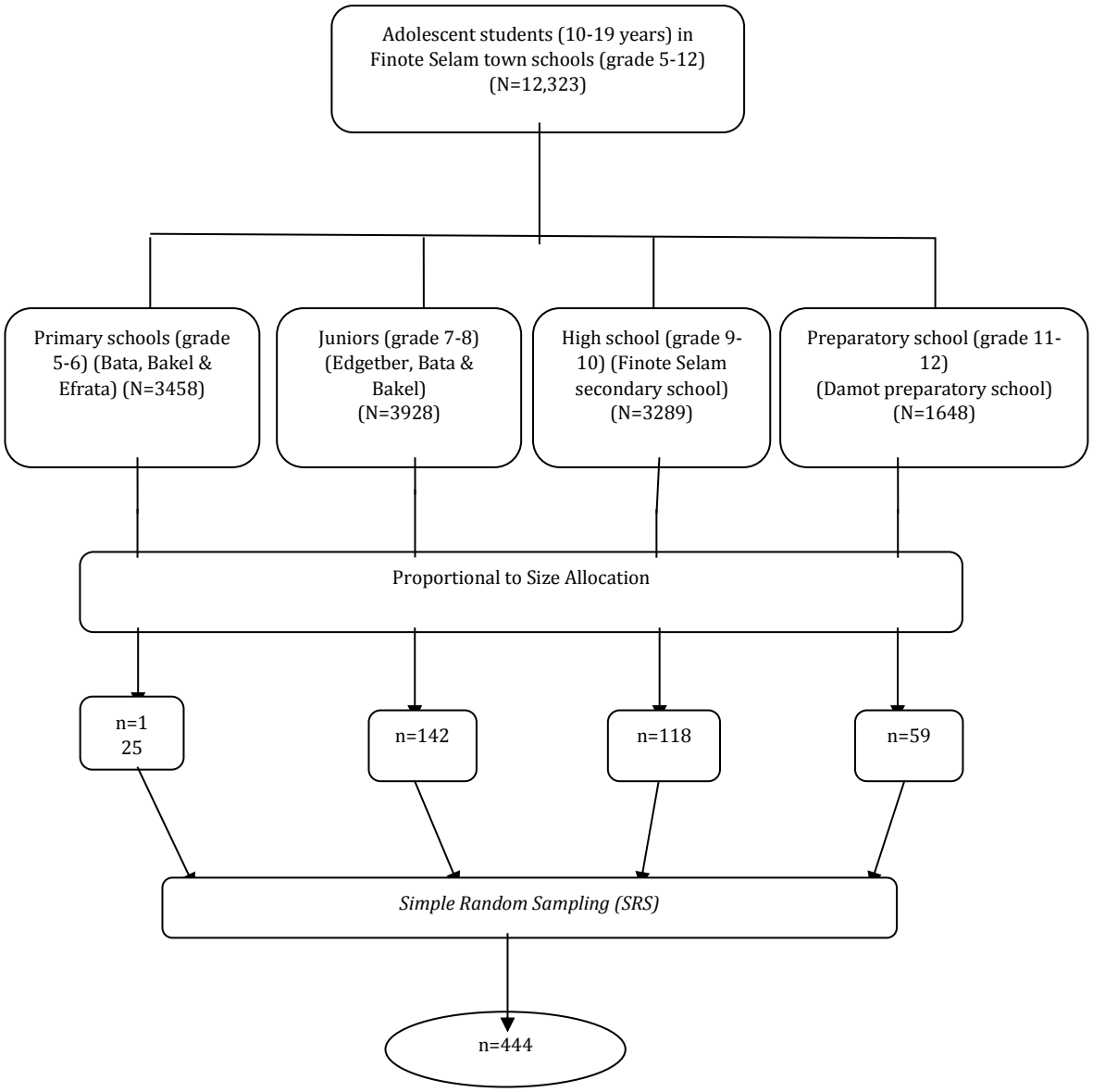

Figure 1. Schematic Presentation of Sampling Procedures

Data Collection

To generate the dataset used in the study, pre-tested structured questionnaires were used for data collection by trained data collectors. These standardized interview questionnaires were adopted and modified based on relevant articles to collect the socio-demographic, nutritional, and health-related data of the subjects (18, 20-23). Each student was interviewed separately for data collection. The recorded parameters were height and weight, and the anthropometric data were collected by trained data collectors who were health extension workers; notably, the procedure was entirely coordinated by the investigators. The height of the adolescent students was measured without shoes in centimeters with an accepted error of 0.1 centimeter. To do so, the adolescent students stood against the wall without footwear, with their head and eyes positioned straight ahead (Frankfurt plane), so that the sightline would be perpendicular to the body. The same measurer was employed in the other anthropometric measurements to avoid variability.

Data Quality Control and Management

To ensure the reliability and validity of the study, training was provided to the data collectors, and data collection was performed by two health extension workers. Moreover, a close follow-up was carried out by the investigator during data collection. The Amharic version of the questionnaire was also tested on 5% of the students of Selamamba Primary School, who were not enrolled in the study, but had similar characteristics with the participants. The data collectors and investigators participated in the pre-testing and standardization of the questionnaires, and the problems highlighted during the preliminary assessment were corrected prior to the actual survey. Each question was properly coded, and continuous supervision was performed during the pretest and data collection processes by the investigator. Furthermore, the completeness and consistency of the recorded data in the questionnaire sheets were evaluated by the investigator at the end of each working day, so that the required corrective measures would be taken the next time.

Statistical Analyses

The socio-demographic, anthropometric, nutritional, and health-related data of the subjects were analyzed in EPI Info version7 and assessed in terms of completeness and consistency, followed by data cleaning and editing in the EPI Info software. Following that, data analysis was performed in SPSS version 20 using descriptive statistics (frequency and proportion) to express the results.

BMI was calculated by dividing weight (kg) by height (m2). Accordingly, underweight, normal weight, and overweight were defined with the BMI of <18.50, 18.50-24.99, and 25.00-29.99 kg/m2, respectively (23). Chi-square and odds ratio (OR) with 95% CI were used to evaluate the strength of the associations between the outcome variable (nutrition status) and independent variables. In addition, bivariate and multivariate binary logistic regression was applied to determine the associations between the dependent variable and independent variables with 95% CI, and the covariates with the P-value of less than 0.25 were retained and analyzed via multivariable logistic regression (24). The P-value of less than 0.05 was considered as the cutoff point for an independent variable to be significantly associated with the outcomes.

Results

Socio-demographic Characteristics of the Participants and Their Families In total, 444 adolescent students were selected as the sample population, and with the response rate of 98.4%, 437 students were enrolled in the study. Out of 437 respondents who were selected for the study, 47 cases (10.8%) were in their early adolescence, 151 (34.6%) were in their mid-adolescence, and 239 (54.7.6%) were in their late adolescence. The male students constituted 257 of the sample population (58.8%), and the females constituted 180 (41.2%). Among the participants, 108 cases (24.7%) were from the primary schools, 132 cases (30.2%) were from the junior high schools, 130 cases (29.7%) were from the high schools, and the others (n=67; 15.3%) attended the preparatory school. In total, 220 students (50.3%) were from urban areas, while 217 cases (49.7%) were from rural areas. The family size of 207 (47.4%) and 230 students (52.6%) was <5 and >5, respectively (Table 1).

Table 1. Sociodemographic Characteristics of Adolescent Students in Finote Selam Town, Amhara Region, Ethiopia (2018)

Variables	Frequency	Percentage
Age Group (year)		
Early Adolescence (10-13)	47	10.8
Mid-adolescence (14-16)	151	34.6
Late Adolescence (17-19)	239	54.7
Gender		
Female	180	41.2
Male	257	58.8
Grade		
5-6	108	24.7
7-8	132	30.2
9-10	130	29.7
11-12	67	15.3
Religion		
Muslim	24	5.5
Orthodox	413	94.5
Place of Residence		
Urban	220	50.3
Rural	217	49.7
Education Level of Father		
Illiterate	96	22.0
Basic	116	26.5
Primary School (1-8)	48	11.0
Secondary School (9-12)	105	24.0
College (or higher)	72	16.5
Education Level of Mother		
Illiterate	103	23.6
Basic	124	28.4
Primary School (1-8)	44	10.1
Secondary School (9-12)	102	23.3
College (or higher)	64	14.6
Occupation Status of Father		
Daily Laborer	59	13.5
Farmer	136	31.1
Merchant	97	22.2
Government/NongovernmentEmployee	145	33.2
Occupation Status of Mother	5	1.1
Daily Laborer	94	21.5
Housewife	127	29.1
Merchant	119	27.2
Farmer	92	21.1
Government/nongovernment Employee	5	1.1
Family Size		
<5	207	47.4
≥5	230	52.6
Source of Drinking Water		
Spring Water	205	46.9
Well Water	114	26.1
Public Tap Water	30	6.9
Tap Water	88	20.1
Presence of Functional Latrine		
Yes	157	35.9
No	280	64.1

Nutritional and Health-related Characteristics of the School Adolescents The majority of the adolescents (n=303; 69.3%) consumed meals three or more times per day, while 134 adolescents (30.7%) consumed meals twice per day. Among the respondents, 68.2% reported illness within the past month. In addition, 233 subjects (53.3%) had a home garden and consumed vegetables (53.3%) and fruits (47.8%) daily (Table 2).

Table 2. Nutritional and Health-related Characteristics of School Adolescents in Finote Selam Town, Amhara region, Ethiopia (2018)

Variables	Frequency	Percentage
Number of Meals per Day		
2	134	30.7
≥3	303	69.3
Illness Reported in Past Month		
Yes	298	68.2
No	139	31.8
Home Garden		
Yes	233	53.3
No	204	46.7
Eat Vegetables at Least Once per Day		
Yes	233	53.3
No	204	46.7
Eat Fruits At Least Once Per Day		
Yes	209	47.8
No	228	52.2
Eat Farm Animal Products At least Once per Week		
Yes	319	73.0
No	118	27.0
Nutritional and Health Information		
Yes	377	86.3
No	60	13.7

Anthropometric Measurements

The minimum and maximum height of the students was 127.50 and 186.70 centimeters, respectively, and the mean total height of the participants was 158±10.67 centimeters. The minimum and maximum weight of the students was 20.5 and 68 kilograms, respectively, and the mean total weight of the participants was 45.99±10.09 kilograms. The mean height of the male and female students was 158.99±12.24 and 156.31±6.94 centimeters, respectively. The mean weight of the male and female students was 45.1±10.97 and 47.53±8.17 kilograms, respectively. The mean age of the participants was 15.54+2.41 years. Underweight, normal weight, and overweight were observed in 202 cases (46.2%), 223 cases (51.1%), and 12 cases (2.7%), respectively.

Factors Associated with the Nutritional Status of the School Adolescents

The results of Chi-square indicated that age, gender, grade, source of drinking water (P<0.0001), occupation status of the father, family size (P=0.003), occupation status of the mother (P=0.01), presence of functional latrine (P=0.024), and the number of the consumed meals per day (P=0.044) were associated with nutritional status (Table 3).

Table 3. Chi-square Analysis of Factors Associated with Nutritional Status in Adolescent School Students in Finote Selam Town, Amhara Region, Ethiopia (2018)

Variables	Nutritional Status by BMI (kg/m²)				
	Underweight N (%)	Normal Weight N (%)	Overweight N (%)	X^2	P-value
Age Group					
Early Adolescent (10-13 years)	40 (85.1)	5 (10.6)	2 (4.3)	65.41	0.000
Mid-adolescent (14-16 years)	89 (58.9)	58 (38.5)	4 (2.6)		
Late Adolescent (17-19 years)	73 (30.5)	160 (66.9)	6 (2.6)		
Gender					
Female	48 (26.7)	124 (68.9)	8 (4.4)		0.000
Male	154 (59.9)	99 (38.5)	4 (1.6)	47.67	
Grade					
5-6	79 (73.1)	27 (25.0)	2 (1.9)	89.87	0.000
7-8	78 (59.1)	48 (36.4)	6 (4.5)		
9-10	30 (23.1)	98 (75.4)	2 (1.5)		
11-12	15 (22.4)	50 (74.6)	2 (3.0)		
Religion					
Muslim	12 (50.0)	10 (41.7)	2 (8.3)	3.40	0.146
Orthodox	190 (46.0)	213 (51.6)	10 (2.4)		
Place of Residence					
Urban	97 (44.1)	115 (52.3)	8 (3.6)	1.85	1.849

Variables	Nutritional Status by BMI (kg/m²)				
Rural	105 (48.4)	108 (49.8)	4 (1.8)		
Education Level of Father					
Illiterate	36 (50.0)	32 (44.4)	4 (5.6)	14.52	0.069
Basic	27 (56.2)	19 (39.6)	2 (4.2)		
Primary School (1-8)	51 (53.1)	45 (46.9)	0 (0.0)		
Secondary School (9-12)	49 (42.2)	65 (56.0)	2 (1.7)		
College (or higher)	39 (37.1)	62 (59.0)	4 (3.8)		
Education Level of Mother					
Illiterate	25 (56.8)	19 (43.2)	0 (0.0)	14.05	0.080
Basic	32 (50.0)	28 (43.8)	4 (6.2)		
Primary School (1-8)	54 (52.4)	49 (47.6)	0 (0.0)		
Secondary School (9-12)	51 (41.1)	69 (55.6)	4 (3.2)		
College (or higher)	40 (39.2)	58 (56.9)	4 (3.9)		
Occupation Status of Father					
Daily Laborer	39 (66.1)	20 (33.9)	0 (0.0)	20.16	0.003
Farmer	65 (47.8)	67 (49.3)	4 (2.9)		
Merchant	40 (41.2)	57 (58.8)	0 (0.0)		
Government/non government Employee	58 (40.0)	79 (54.5)	8 (5.5)		
Occupation Status of Mother					
Daily Laborer	3 (60.0)	2 (40.0)	0 (0.0)	21.87	0.010
Farmer	52 (55.3)	36 (38.3)	6 (6.4)		
Housewife	47 (37.0)	80 (63.0)	0 (0.0)		
Merchant	53 (44.5)	64 (53.8)	2 (1.7)		
Government/non government Employee	47 (51.1)	41 (44.6)	4 (4.3)		
Family Size					
<5	80 (38.6)	123 (59.4)	4 (1.9)	11.26	0.003
≥5	122 (53.0)	100 (43.5)	8 (3.5)		
Source of Drinking Water					
Well Water	52 (59.1)	36 (40.9)	0 (0.0)	27.81	0.000
Spring Water	103 (50.2)	92 (44.9)	10 (4.9)		
Public Tap Water	40 (35.1)	72 (63.2)	2 (1.8)		
Tap Water	7 (23.3)	23 (76.7)	0 (0.0)		
Presence of Functional Latrine					
Yes	131 (46.8)	137 (48.9)	12 (4.3)	7.46	0.024
No	71 (45.2)	86 (54.8)	0 (0.0)		
Number of Meals per Day					
2	152 (50.2)	143 (47.2)	8 (2.6)	6.21	0.044
≥3	50 (37.3)	80 (59.7)	4 (3.0)		
Illness Reported in Past Month					
Yes	71 (51.1)	62 (44.6)	6 (4.3)	4.52	0.104
No	131 (44.0)	161 (54.0)	6 (2.0)		
Home Garden					
Yes	143 (44.1)	173 (53.4)	8 (2.5)	2.91	0.230
No	59 (52.2)	50 (44.2)	4 (3.5)		
Eat Vegetables at Least Once per Day					
Yes	82 (40.2)	116 (56.9)	6 (2.9)	5.61	0.058
No	120 (51.5)	107 (45.9)	6 (2.6)		
Eat Fruits at Least Once per Day					
Yes	95 (41.7)	125 (54.8)	8 (3.5)	4.50	0.105
No	107 (51.2)	98 (46.9)	4 (1.9)		
Eat Farm Animal Products At least Once per Week					
Yes	52 (44.1)	60 (50.8)	6 (5.1)	3.38	0.187
No	150 (47.0)	163 (51.1)	6 (1.9)		
Nutritional and Health Information					
Yes	26 (43.3)	34 (56.7)	0 (0.0)	2.47	0.272
No	176 (46.7)	189 (50.1)	12 (3.2)		

Factors Associated with the Underweight School Adolescents

According to the multivariable logistic regression analysis, the factors of gender, place of residence, education level of the father and mother, family size, number of the meals per day, and illness reported in the past one month were significantly associated with being underweight.

According to the findings, the OR of nutritional status was 1.64 times higher in the adolescent students in their early adolescent stage compared to those in the late adolescent stage (AOR=1.64; 95% CI: 0.87-3.08). Furthermore, the OR of the male adolescent students was 1.58 times higher in terms of being underweight (AOR=1.58; 95% CI: 1.04-2.39) compared to the female adolescent students. The OR of underweight was also 1.68 times higher in the adolescent students living in rural areas compared to those living in urban areas (AOR=1.68; 95% CI: 1.22-2.47). The adolescent students with illiterate fathers were more likely to become underweight compared to those whose fathers had college (or higher) education levels (AOR=2.54, 95% CI: 1.16-4.81). Similarly, the adolescents with uneducated mothers were more likely to become underweight compared to those whose mothers had college (or higher) education levels (AOR=2.84; 95% CI: 1.02-7.94). The adolescent students whose family size was ≥5 were 1.58 times more likely to become underweight compared to those with the family size of <5 (AOR=1.58; 95% CI: 1.06-2.48). In addition, the adolescent students who consumed less than three meals per day were 1.67 times more likely to become underweight compared to those who consumed three or more meals per day. Furthermore, the risk of underweight was 1.62 times higher in the adolescent students with illnesses within the past one month compared to those who were apparently healthy (Table 4).

Table 4. Bivariate and Multivariable Logistic Regression of Factors Associated with Underweight in Adolescent School Students in Finote Selam Town, Amhara Region, Ethiopia (2018)

Variables	Underweight		COR (95% CI)	AOR (95% CI)
	Yes N (%)	No N (%)		
Age Group				
Early Adolescent (10-13 years)	40 (85.1)	7 (14.9)	3.06 (1.68-5.67)	1.64 (0.87-3.08)
Mid-adolescent (14-16 years)	89 (58.9)	62 (41.1)	1.73 (1.12-2.71)	1.23 (0.59-2.54)
Late Adolescent (17-19 years)	73 (30.5)	166 (69.5)	1	1
Gender				
Female	48 (26.7)	132 (73.3)	1	1
Male	154 (59.9)	103 (40.1)	2.43 (1.42-4.06)	1.58 (1.04-2.39)*
Grade				
5-6	79 (73.1)	29 (26.9)	1.97 (1.19-3.25)	1.79 (0.85-3.67)
7-8	78 (59.1)	54 (40.9)	1.38 (0.76-2.48)	1.11 (0.63-1.97)
9-10	30 (23.1)	100 (76.9)	1.28 (0.62-2.74)	0.82 (0.33-2.03)
11-12	15 (22.4)	52 (77.6)	1	1
Religion				
Muslim	12 (50.0)	12 (50.0)	1.05 (0.31-1.73)	0.92(0.53-1.76)
Orthodox	190 (46.0)	223 (54.0)	1	1
Place of Residence				
Urban	97 (44.1)	123 (55.9)	1	1
Rural	105 (48.4)	112 (51.6)	2.12 (1.30-3.42)	1.68 (1.22-2.47)*
Education Level of Father				
Illiterate	51 (53.1)	45 (46.9)	2.84 (0.78-11.72)	2.54 (1.16-4.81)*
Basic	27 (56.2)	21 (43.8)	1.61 (0.44-5.91)	1.78 (0.76-3.86)
Primary School (1-8)	36 (50.0)	36 (50.0)	1.21 (0.23-6.74)	1.53 (0.99-2.36)
Secondary School (9-12)	49 (42.2)	67 (57.8)	1.40 (0.83-2.35)	0.98 (0.16-5.82)
College (or higher)	39 (37.1)	66 (62.9)	1	1
Education Level of Mother				
Illiterate	25 (56.8)	19 (43.2)	2.92 (1.53-5.57)	2.84 (1.02-7.94)*
Basic	54 (52.4)	49 (47.6)	2.39 (1.55-3.68)	2.03 (0.68-5.82)
Primary School (1-8)	32 (50.0)	32 (50.0)	1.86 (1.15-3.00)	2.26 (0.85-6.03)
Secondary School (9-12)	51 (41.1)	73 (58.9)	0.98 (0.47-1.86)	1.60 (0.90-2.84)
College (or higher)	40 (39.2)	62 (60.8)	1	1
Occupation Status of Father				
Daily Laborer	39 (66.1)	20 (33.9)	3.07 (1.67-5.68)	2.85 (1.06-7.98)*
Farmer	65 (47.8)	71 (52.2)	2.63 (1.58-4.33)	2.27 (0.89-6.06)
Merchant	40 (41.2)	57 (58.8)	1.74 (1.12-2.72)	2.03 (0.69-5.83)
Government/non-government Employee	58 (40.0)	87 (60.0)	1	1
Occupation Status of Mother				

Daily Laborer	3 (60.0)	2 (40.0)	1.69 (0.98-2.49)	1.66 (0.56-5.04)
Farmer	52 (55.3)	42 (44.7)	1.15 (0.67-1.96)	0.79 (0.33-2.06)
Housewife	53 (44.5)	66 (55.5)	0.92 (0.55-1.54)	0.64 (0.18-2.22)
Merchant	47 (37.0)	80 (63.0)	0.82 (0.15-4.86)	0.44 (0.16-1.29)
Government/non-government Employee	47(51.1)	45 (48.9)	1	1
Family Size				
<5	80 (38.6)	127 (61.4)	1	1
≥5	122 (53.0)	108 (47.0)	1.73 (1.04-2.81)	1.58 (1.06-2.48)*
Source of Drinking Water				
Well Water	52 (59.1)	36 (40.9)	2.78 (1.23-11.79)	1.73 (0.96-3.14)
Spring Water	103 (50.2)	102 (49.8)	2.37 (1.58-7.23)	1.05 (0.63-1.87)
Public Tap Water	40 (35.1)	74 (64.9)	1.38 (0.60-2.88)	0.83 (0.48-1.44)
Tap Water	7 (23.3)	23 (76.7)	1	1
Presence of Functional Latrine				
Yes	131 (46.8)	149 (53.2)	1	1
No	71 (45.2)	86 (54.8)	0.96 (0.51-1.84)	0.79(0.31-2.06)
Number of Meals per Day				
2	152 (50.2)	151 (49.8)	1.88 (1.25-3.20)	1.67 (1.22-2.48)*
≥3	50 (37.3)	84 (62.7)	1	1
Illness Reported in Past Month			2.92 (2.04-7.57)	1.62 (1.03-2.57)*
Yes	71 (51.1)	68 (48.9)		
No	131 (44.0)	167 (56.0)	1	1
Home Garden				
Yes	143 (44.1)	181 (55.9)	1	1
No	59 (52.2)	54 (47.8)	1.55 (0.98-2.37)	1.28 (0.69-2.56)
Eat Vegetables at Least Once per Day				
Yes	82 (40.2)	122 (59.8)	1	1
No	120 (51.5)	113 (48.5)	1.63 (0.91-2.89)	1.28 (0.89-1.78)
Eat Fruits at Least Once per Day				
Yes	95 (41.7)	133 (58.3)	1	1
No	107 (51.2)	102 (48.8)	1.45 (0.85-2.43)	1.28 (0.86-1.88)
Eat Farm Animal Products At least Once per Week				
Yes	52 (44.1)	66 (55.9)	1	
No	150 (47.0)	169 (53.0)	1.87 (1.05-3.39)	1.25 (0.69-2.56)
Nutritional and Health Information				
Yes	26 (43.3)	34 (56.7)	1	1
No	176 (46.7)	201 (53.3)	1.42 (0.85-2.56)	1.24 (0.98-1.79)

Discussion

In the present study, the prevalence of underweight and overweight and the influential factors were investigated in the adolescent students of Finote Selam town. According to the findings, the prevalence of underweight was 46.2%, which is significantly higher compared to the studies in Chiro town (24.4%) (25), Ambo town (27.5%) (26), and Arba Minch town (19.7%) (27). In the present study, the prevalence of underweight was higher compared to the studies in West Bengali, India (42.2%) (28), Djibouti (31.9%) (29), and Egypt (12.6%). The discrepancy might be due to the differences in the socioeconomic background, sample size, dietary habits, and type of meals.

In the current research, the prevalence of overweight in the participants was 2.7%, which is similar to the study in Chiro town (2.7%) (25) due to the almost similar demographic conditions. On the other hand, the prevalence of overweight in the present study was higher compared to Mekelle City (2%) (10), while lower than Addis Ababa (8.5%) (30). Furthermore, the prevalence of overweight in the current research was significantly lower compared to Arba Minch town (8.7%) (27), Ghana (8.7%), and Egypt (31.4%) (29), which might be due to the differences in the barriers to under-nutrition, such as cultural differences and other socio-demographic characteristics.

Among the studied factors in the current research, gender, place of residence, education level of the father, education level of the mother, family size, number of the meals per day, occupation status of the father, and illness in the past one month were associated with underweight. The OR of underweight was 1.64 times higher in the early adolescent stage compared to the late adolescent stage, which is

consistent with the results obtained in Chiro town in eastern Ethiopia (25). This might be due to the fact that with increased age, adolescents are likely to access food easily and become more matured. In the present study, the risk of underweight was 1.58 times higher in the male adolescent students compared to the females. This finding is consistent with the studies in Chiro town in eastern Ethiopia (25) and Arba Minch town (27). The difference might be due to the fact that boys are more influenced by environmental stress compared to girls.

In the current research, the risk of underweight was 1.68 times higher in the adolescent students living in rural areas compared to those living in urban areas, which is in line with the study conducted in Gobu Seyo District in East Wollega Zone, Oromia regional state of West Ethiopia (31). The difference might be due to the food preferences, food consumption patterns, and inequalities in dietary diversity between urban and rural areas.

In the present study, the adolescent students with illiterate fathers were more likely to become underweight compared to those whose fathers had college or higher education levels; this is consistent with the studies in Arba Minch town in Ethiopia (27) and Chiro town in eastern Ethiopia (25). Paternal education level might be an essential factor in food security and feeding practices as educated fathers might also have a better income than non-educated fathers. On the other hand, the risk of underweight in the school adolescents whose mothers were illiterate was 2.84 times higher compared to those whose mother had college or higher education levels. This finding is in line with a study conducted in south Ethiopia (27) possibly due to the fact that if the education level of the mother is low, her decision-making and contribution to the total family income may be inadequate.

The risk of being underweight in the school adolescents whose fathers had a daily laborer occupation was 2.85 times higher compared to those whose fathers had a government/non-government job. This is consistent with the finding in Chiro town and west Hararge (25). Having government/non-government jobs might be an essential factor for the family to have a permanent income source for livelihood.

According to the results of the present study, the school adolescents who had meals twice per day were 1.67 more likely to become underweight compared to those who had three meals. The risk of being underweight in the school adolescents who had reported illnesses in the past one month was 1.62 times higher compared to the others. Furthermore, the adolescent students with the family size of ≥5 were 1.58 times more likely to become underweight compared to those with the family size of <5. This is in line with the studies in Addis Ababa, Ethiopia (32), Arba Minch town, Ethiopia (27), and Osun State, Nigeria (33). The probable reason is that with many children living together in the family, there may be food competition and tendency toward under-nutrition.

Limitations of the Research

The major limitations of the study were the lack of a fund source and the fact that the age of the children obtained from the school records might have been underestimated and there also might have been measurement bias in the anthropometric measurements. Moreover, the study was conducted on school adolescents, and the findings cannot be generalized to the adolescent population.

Conclusion

According to the results, underweight was the major prevalent issue in the study area. Factors such as gender, place of residence, parental education level, family size, number of the meals per day, and illness reported in the past one month largely influenced underweight in the adolescent students. Therefore, the effects of these factors should be considered in the adoption of strategies for the reduction of malnutrition in the town.

Ethical Approval and Consent to Participate

The study protocol was approved by the Ethics Committee of the Faculty of Chemical and Food Engineering at Bahir Dar University (protocol No. 12/2010). Supportive letters were also obtained from Amhara Public Health Institute to the West Gojjam Health Office, Finote Selam Town Administration Health Office, and the target schools. In addition, written informed consent was obtained from the parents or legal guardians after clearly explaining the research objectives.

Conflicts of interest

None declared.

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Determination of the pH, Salt, Sodium and Potassium Content in the Traditional Bread in Western Iran

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ABSTRACT

Introduction: Bread is frequently used worldwide and provides a significant portion of the energy, protein, minerals, and vitamins needed by the body. The present study aimed to determine the pH, salt, sodium, and potassium content in the traditional breads in Chaharmahal and Bakhtiari province, Iran.

Methods: This study was conducted on 451 traditional bread samples of various types, which were randomly collected by the bakery health inspectors in Chaharmahal and Bakhtiari province in 2016**.** The pH, salt, sodium, and potassium content of the samples were measured using a pH metric and potentiometric and flame photometric methods at the Food Control Laboratory of Shahrekourd University of Medical Sciences.

Results: The mean pH, salt, sodium, and potassium content of the bread samples were 5.85, 1.95%, 765, and 108 mg/100 g, respectively. At least 7.7% of the bread samples were positive for sodium bicarbonate use, and 54% had higher levels than the recommended maximum of salt content. In addition, the ratio of sodium to potassium was 12.07.

Conclusion: According to the results, salt use was high in bread production, and a significant portion of the bread samples were positive for sodium bicarbonate use. This could be a major health threat to the community. Therefore, strong control and proper supervision are essential in bread production units.

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Introduction

Bread is frequently used throughout the world as a major food and plays a key role in the provision of the energy, protein, minerals, and vitamins needed by the human body. Previous findings have indicated that in urban and rural communities, 42% and 47% of the daily energy is provided through bread consumption, respectively (1). Flour, water, yeast, and sodium chloride are the main ingredients of bread. The addition of sodium chloride to dough causes the gluten structure to become firm and strong, which improves the ability of the dough to retain the carbon dioxide that is produced during the fermentation process; as a result, bread gains proper volume.

Salt is a natural antioxidant (2). If sodium chloride is not added to bread dough, most of the carotenoid pigments are eliminated, and the effective substances in the flavor reduce (2). Sodium chloride is a hygroscopic material that tends to absorb water from the surrounding environment, serving two main purposes; the first purpose is to slow the fermentation rate, and the second is to preserve the moisture and softness of bread.

Today, the maximum limit of salt use in bread production is 2±0.2% of the flour consumption (2). According to the National Standard No. 2628 of Iran, the maximum salt content in various types of traditional breads (Tafton, Barbari, Lavash, and Sangak) is 1.8% of the dry matter (3). In addition, the National Standard No. 2628 of Iran has introduced pH as an appropriate indicator for the quality assessment of the bread production process. This indicator could also

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detect the illegal use of sodium bicarbonate (baking soda) in bread production. According to the same standard, the pH limit for Tafton, Barbari, and Lavash breads is 5-6, while it is 4.6-5.6 for Sangak bread (3). In the previous studies in this regard, the pH value has been considered the main criterion for the detection of sodium bicarbonate in bread; if the pH of bread is ≥6.2, the bread is positive for sodium bicarbonate (4).
Sodium and potassium are the major cations of the extracellular and intracellular fluids of the human body, respectively (5). These elements play a pivotal role in the osmotic balance, neuromuscular function, and balance of the acidic and alkaline fluids in the body. High sodium and low potassium intake are associated with the risk of hypertension, stroke, cardiovascular diseases, osteoporosis, obesity, and diabetes, while gastric cancer is also associated with long-term adherence to high-sodium diets (5). According to the World Health Organization (WHO) guidelines, these cations could have positive functions in the body when the sodium-to-potassium ratio of the daily diet is equal to one (6-8).
Studies have indicated that in the world's diet, more than 75% of the body's sodium intake is obtained through the consumption of processed food (e.g., bread), and only 10-15% of sodium is obtained by added salt during cooking and at the table (9, 10). The British Standard Food Agency has set the average sodium chloride content in various types of bread at 1% (range: 0.75-1.2%) (11).
Previous studies in different regions of Iran have shown that the sodium chloride content in various types of bread is approximately 1.2-2%. Since bread is an Iranian staple food, it could be the main source of sodium in the Iranian diet. The present study aimed to evaluate the quality of the bread used in Chaharmahal and Bakhtiari province, Iran based on pH, salt, and sodium and potassium content.

Materials and Methods

This cross-sectional, descriptive-analytical study was extracted from the first part of an HSR study (code: 568), registered at Shahrekord University of Medical Sciences. Ethical criteria were implemented in accordance with the Code of Ethics No. 87-12-10, and the study protocol was approved by the Ethics Committee of Shahrekourd University of Medical Sciences.

The study was conducted on 451 traditional bread samples, including Tafton, Barbari, Sangak, Lavash, and local breads. This sample size was equivalent to half of the total bread samples collected by the health inspectors of Shahrekourd University of Medical Sciences from the bakeries in Chaharmahal and Bakhtiari province in 2016. The bread samples were randomly collected and sent to the Food Control Laboratory for health control.
The pH, salt, and sodium and potassium content of the bread samples were measured using valid methods. In addition, chemicals such as silver nitrate, nitric acid, and sodium chloride were measured using the laboratory-grade by Merck, Germany.

Determination the pH of the Bread

The pH of the bread samples was measured in accordance with the Iranian National Standards No. 2628 and 37. In order to determine the pH of the bread samples, a pH meter was first adjusted using the buffer solutions 7 and 4, respectively. Afterwards, 10 grams of the samples was placed in a 100-milliliter container of distilled water, and the pH was measured after 30 minutes. Similar to previous studies, the criteria for the detection of sodium bicarbonate in bread was considered to be pH≥6.2 in the current experiment (4).

Sample Preparation for the Determination of the Salt, Sodium, and Potassium Contents of the Bread

To determine the salt, sodium, and potassium contents of the bread samples, 10 grams of each sample was placed in an electric oven at the temperature of 105°C for a minimum of three hours to remove moisture.

Determination of the Salt Content of the Bread

The sodium chloride content of the bread samples was measured using the potentiometric method (model: Titrando 835 Metromh, made in Switzerland), with the device functioning based on the potentiometric method. The bread salt content was measured using the 0.1 M silver nitrate solution (16.98 grams of dry silver nitrate dissolved in 1 liter of distilled water). To determine the salt content of the bread samples, approximately 1.000 gram of the dried bread samples was placed in a container with 100 milliliters of distilled water, and one drop of nitric acid was added to acidify the reaction medium. Following that, the container was

attached to a Tetrando machine, and the salt content of the bread was calculated based on the amount of the consumed titrant in grams/percentage using the following equation.

Percentage of Bread Salt = (0.585 × Consumed Titrant (ml) / Sample Weight (g))

Determination of the Sodium and Potassium Contents of the Bread

Sample Preparation

The sodium and potassium contents of the bread samples were measured based on the amount of the released ions in the flame using a flame photometer (model: 405, made in USA) (12). To determine the levels of sodium and potassium in the bread samples, 1.000 gram of the dried powder was placed in a capsule and burned with a flame for conversion into black ash. In the next step, the black ash was converted into white ash in an electric furnace at the temperature of 600°C for one day. The obtained ash was dissolved in a 100-milliiter balloon containing distilled water and prepared for injection into the flame photometer apparatus (12).

Preparation of the Standard Curves

To determine the standard curve of sodium and potassium, a stock solution and standard working solutions were prepared using the AOAC 969.23 method and laboratory-grade sodium chloride and potassium chloride (12). The sodium and potassium contents of the bread samples were measured using the atomic absorption method with a flame photometer (model: 405, USA) (12).

In accordance with the Instructions of the manufacturer of the device, the device was switched on, and the flame was set to the blue color by adjusting the amount of gas and air entering the device. Afterwards, pure water was injected into the device as a blank solution, and the device was set to zero in millivolts. In the next step, the working standards of the sodium and potassium solutions were injected into the device from the highest to the lowest concentration. Data were obtained based on the ions released into the flame (mV).

Statistical Analyses

The general equation $y=ax+b$ was obtained after the regression analysis of the obtained data from the injection of the standard sodium and potassium working solutions into the flame photometer using the IBM SPSS version 19. In the equation, y shows the output data of the flame photometer (mV), x is the concentration of the substance in the sample solution (mg/ml), a represents the standard curve slope, and b is the constant value of the standard curve. In addition, the following equation was applied to calculate the sodium and potassium concentrations in the bread samples:

Sodium and Potassium Contents of Bread (mg/100 g) = 100 x x/Sample Weight

Results

In total, 451 samples of various traditional breads were assessed, including 297 samples of Tafton bread (65.9%), 66 samples of local bread (14.4%), 35 samples of Barbari bread (7.7%), 29 samples of Lavash bread (6.4%), and 25 samples of Sangak bread (5.5%). The pH, salt, and sodium and potassium contents of the samples were measured, and the results of the experiments are presented in Table 1.

Table 1. Values of pH, Salt, and Sodium and Potassium Ions in Bread Samples

Types of Bread	Number of Tested Samples	Mean Moisture	Mean pH	Salt (g/100)			Sodium Content (mg/100 g)	Potassium Content (mg/100 g)
				Mean	Maximum	Minimum		
Tafton	297	27.77±2.51	5.66±0.42	1.98±0.65	3.81	0.38	787±287	106±18
Lavash	29	26.39±3.58	6.08±0.21	1.52±0.21	4.17	0.29	608±441	103±11
Local	65	26.72±1.79	5.79±0.24	2.06±0.64	3.22	1.01	801±323	109±15
Barbari	35	27.41±1.94	5.90±0.28	1.36±0.66	2.22	0.41	550±588	112±14
Sangak	25	29.12±1.93	5.50±0.11	1.67±0.85	2.82	0.9	663±338	110±10
Total	451	28.27±2.27	5.85±0.4	1.95±0.63	6.30	0.29	769±248	108±13

ά: standard deviation

According to the results, the mean pH of various types of bread was 5.84±0.44, and the pH of 51.9% of the samples was within the permissible range of 5-6. In addition, the mean pH of all types of traditional bread (other than Lavash bread) was within the permissible range.

According to the information in Table 2, the pH of 41.9% of the bread samples was above the maximum permissible limit, while the pH of 5.8%

of the samples was below the minimum permissible limit (5). Based on the pH of ≥6.2 as the diagnostic criterion of baking soda in bread, only 7.3% of the bread samples were positive for baking soda.

Table 2. pH Status of Bread Samples

Sample Number	pH<5		pH: 5-6		6<pH≤6.2		pH>6.2	
Total	N	%	N	%	N	%	N	%
451	26	5.8	234	51.9	157	34.8	33	7.3

The mean sodium chloride in various types of bread was 1.95±0.63%, which was above the maximum permissible limit (1.8%). However, the salt content of all types of the traditional bread (except the local bread) was within the permissible range. As can be seen in Figure 1, the salt content of 54% of the samples was above the maximum permissible limit.

The mean potassium in various bread samples was 108±13 mg/100 g of bread, and since the potassium source of bread was quite natural, no significant difference was observed in the potassium content of various types of bread. The mean sodium in various types of bread was 769±248 mg/100 g, with the lowest value observed in Sangak bread and the highest value observed in local bread. Since bread formula salt content is the main source of sodium in bread, a significant difference was observed in the sodium content of various bread types.

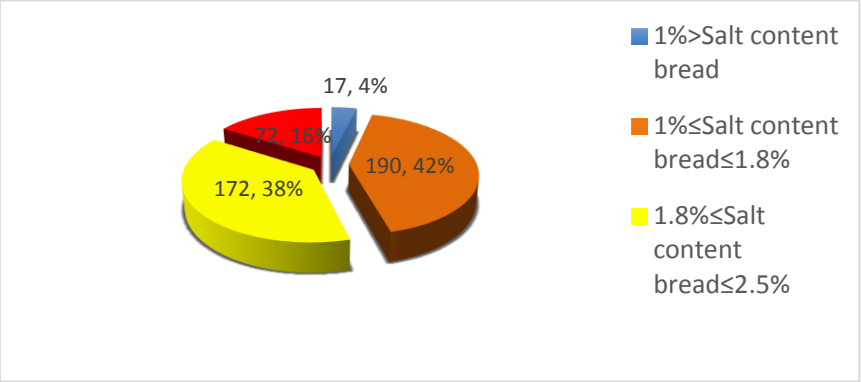

Figure 1- The frequency of salt content in bread samples tested

Discussion

Excess sodium chloride used in bread production leads to high sodium intake, which is a major cause of several diseases. The results of the present study showed that only 52% of the bread samples had proper fermentation since the pH of the samples was within the permissible range of 5-6. A study conducted in Sabzevar (Iran) indicated that 76% of the bread samples were within the permissible range, and the fermentation status of the samples in the mentioned study was better than the samples in the present study (4).

Bread pH indicates the fermentation status, and fermentation rate has a significant effect on the micronutrient availability of bread (6). In the current research, 42% of the bread samples with higher pH than the maximum permissible limit was insufficiently fermented. As a result, the bioavailability of micronutrients such as iron and zinc in these samples was low (5, 6). When dough is not fermented sufficiently, yeast cannot produce the phytase enzyme, and the phytic acid in the dough is not degraded sufficiently, which in turn disrupts the absorption of these cations (5, 6).

Similar to previous studies, we considered the pH of ≥6.2 in the current research as the criterion for the detection of sodium bicarbonate in bread. Since the pH of 7.3% in the bread samples was higher than 6.2, these samples were considered positive for sodium bicarbonate. The use of sodium bicarbonate in bread production in Chaharmahal and Bakhtiari province has been on the rise as it has been reported that 5% of the bread samples in the area had higher pH than 6.2

in a study (14). However, the use of sodium bicarbonate in this province is less common than the average level of the country (9.1%), as well as the reported values in Najafabad (8.5%) and Isfahan (8%) (15, 16). Bread iron could be absorbed in the intestine when it is first released from the insoluble complexes in bread, reducing from the trivalent form (ferric) to the bivalent form (ferrous) in the presence of gastric acid. This process is disrupted in the presence of sodium bicarbonate and decreases the iron absorption in the gut and lead to the loss of iron-enriched flour costs and gastric inflammation. These events occur due to the inadequate supervision over bread production units (5).

According to the results of the present study, the salt content in various types of bread exceeded the maximum permissible limit (1.8%) (3). Previous studies in different regions of Iran have also shown that bread salt content is within the range of 1.31-2.19% (17-19). In the current research, the salt content of 54% of the bread samples was above the maximum permissible limit, while the studies in Najafabad, Isfahan, and Shiraz (Iran) have indicated that the bread salt content was higher than the maximum permissible limit in 64.5%, 13%, and 9% of bread samples, respectively (15, 16, 19). The Ministry of Health of the Islamic Republic of Iran has recently set the maximum salt level of 1% for different types of bread. Accordingly, the mean salt content of bread in the present study was almost twice higher than the permissible limit.

The current research and previous studies conducted in Iran have shown that the sodium chloride content in bread is higher compared to developed countries, such as the United States and United Kingdom. In a study in this regard, the sodium chloride content of bread in the United States was reported to be 1.28% (20), while in the other studies in the United Kingdom, Spain, France, and Turkey, this value has been estimated at 0.98%, 1.28%, 1.80%, and 1.8%, respectively (10). The issue of reducing sodium chloride consumption in developed countries has received more attention as the United Kingdom managed to reduce the salt content of bread from 1.23% in 2001 to 0.98% in 2011 by 20% (21).

Considering that bread is the staple food of Iranians, it could be a major source of dietary sodium. A study performed in Isfahan showed that the sodium content in more than 53% of bread samples was higher than 800 mg/100 g, which is in line with the present study (17). While the main source of sodium in bread is sodium chloride added during the production process, the origin of potassium in bread is quite natural. The results of the present study indicated that the mean sodium content of bread was 769 mg/100 g (33.4 millimoles), and the potassium content of bread was 108 mg/100 g (2.7 millimoles). According to the findings in Sweden, Finland, and Norway, the sodium content of bread was 420, 455, and 467 mg/100 g, respectively, while in the present study, the sodium content was almost twice higher (22). Salt plays a key role in the strengthening the gluten network of bread dough, and low-gluten flour needs more salt for good strength in the gluten network (2).

Since sodium is the main extracellular fluid cation and potassium is the intracellular fluid cation, the sodium-to-potassium ratio (molar) of these fluids indicates the state of function of the cell; optimally, this ratio is equal to one (23). Since no additives containing potassium are added to bread during the production process, the potassium content of bread is approximately equal to the potassium content of the flour. If the potassium content of British bread is considered to be 108 mg/100 g, the ratio of sodium to potassium is 1.6, while the ratio in the present study was 12.07. Therefore, British breads are healthier compared to the traditional breads in Chaharmahal and Bakhtiari province.

Conclusion

Based on chemical indicators (pH, salt, and sodium bicarbonate), the bread consumed in Chaharmahal and Bakhtiari province is not nutritionally appropriate. For the most part, the consumed bread in this region is made of dough with insufficient fermentation. The excessive use of sodium chloride and illegal use of sodium bicarbonate in the bread production process significantly increases the sodium content in bread. Consequently, the consumption of such breads causes numerous health problems. To address this issue, proper Training and continuous monitoring of bread production units are recommended for the reduction of the salt used in bread production. Furthermore, the provision of infrastructures for industrial bread production could be an effective and safe approach to the production of healthy bread. The use of herbs such as fenugreek and fennel could also be effective in reducing salt use in bread

production; herbs and spices are often recommended for salt reduction as they could improve the flavor of bread while exerting healthful effects (24). Therefore, the policies for the reduction of salt consumption should be updated by the authorities.

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