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The effects of date seed (Phoenix dactylifera) supplementation on exercise-induced oxidative stress and aerobic and anaerobic performance following high-intensity interval training sessions: a randomised, double-blind, placebo-controlled trial

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Abstract

High-intensity interval training (HIIT) is an efficient method to improve vascular function, maximal oxygen consumption, and muscle mitochondrial capacity. However, acute HIIT overstresses the oxidative system and causes muscle soreness and damage. The aim of the present study was to investigate the effects of date seeds on exercise-induced oxidative stress and aerobic and anaerobic performance following HIIT sessions. Thirty-six physically active men and women aged 18–35 years were assigned to take 26 g/d of date seed powder (DSP, *n* 18) or wheat bran powder (placebo, *n* 18) before and after HIIT workouts for 14 d. Total antioxidant capacity (TAC), oxidative stress index (OSI), total oxidant status (TOS), superoxide dismutase (SOD), glutathione peroxidase (GPx), uric acid, malondialdehyde (MDA), and 8-iso-PGF2α were determined at baseline, at the end of the intervention, and 24-h post-intervention. We used the Cooper and running-based anaerobic sprint test to assess aerobic and anaerobic performance at the study's beginning and end. Independent-samples Student's *t* tests, ANCOVA and repeated-measures ANOVA were used to compare the quantitative variables. Positive changes were observed in TAC, TOS, OSI, GPx, MDA and visual analogue scale after intervention and at 24-h post-exercise ($P < 0{\cdot}05$). Likewise, peak power and fatigue index were significantly improved in DSP in comparison with the placebo group. Levels of SOD, uric acid, 8-iso-PGF2α, VO_2 max and average power were not changed after training. Our results showed that date seed supplementation in active participants performing HIIT bouts ameliorated oxidative stress and improved performance parameters.

Keywords: **Date seed: HIIT: Exercise performance: Oxidative stress: Polyphenol: Functional food: Prebiotic**

As a highly nutritious food, dates (Phoenix dactylifera) are grown mainly in North Africa and the Middle East. Iran is the second largest producer of date fruits, accounting for 21 % of world production[1]. Date seeds are waste products generated in large quantities in the production process of dates. Recently, it has been the focus of growing interest regarding its application as a functional food in both animal studies and clinical trials, owing to its high content of dietary fibre (75–80 g/100 g), antioxidants (phenolic acids (2697–5342 mg of gallic acid equivalents/100 g), total flavonoids (1224–1844 mg of rutin equivalents/100 g) and carotenoids) and considerable amounts of minerals, vitamins, protein and fat[2,3]. Earlier studies highlighted flavanols (catechin, epicatechin and procyanidins) as the most important polyphenolic compounds found in date seeds[4]. Several studies have reported health benefits of date seeds, including antioxidant activity, anti-carcinogenic, antimutagenic and anti-inflammatory effects, as well as the amelioration of hyperglycaemia, hyperlipidaemia,

Abbreviations: DSP, date seed powder; FI, fatigue index; GPx, glutathione peroxidase; HIIT, high-intensity interval training; HR, heart rate; MDA, malondialdehyde; OSI, oxidative stress index; ROS, reactive oxygen species; SOD, superoxide dismutase; TAC, total antioxidant capacity; TOS, total oxidant status; VAS, visual analogue scale.

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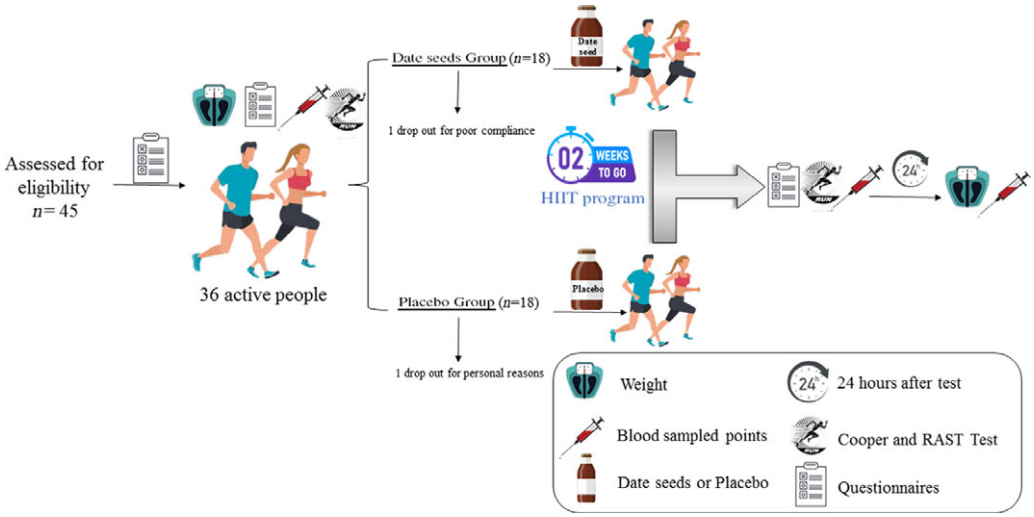

Image 1. Experimental design.

and memory, and learning disorders[5–9]. The safety of date seeds has been reported at a dose of 0·5 g/kg/d[10].

High-intensity interval training (HIIT) is a useful exercise condition that facilitates metabolic adaptations improves muscle mitochondrial capacity, vascular function, maximal oxygen consumption, and alleviates hyperglycaemia, cardiometabolic risk factors, and body fat[11–14]. However, during a HIIT session, exercise-related physiological responses incline muscles to produce more reactive oxygen species (ROS), which results in the development of oxidative stress[15]. Oxidative stress is regarded as an imbalance between the generation and degradation of reactive molecules that results in predomination of pro-oxidants over antioxidants and disruption of redox signalling and molecular damage[16,17]. In HIIT sessions, oxidative stress reduces Ca uptake by the sarcoplasmic reticulum, influences muscle contraction, and consequently, causes an acute decrease in physical performance (namely muscle soreness and fatigue)[18].

Antioxidant supplements can reduce exercise-induced oxidative stress. However, the antioxidants effects on exercise performance can be antagonistic which depends on the dose, type and duration of the antioxidant administration. Several studies have shown that isolated bioactive compounds (e.g. vitamin C, vitamin E and lipoic acid) may have adverse effects on signs and adaptive responses to exercise[19,20]. In contrast, no evidence of such relations following the consumption of antioxidant-rich foods and/or extracts was reported[21,22]. Furthermore, studies suggest that consuming polyphenols during exercise, regardless of the length of the intervention[23], may provide antioxidant protection and thus may minimise the negative physiological responses that occur during and following exercise, like fatigue and muscle pain[24]. A study reported a significant increase in urinary polyphenol metabolites as biomarkers of date seed polyphenol intake for up to 24 h in the urine samples[10]. Limited clinical trials[6,8,10,25] and animal studies[26–31] have reported modulating effects of date seed on metabolic parameters, oxidative stress and inflammation. To our knowledge, no previous study has investigated the impact of date seed powder (DSP) supplementation on oxidative stress and aerobic and anaerobic performance in humans performing HIIT. Therefore, this study aimed to examine the effects of date seeds as a rich source of polyphenol antioxidants on oxidative stress markers, muscle pain, and aerobic and anaerobic performance following HIIT.

Materials and methods

Ethical approval

This research was performed following the principles of the Declaration of Helsinki. After a detailed explanation of the study methodology, all volunteer participants signed an informed consent form at the beginning of the study. The ethics committee at Tabriz University of Medical Sciences approved the study plan (IR.TBZMED.REC.1399·1011), which was then registered on the Iranian Registry of Clinical Trials (https://www.irct.ir/) with the number IRCT20150205020965N9.

Participants

Between October and November 2021, thirty-eight healthy and physically active men and women (recreational runners, Image 1) participated in this study. Their eligibility to participate actively in the study was determined by the Physical Activity Readiness Questionnaire (PAR-Q)[32] under the supervision of a physician. The study protocol followed the Consolidated Standards of Reporting Trials (CONSORT) checklist, and Fig. 1 shows the diagram of the study protocol[33]. The inclusion criteria were as follows: male and female aged between 18 and 35 years; physically active subjects based on a) 3 d of vigorous activity (minimum of 20 min/d), or b) 5 d of any combination of walking, vigorous-intensity or moderate-intensity activities (minimum of 600 MET-min.week^{-1}), or c) 5 d of walking or moderate-intensity exercise (for the minimum of 30 min/d); stable BMI (changes of < 3 kg were acceptable) within the last 5 months; BMI of 18·5–25 kg/m^2; no HIIT in the past 3 months; and willingness of subjects to participate in this study. The

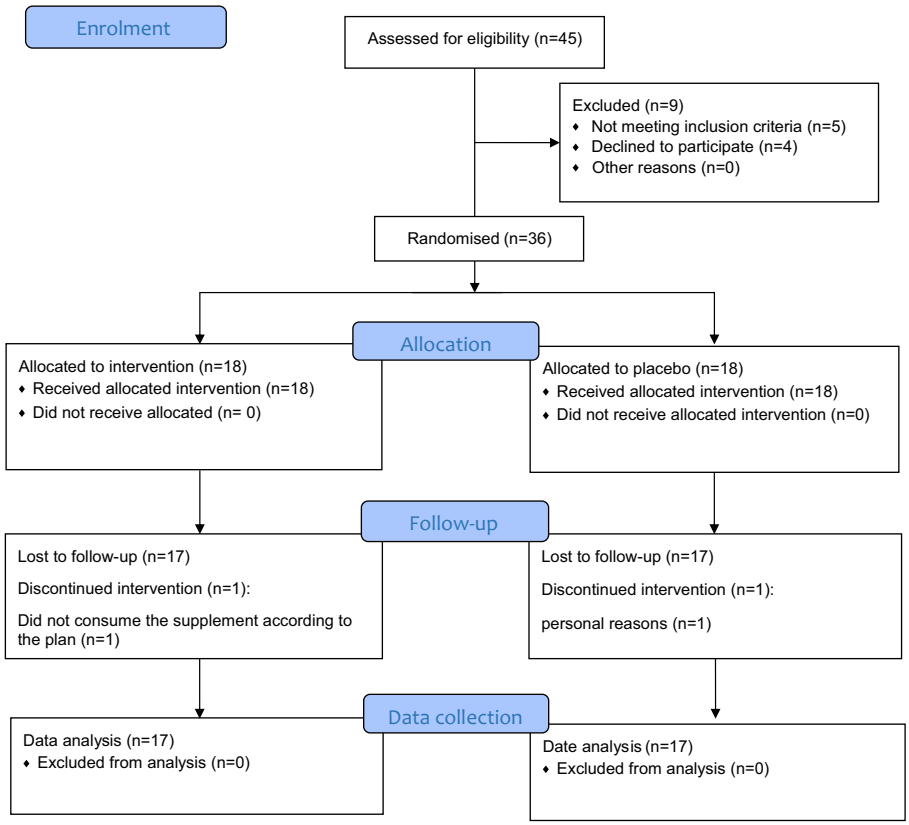

Fig. 1. Flowchart of study.

exclusion criteria were as follows: light-intensity activities; a history of chronic diseases such as CVD, thyroid, gastrointestinal, kidney, diabetic, cancer or pancreatic disease; infectious diseases; cognitive disorders; smoking; pregnancy or lactation; anaemia (Hb < 13 g/dl); musculoskeletal injuries; antacids; taking antibiotics; antidiarrhoeals; anti-inflammatory; antihypertensive, or laxative medications, anabolic steroids, ergogenic agents (arginine, carnitine, creatine and caffeine), or other medications during the previous month; subjects with special diets or dietary restrictions; and recent consumption of antioxidants. Also, during the study, if an individual lost more than 10 % of the supplementation packets and did not attend at least 90 % of each week's training sessions, he/she was considered non-compliant and was consequently excluded from the study.

Sample size

The sample size was estimated based on the changes in the parameter malondialdehyde (MDA) as the main outcome following Platat et al.[10]. Using Stata software (version 16), the sample size in this study was estimated to be at least sixteen subjects for each group, with a power of 90 % and 95 % CI. Based on a 25 % decrease in the level of expected MDA through supplementation and a 10 % dropout rate for each group, the sample size in each group increased to 18.

Randomisation and allocation concealment

After a run-in period, we used the PAR-Q, which is a pre-study screening questionnaire to assess a person's medical history, lifestyle, eating habits and physical fitness in several areas[32]. When the participant answered 'yes' to a question on the questionnaire, he or she was excluded from the study. According to the PAR-Q analysis, thirty-six eligible volunteers from Tabriz stadiums were randomly allocated to the intervention group (DSP, *n* 18) and the placebo group (*n* 18) for 14 d. The randomisation procedure was performed into the two groups (1:1) following stratified randomisation based on sex and VO_2 max. We used random allocation software to perform randomised blocks of sizes 2 and 4. To ensure blinding of the study, a third person allocated the subjects into groups. The main researcher was blinded to the groups of subjects until the end of the analysis.

Intervention

The intervention group received DSP at a dosage of 26 g/d (date seeds, Flavinea Co.) for 14 d according to a pilot study of DSP for 2 weeks in active people (data not reported). The placebo group received the same amount of placebo (wheat bran powder, Nazhvangiah Co.). The powder was divided into two 13-g packages, and it was given to subjects 1 h before HIIT activity and 1 h after HIIT with a cup of water. Both date seed and placebo powder were tasteless, odourless brown powders distributed to participants in identical opaque packages. The powders were delivered to participants weekly for 2 weeks. The main researcher was in daily contact with the subjects via text message to emphasise physical activity maintenance, clarify supplement use problems and ensure compliance. Participants were also given a checklist to tick off after each powder intake to check for non-compliance.

Exercise protocol

Based on the American College of Sports Medicine (ACSM) recommendations for physical activity, a HIIT exercise protocol was established for each participant in both groups[34]. The first phase involved habituation to the intensity of the HIIT programme. After consuming DSP or a placebo, the HIIT sessions in the following phases followed the same pattern. The subjects ate a standardised breakfast 2 h before the HIIT session. The intervention was given to subjects 1 h prior to HIIT activity and 1 h later. Subjects participated in a 2-week HIIT programme (five exercise sessions per week; ten sessions during the study period). Each session began with a 15-min warm-up at 50 % heart rate (HR) reserve (including various stretches, flexibility, walking and running). Both groups' main actives comprised two sessions of three to four repetitions and 30 s of running at 90–100 % of the HR reserve on each repetition. There were 90–180 s of active rest after each repetition and 2·5–4 min of active recovery after each phase, respectively. Also, each session finished with a 5-min cool-down with 45 % HR reserve[35]. This strategy has been demonstrated to be effective in causing oxidative stress in physically active individuals[36].

Also, HR was continuously monitored during the supervised exercise intervention, and participants' HR was recorded using a Polar heart rate (Polar, RS800CX) to ensure training at the intended intensity. The Karvonen formula was used to calculate the target heart rate zone for each participant, and mean target heart rate was reported:

Max HR = 220 – age

Target Heart Rate = [(max HR – resting HR) × % Intensity)] + resting HR[37].

Throughout the trial, the research team kept in touch with individuals daily. A blinded researcher conducted the 17-min HIIT sessions about the consumption of DSP or a placebo. All participants were guided to make their cooperative decisions during high-intensity exercise training. The number of sessions attended is used to assess adherence to the training programme.

Primary and secondary outcomes

Changes in total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), superoxide dismutase (SOD), glutathione peroxidase (GPx), uric acid, MDA, 8-iso-PGF2α and muscle pain are the primary outcomes of the current research. VO_2 max, peak power, average power and fatigue index (FI) were considered the secondary outcomes.

Assessment of dietary intake

A dietary protocol was used to check the intake of macronutrients and antioxidants (vitamin A, vitamin C, vitamin E, α- tocopherol, β-carotene, lycopene, β-cryptoxanthin, Zn and Se) to ensure that they were having their habitual diet and were not consuming additional antioxidants via diet. The regular food and beverage intake was assessed using 3-d food records (two weekdays and one weekend) before starting the HIIT protocol and supplements and at the study's end during the last week[38]. At the beginning of the interview, a dietitian informed the participants about the recording procedure and asked them to record their consumed beverages and foods. The information recorded by the subjects during home measurements was standardised and converted to grams and/or millilitres of the foods and/or drinks. The 'Nutritionist 4' software (First Databank Inc., Hearst Corp.) was used to analyse the nutritional data.

Characterisation of study subjects

Anthropometric parameters (height, weight and BMI) were assessed at the baseline and at the end of the study. A reliable scale (Seca) was used to measure weight while barefoot and wearing light clothing to the nearest 0·5 kg. A centimetre tape with a precision of 0·1 cm was used to measure height with no shoes. The BMI was calculated by the weight (kg) divided by the height (m) squared. Based on the WHO's classifications, BMI has been adopted as a measure of nutritional status[39].

We determined their physical activity level using the International Physical Activity Questionnaire (IPAQ – short version)[40]. The following formula was used to calculate the physical activity rate: moderate activity = (4·0 × moderate activity minutes' × d) and vigorous activity = (8·0 × vigorous activity minutes × d). The cut-off levels were divided into three groups based on the IPAQ scoring protocol's current PA guidelines: 1. Low: some activities were reported but not enough to meet categories 2 or 3, 2. Moderate: 5 or more days of any combination of walking, moderate- or vigorous-intensity activities (at least 600 MET-min.wk^{-1} accumulatively), and 3. High: 7 or more days of any combination of walking, moderate-intensity, or vigorous-intensity activities (at least 3000 MET-min.wk^{-1} accumulatively)[41]. The intensity of pain was quantified using a visual analogue scale (VAS). On a 10-cm ruler, '0' represented the absence of pain and '10' the maximum pain level that active men or women can tolerate[42]. Each participant was instructed to record the VAS at baseline (after the Cooper 12-min run test and running-based anaerobic sprint test), after the intervention, and 24 h later.

Blood samples

Blood samples (10 ml) were collected at three time points: at the beginning, at the end of the study (day 14) and 24 h after that. Blood samples were taken at each time point from the intermediate vein of the forearm into tubes with/without EDTA. Aliquots of plasma were used to analyse TAC and TOS using the colorimetric method (TAC: RANDOX kits; TOS: ZellBio GmbH), and the OSI was calculated using TAC and TOS as follows: OSI = 100 × (TOS/TAC)[43]. The SOD and GPx activities were determined via a commercially available kit (Randox Laboratories Ltd). Uric acid was measured by using the enzymatic method by an autoanalyser using kits (Pars-Azmoon Co.). Serum levels of 8-iso-PGF2α were determined using an ELISA kit (Abcam). The spectrofluorimeter was used to determine MDA levels (Kontron, model SFM 25A)[44].

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Aerobic and anaerobic performance parameters

The Cooper 12-min run test was used to measure aerobic endurance. Participants ran continuously for 12 min in the 400-m running track before and after the intervention period, and the next covered distance was used to determine VO_2 max using Cooper's equation[45]:

VO_2 max (ml/kg/min) = (Distance − 504·9) ÷ 44·73.

We also measured anaerobic endurance prior and subsequent to the intervention using the running-based anaerobic sprint test. The running-based anaerobic sprint test consists of six parallel 35-m sprints separated by a 10-s rest period. Participants completed the running-based anaerobic sprint test, and an electronic timing device automatically recorded the time spent on each attempt. Then, we calculated anaerobic power output using the formula, as well as FI as a measure of performance degradation:

- Power (watts) = (Weight ×Distance^2) ÷ Time^3
- Peak Power (watts) = the highest power (the fastest sprint)
- Average Power (watts) = the sum of all six values ÷ 6
- FI (watts/s) = (Maximum Power − Minimum Power) ÷ Time spent in six sprints[46].

During the orientation session, all participants were reminded of the importance of performing tests and encouraged to give their all. In the 24 h leading up to the aerobic and anaerobic tests, they were also forbidden from engaging in any strenuous physical activity. Before undertaking exercise tests, all subjects were given a warm-up session for 5 min.

Date seed powder chemical characterisation and antioxidants

A specialised company produced the commercial DSP samples (date seeds, Flavinea Co.). The recommended procedures by the Association of Official Analytical Chemicals were used to determine the chemical analysis[47]. The Folin–Ciocalteu colorimetric technique was used to measure the total phenolic content of the samples[48]. Likewise, the aluminium chloride colorimetric method was used to assess flavonoid levels[49]. Table 1 shows the average of the composition and antioxidant properties of the DSP (100 g) that were determined three times.

Statistical analysis

The SPSS program version 24 was used to analyse the data. The Shapiro–Wilks test was used to determine the data's normality. We presented qualitative data as frequency (percent), whereas quantitative data as mean and standard deviation. The independent-samples Student's *t* test was used to compare the quantitative variables between groups according to time. Also, ANCOVA was used to compare after intervention between two groups, adjusting for baseline value.

For oxidative stress biomarkers and VAS parameter, which were assessed three times, a repeated-measures ANOVA test was performed and *P*-value supplement × time in the repeated-measures analysis reflect the effect of the intervention. Statistical significance was defined as a *P*-value of $P < 0{\cdot}05$.

Results

Of thirty-six participants enrolled for trial eligibility, thirty-four subjects completed the study (seventeen in the placebo group and seventeen in the date seed group). One subject was dropped out for poor compliance in the date seed group, and another dropped out for personal reasons in the placebo group. The dropouts did not differ between the two groups, and the participation rate was 94·44 %. No side effects were reported after supplementation with date seeds and placebo. The characteristics of volunteers at baseline were similar in both groups in terms of sex, age, physical activity, HR, BMI and body weight (Table 2), dietary nutritional intake (Table 3), TAC, OSI, SOD, GPx, uric acid, MDA, 8-iso-PGF2*α*, VAS (Table 4), VO_2 max, peak power, average power, and FI (Table 5) ($P > 0{\cdot}05$). No significant differences were observed in weight, BMI and the dietary antioxidant intake, as efficient factors on oxidative stress status between groups at the end of the trial (ANCOVA, $P > 0{\cdot}05$) (Table 2, Table 3). Based on Table 4, there was a significant supplement × time interaction for TAC, TOS, OSI, GPx, MDA and VAS (for all $P < 0{\cdot}05$) and no effect for supplement × time in SOD, uric acid, and 8-iso-PGF2*α*. Furthermore, we observed a significant main effect for supplementing in TAC, TOS, OSI, GPx, MDA, 8-iso-PGF2*α* and VAS (for all $P < 0{\cdot}05$), whereas there were no changes for SOD and uric acid. TAC, TOS, OSI, SOD, GPx, uric acid, MDA, 8-iso-PGF2*α* and VAS all showed a significant main effect for time (all $P \leq 0{\cdot}001$).

Our findings showed statistically significant differences in TAC, TOS, OSI, SOD (24 h), GPx, MDA, 8-iso-PGF2*α* (after) and VAS (24 h) in the date seed group when compared with the placebo. However, there were no differences in SOD (after), uric acid, 8-iso-PGF2*α* (24 h) and VAS (after) in the date seed group in comparison with the placebo group (Table 4). Figure 2 also, illustrates oxidative stress marker concentrations and VAS from pre- to post-intervention and 24 h after HIIT.

In aerobic and anaerobic performance, just peak power and FI showed significant differences between the two groups at the end of the study (ANCOVA, $P < 0{\cdot}05$) (Table 5).

Discussion

The present study investigated the role of DSP on exercise-induced oxidative stress and parameters of performance following HIIT sessions. To our knowledge, this is the first randomised, double-blind, placebo-controlled clinical trial of its kind. Our findings demonstrated that supplementation with DSP for the duration of 2 weeks attenuated oxidative stress and improved exercise performance in men and women performing HIIT bouts.

It has been postulated that exercise, which enhances oxygen consumption considerably, can increase free radicals and oxidative stress[50]. HIIT provokes oxidative stress and lipid peroxidation by increasing NADPH oxidase, xanthine oxidase, phospholipase A2 activity, cytochrome c from the mitochondria and catecholamine oxidation[36,51]. Many adaptations, such as redox signalling cascades and endogenous antioxidant enzyme up-regulation, muscle hypertrophy, glucose uptake by the

Table 1. Chemical composition, total phenolic acid and flavonoid content of date seeds and placebo (100 g) and each supplement package

	Date seeds (*n* 3)		Placebo (*n* 3)			
Name	Mean	SD	Mean	SD	Date seeds package (13 g)	Placebo package (13 g)
Energy (kcal)	270·79	21·65	252·30	19·64	35·20	32·79
Carbohydrate (g)	13·12	2·56	13·15	2·81	1·70	1·70
Protein (g)	6·10	0·82	7·60	0·93	0·79	0·98
Total lipids (g)	5·70	1·03	6·59	1·37	0·74	0·85
Fibre total (g)	66·76	10·43	50·65	11·02	8·67	6·58
Soluble dietary fibre (g)	57·12	11·48	45·67	9·43	7·42	5·93
Insoluble dietary fibre (g)	9·64	2·06	4·98	0·97	1·25	0·64
Ash (g)	1·30	0·11	7·59	1·13	0·16	0·98
Moisture (%)	7·02	1·42	14·42	2·87	0·91	1·87
Antioxidants						
Total phenolic acid (mg GAE/g)	3456·86	522·71	1033·46	231·40	449·39	134·34
Flavonoid content (mg QE/g dry weight)	1624·54	352·12	28·67	5·22	211·19	3·72

GAE, gallic acid equivalent; QE, quercetin equivalent.

Table 2. Characteristics of the study participants

	Date seeds (*n* 17)		Placebo (*n* 17)	
Variables	*n*	%	*n*	%
Age (years)				
Mean	21		23	
SD	2		2	
Range	19–26		18–27	
Sex, *n* (%)				
Male	9	52·9	8	47·1
Female	8	47·1	9	52·9
PAL, *n* (%)				
Moderate (600–3000 MET-min.week^{-1})	11	64·7	10	58·8
Vigorous (>3000 MET-min.week^{-1})	6	35·3	7	41·2
	Mean	SD	Mean	SD
Maximum HR (beats/min)	198	2	196	2
Mean THR (beats/min)	191	10	190	10
Anthropometric indices				
Weight at baseline (kg)	67·80	10·90	67·60	9·00
Weight at the end of trial (kg)	67·40	11·00	67·20	8·80
Height (cm)	171·90	12·45	172·20	12·30
BMI at baseline (kg/m^2)	22·80	1·10	22·75	1·05
BMI at end of trial (kg/m^2)	22·70	1·00	22·60	1·00

PAL, physical activity level, HR, heart rate, THR, target heart rate.

skeletal muscle, and mitochondrial biogenesis, contribute to the attenuation of oxidative stress following HIIT[52]. Nonetheless, to achieve ideal recovery time and to strengthen an impaired antioxidant capacity (that accompanies poor performance), supplementation with an antioxidant-rich source like DSP that protects the body from oxidative damage is necessary. In our study, attenuated oxidative stress response following DSP supplementation was indicated by decreased TOS, OSI, MDA, and increased TAC and GPx in the DSP group that can be due to the antioxidative effects of its polyphenolic compounds like flavonoids and/or its ability to enhance endogenous antioxidants. The decreased OSI in the DSP group is indicative of the beneficial effects of date seed on increasing TAC and decreasing TOS in this group. Serum GPx activity was significantly increased in both groups at the end of the study and 24 h after that; however, the increments were more profound ($P < 0·05$) in the DSP group than the placebo group. GPx is considered a key barrier against ROS as it converts H_2O_2 to H_2O. It is plausible that elevated levels of H_2O_2 following HIIT sessions stimulated GPx production, which is, in fact, the body's response to increased oxidative stress. Moreover, our findings demonstrated a significant reduction in MDA concentration in the DSP group in comparison with the placebo group. In summary, GPx defends cellular membranes against peroxidation by eliminating lipid peroxides; likewise, in our study, reduced MDA levels can be linked to enhanced GPx levels[53]. What is more, uric acid, as the component of plasma's antioxidant capacity and a powerful eliminator of peroxynitrite and peroxyl radicals[54] did no change in this study. In line with our findings, several animal studies[26,29,55,56], human studies[10,25] and a systematic review[27] have reported the beneficial effects of date seed supplementation on the antioxidant defence system under various health

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Table 3. Nutritional intakes of subjects at baseline and at the end of the study (Mean values and standard deviations)

	Baseline		After 2 weeks		Change		
Variables	Mean	SD	Mean	SD	Mean	SD	*P**
Energy (kcal/d)							0·921
Date seeds	2555·50	585·60	2638·50	590·70	83·00	137·90	
Placebo	2599·55	545·60	2680·10	468·85	80·60	139·40	
Protein (g/d)							0·284
Date seeds	141·00	47·45	155·60	35·25	14·60	31·30	
Placebo	139·55	32·90	147·35	24·30	7·80	18·50	
Carbohydrate (g/d)							0·158
Date seeds	321·40	75·00	336·80	75·40	15·35	23·30	
Placebo	334·45	86·60	364·80	88·40	30·35	40·25	
Fat (g/d)							0·959
Date seeds	84·00	23·20	79·30	29·60	−4·70	16·75	
Placebo	84·10	23·35	79·10	16·70	−5·10	12·40	
Vitamin A (RAE/d)							0·668
Date seeds	436·20	159·70	472·70	91·80	36·50	165·20	
Placebo	487·00	93·20	470·20	83·95	−16·80	91·60	
Vitamin C (mg/d)							0·215
Date seeds	163·05	26·50	180·40	32·60	13	30·70	
Placebo	153·80	27·10	166·80	25·50	17·30	45·30	
Vitamin E (mg/d)							0·561
Date seeds	18·05	3·80	19·25	5·15	1·20	6·70	
Placebo group	18·50	4·50	18·30	3·30	−0·20	6·50	
α-tocopherol (mg/d)							0·253
Date seeds	25·75	3·10	26·50	4·60	0·80	4·80	
Placebo	25·65	3·05	24·80	3·80	−0·90	5·10	
β-Carotene (mg/d)							0·733
Date seeds	533·90	90·20	532·40	117·90	−1·40	157·10	
Placebo	556·55	115·40	516·60	132·90	−39·90	171·90	
Lycopene (μg/d)							0·118
Date seeds	1318·55	371·90	1237·20	252·30	−81·35	501·20	
Placebo	1300·40	247·90	1373·00	230·60	72·60	337·40	
β-Cryptoxanthin (μg/d)							0·196
Date seeds	120·25	18·90	127·95	17·20	7·70	26·70	
Placebo	114·00	19·30	118·80	23·50	4·74	31·10	
Zn (mg/d)							0·739
Date seeds	17·10	4·40	14·30	3·20	−2·80	5·30	
Placebo	16·05	2·80	14·60	2·20	−1·40	3·60	
Se (mg/d)							0·477
Date seeds	64·10	7·85	62·00	14·20	−2·20	15·90	
Placebo	64·70	8·60	65·20	11·55	0·50	15·00	

* $P < 0·05$, ANCOVA for comparison of data after 2 weeks between groups after adjusting for baseline values.

conditions. In a study, supplementation of Wistar rats with date seeds (0·75 g/kg, for 7 d) was shown to significantly increase SOD and GPx levels[56]. Moreover, Hasan et al. observed significant changes in MDA and SOD levels following 10 ml of aqueous date seed extract/d supplementation in diabetic Wistar rats at the end of 8 weeks[29]. Following 13 weeks of treatment with a diet comprising 2, 4 or 8 g/kg date seeds, MDA levels attenuated dose-dependently in male Wistar rats[26]. In a study by Saryono et al., date seed supplementation (2·5 g/d for 2 weeks) in postmenopausal women significantly improved MDA, SOD and GPx enzyme activities[25]. Platat et al. reported that administration of 0·25 g and 0·5 g date seeds/kg acute dose reduced MDA in healthy participants dose-dependently[10]. The differences in genotype, the dosage and kind of supplementation, basal oxidative stress status, supplementation duration, and difference in study design explicate the differences in findings.

Date seeds are rich sources of polyphenols[26,55,56], and their polyphenols, particularly their flavonoids, can be responsible for attenuation of the exercise-induced oxidative stress observed in the present study. Although the exact mechanisms through which date seed polyphenols modulate oxidative stress were mainly unexplored, some of the proposed mechanisms can be as follows: scavenging free radicals through chelating and/or reducing metal ions by the OH groups attached to the aromatic ring of polyphenol[57], enhancing the expression of nuclear transcription factor-erythroid 2-related factor 2 as a major transcriptional regulator of antioxidant enzymes such as SOD, CAT, and GPx[58], impeding the NF-κβ cascade pathway at different steps as inducer of the expression of the target genes including IL-6, IL-2, IL-8, cyclo-oxygenase-2, and inducible nitric oxide synthase[59], activating sirtuins 1 to suppresses pro-apoptotic factors and proinflammatory factors by downregulating p53 and NF-κB[60], and modulating metabolic endotoxemia involved in oxidative stress and MAPK–NF-κβ cascade pathway[61]. High oxygen uptake during exercise stimulates ROS production[62]. While ROS can support the recovery process[63,64], it has been demonstrated that in the state of impaired antioxidant

Table 4. Markers of oxidative stress and visual analogue scale status of subjects at baseline, the end of the study and 24 h after intervention (Mean values and standard deviations)

	Baseline		After 2 weeks		24 h		
Variables	Mean	SD	Mean	SD	Mean	SD	*P* supplement × time*
TAC (mmol/l)							< 0·001
Date seeds	1·32	0·04	1·56	0·06	1·65	0·05	
Placebo	1·30	0·05	1·41	0·05	1·37	0·05	
P†	0·222		< 0·001		< 0·001		
TOS (μmol/l)							0·019
Date seeds	11·87	0·64	12·90	0·85	12·01	0·24	
Placebo	12·39	0·56	13·95	0·52	13·24	0·05	
P†	0·020		< 0·001		< 0·001		
OSI							< 0·001
Date seeds	0·89	0·07	0·82	0·06	0·72	0·03	
Placebo	0·95	0·08	0·98	0·06	0·96	0·04	
P†	0·048		< 0·001		< 0·001		
SOD (U/ml)							0·085
Date seeds	0·17	0·02	0·24	0·04	0·23	0·04	
Placebo	0·18	0·02	0·22	0·05	0·20	0·04	
P†	0·066		0·411		0·049		
GPx (U/ml)							0·001
Date seeds	4·09	0·36	6·16	0·88	5·64	1·25	
Placebo Group	4·36	0·57	5·14	0·59	4·82	0·58	
P†	0·113		0·001		0·019		
Uric acid (mg/dl)							0·748
Date seeds	4·17	0·41	5·03	0·62	4·31	0·55	
Placebo group	4·21	0·40	5·20	0·91	4·58	0·61	
P†	0·785		0·546		0·180		
MDA (nmol/ml)							0·046
Date seeds	2·05	0·12	2·19	0·26	2·12	0·28	
Placebo group	2·03	0·24	2·42	0·31	2·39	0·32	
P†	0·754		0·027		0·015		
8-iso-PGF2α (pg/ml)							0·518
Date seeds	26·81	2·56	43·92	2·29	35·13	6·12	
Placebo group	27·11	3·41	46·97	5·21	36·82	6·18	
P†	0·777		0·034		0·431		
VAS							< 0·001
Date seeds	6·35	1·36	5·06	1·14	3·35	1·05	
Placebo group	6·41	1·46	5·82	1·23	5·00	1·11	
P†	0·904		0·070		< 0·001		

TAC, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index, SOD, superoxide dismutase; GPx, glutathione peroxidase; MDA, malondialdehyde; VAS, visual analogue scale.
* $P < 0·05$, repeated-measures ANOVA and *P*-value supplement × time reflect the effect of the intervention.
† $P < 0·05$, independent-samples Student's *t* test for comparison of data between groups at different times of baseline, after 2 weeks and 24 h later.

capacity[65], excessive ROS can damage muscles, thereby decreasing exercise performance[66,67]. Theoretically, supplementation with antioxidants would reinforce the antioxidant system of the body, would decrease oxidative stress-induced damage, and consequently would improve performance. Consistently, in the current study, we investigated the effect of DSP supplementation on the markers of performance following HIIT bouts. Our findings indicate that DSP supplementation can significantly increase peak power and decrease pain, which implies enhanced performance following DSP supplementation. Decreased pain and improved power and performance following date seed supplementation can be related to its role in speeding up the recovery from ROS generation.

We also found that DSP can lessen fatigue, as indicated by decreased FI. The positive effect of DSP on exercise-induced fatigue can be related to the ability of its antioxidant content (i.e. polyphenols) to neutralise the reactive species produced by HIIT bouts. In a systematic review and meta-analysis by Blake et al., it was reported that foods rich in polyphenols improve endurance exercise performance in subjects[68]. Morgan et al. found that daily consumption of 330 ml of cacao juice (containing high flavanols levels) in recreationally active males for 8 d significantly decreased VAS[69]. Roberts et al. reported that decaffeinated green tea extract supplementation as a flavanols polyphenol source (571 mg/d for 4 weeks) alongside cycle exercise in recreationally active males improved average power[70]. Jo´wko et al. showed non-significant changes in peak power, average power and FI in subjects with cycle sprint following green tea extract (245 mg/d for 4 weeks)[71]. In another study, da Silva et al. reported that green tea supplementation (500 mg/d for 15 d) reduced muscle damage, but not muscle pain (VAS) in non-trained male subjects[72]. A double-blind, randomised clinical trial indicated that epicatechin supplementation (200 mg daily for 4 weeks) resulted in significant VO_2 max, average power, peak power and FI changes in recreationally active men and women[73]. The different findings may be related to various types of polyphenol, the dosage of supplements, metabolic and psychological characteristics of study participants, the use of different exercise protocols, and fitness levels. It is noteworthy that our analysis on the intake of other dietary antioxidants, like vitamins A, C, E and

Table 5. Changes in performance markers of subjects at baseline and the end of the study (Mean values and standard deviations)

Variables	Baseline		After 2 weeks		Change		*P**
	Mean	SD	Mean	SD	Mean	SD	
VO_2 max (ml/kg/min)							0·301
Date seeds	46·40	3·49	49·09	2·34	2·69	4·87	
Placebo	45·06	3·99	47·60	3·55	2·54	1·45	
P†	0·308		0·160		–		
Average power (W)							0·070
Date seeds	456·91	24·59	497·41	17·31	40·50	26·56	
Placebo	460·13	30·26	481·49	36·02	21·35	35·16	
P†	0·735		0·110		–		
Peak power (W)							< 0·001
Date seeds	654·48	25·24	693·21	27·73	38·72	15·12	
Placebo	665·96	41·05	682·58	37·58	16·61	13·58	
P†	0·333		0·355		–		
Fatigue index (W/s)							0·006
Date seeds	45·27	8·72	37·20	5·54	–8·07	11·99	
Placebo group	49·75	9·75	42·93	5·76	–6·81	10·33	
P†	0·168		0·006		–		

W, watts; W/s, watts/seconds.
* $P < 0·05$, ANCOVA for comparison of data after 2 weeks between groups and adjusting for baseline values.
† $P < 0·05$, independent-samples Student's *t* test for comparison of data between groups at different times of baseline and after 2 weeks.

α-tocopherol, lycopene, *β*-carotene, *β*-cryptoxanthin, Zn, and Se, did not show any significant difference between the two groups either at the baseline or at the end of the study, which eliminates any probable intervening effects of dietary antioxidants and endorses the pivotal role of date seed on the amelioration of oxidative stress in individuals performing intensive activities.

HIIT sessions may decline aerobic and anaerobic performance via several mechanisms like changing in stretch fibres, collapsing membrane surrounding the sarcoplasmic reticulum and muscle fibres, damaging excitation–contraction coupling, stimulation of proteolytic enzymes, activating inflammatory response, which can result in muscle edema and pain, and exacerbated muscle function[74,75]. However, improved oxidative stress and inflammation have been mentioned to decrease the amount of pain and improve exercise performance following HIIT sessions. Other non-oxidative mechanisms can be involved in the performance improvement following date seed supplementation in the present study. For instance, in vitro studies show that polyphenols can act as an adenosine A1-receptor antagonist and present analgesic effects that result in the reduction of effort perception or muscle aches and pains during exercise[76]. Furthermore, it was previously found that polyphenols can reduce the conversion of nitric oxide to peroxynitrite, which probably contributed to increased vasodilation response, improved muscle perfusion and increased oxygenation by increasing nitric oxide bioavailability[77]. While these can explain the favourable effects of date seeds on aerobic and anaerobic performance, further research is required to investigate mechanisms that link polyphenol date seeds with increased exercise performance.

Strengths and limitations

This study's strengths include its double-blind, placebo-controlled, randomised clinical design, as well as stratification by sex and VO_2 max variables, which eliminate inter-individual variance and the participation of volunteers. Another strength of the present study was its novelty, as it is the first clinical experiment that exploited date seed (which could be a waste product) to determine the effects of its supplementation on recreational runners. Application of date seeds as alternative sources for functional foods can minimise the cost of waste management as well as the waste of these valuable by-products. The study's limitations include using a fixed supplement dose, a short duration and the lack of assessment of the level of polyphenols or flavonoid content, glycemic indices, other hormones, and inflammatory biomarkers. This is knowing that longer-term studies testing DSP, its polyphenols extract, or by-products such as bread or chocolate should be conducted to confirm our findings. In addition, the sample size was calculated based on the decrease in MDA, with 90 % power and 95 % CI. The power achieved for this particular variable was sufficient to provide significant results. However, it appears that a larger sample size and higher power are required to achieve statistical significance for some other variables.

Conclusion

The present double-blind, placebo-controlled clinical trial revealed that date seed supplementation can improve exercise-induced oxidative stress and performance parameters in healthy active subjects. Its findings provided new insights into date seed consumption. Further studies that investigate the effects of DSP in different doses, with longer intervention periods, and with other exercises are needed.

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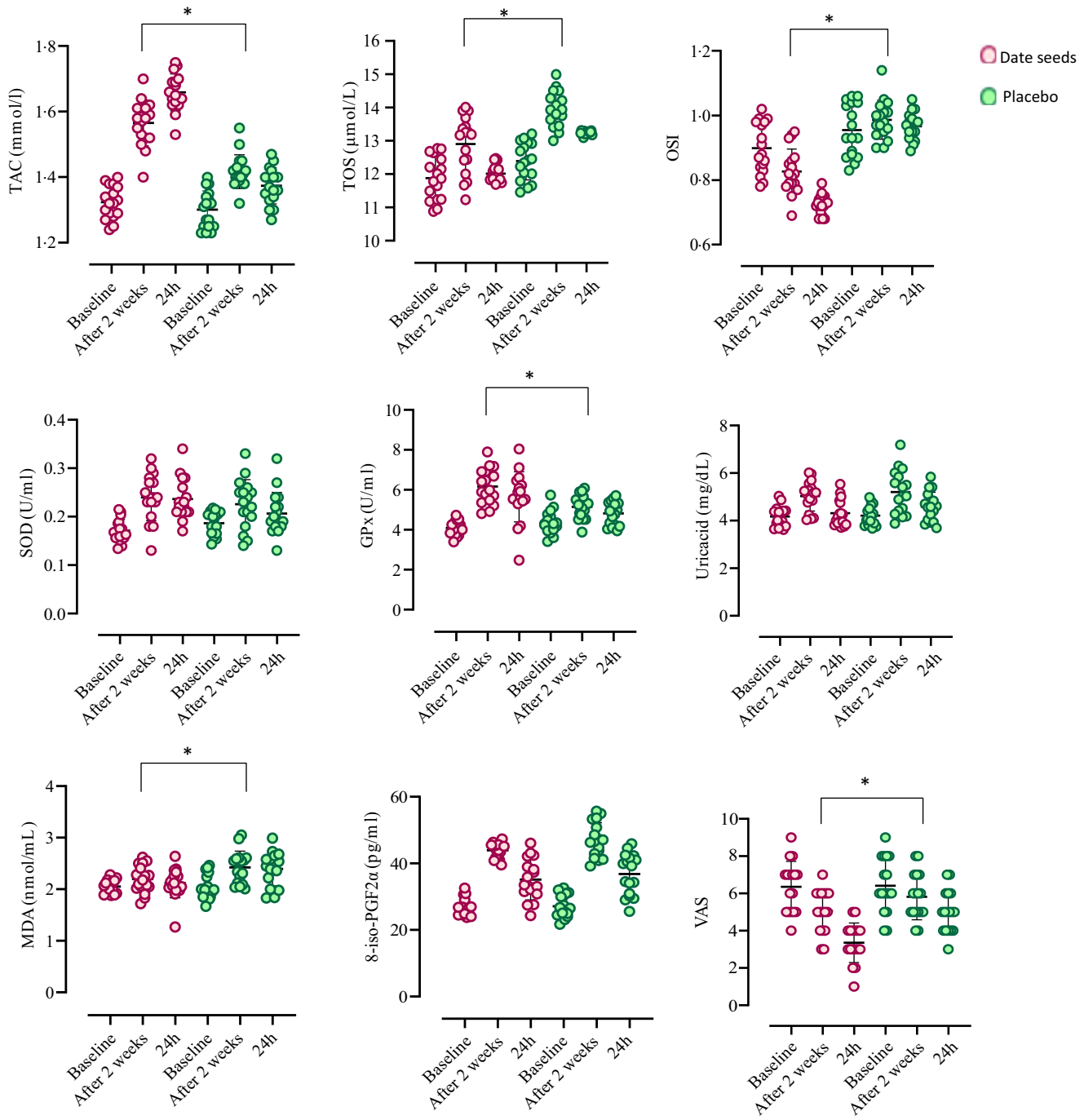

Fig. 2. Concentrations of oxidative stress and muscle pain markers from pre- to post-intervention and after that. TAC, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index; SOD, superoxide dismutase; GPx, glutathione peroxidase; MDA, malondialdehyde; VAS, visual analogue scale. Data are presented as mean ± SD. Error bars represent the standard deviation of the mean. *$P < 0{\cdot}05$, repeated-measures ANOVA.

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