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Assessment of *Opuntia ficus-indica* supplementation on enhancing antioxidant levels

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Opuntia ficus-indica (OFi) **is a major fruit source prevalent in semiarid and arid regions across various countries worldwide. It is widely recognised for its potential health benefits; however, most studies investigating its effects have been limited to pre-clinical models, highlighting the need for further validation through clinical trials. This study aimed to evaluate the effectiveness of OFi supplementation in enhancing antioxidant levels. Fifty healthy participants, aged 18 years and older, including males and females, received a daily OFi supplement of 1500 mg for 3 months. These findings revealed a significant 48.1% increase in salivary total antioxidant capacity (TAC) ($P < 0.001$), indicating improved antioxidant activity. Simultaneously, oxidative stress biomarkers showed substantial reductions: malondialdehyde (MDA) decreased by 28.3%, nitrotyrosine (3-NT) decreased by 51.5%, and 8-hydroxy-2′-deoxyguanosine (8-OHdG) decreased by 59.8% ($P < 0.001$). Furthermore, participants reported a 20.1% improvement ($P < 0.001$) in Visual Analogue Scales (VAS), reflecting a notable enhancement in overall well-being. In conclusion, OFi exhibited promising efficacy in elevating antioxidant levels and mitigating oxidative stress. These findings suggest its potential as an adjuvant therapy for managing chronic conditions associated with oxidative stress.**

Keywords *Opuntia ficus-indica*, Supplement, Antioxidant, Reactive oxygen species (ROS), Clinical trial

Opuntia ficus-indica (OFi), known as nopal cactus or prickly pear, is a well-established source of natural antioxidants and is classified within the Cactaceae family (Fig. 1). Traditionally, the plant and its derivatives have been used to prepare various foods and beverages, such as jams, jellies, and syrups. Beyond its culinary applications, OFi is also renowned in traditional herbal medicine for its diverse medical properties[1,2]. The stems of OFi have been utilised as natural remedies for various chronic conditions, including diabetes[3–5], hypercholesterolemia[3–5], hypertension[5] and pulmonary diseases such as asthma[2], all of which are linked to oxidative stress[6,7]. OFi is predominantly native to Latin America, particularly Mexico[8]. Phytochemical analyses of OFi extracts reveal high concentrations of bio-reactive compounds, including polyphenols, vitamins C and E, β-carotene, glutathione, and a combination of betaxanthin and betacyanin pigments. These compounds act as potent antioxidants, effectively combatting free radicals and mitigating oxidative stress[9].

Reactive oxygen species (ROS) are a common by-product of cellular metabolism and are required in trace amounts for essential cellular functions, including roles in immune responses[10,11]. However, overproduction and accumulation of free radicals can result in cellular damage. Excessive free radical production is triggered by both endogenous factors, such as mental stress[10] and exogenous factors, including heavy metal exposure[10], radiation[10], strenuous exercises[12], smoking[12], and diets high in fat[13–15] and carbohydrates[13,15,16]. Additionally, specific anti-cancer, anti-inflammatory and psychiatric medications have been associated with the induction of free radical production[10,17]. Lipid peroxidation, a process triggered by oxidative stress, plays a dual role as both a contributor to and a driver of the progression of various pathological conditions. For instance, oxidative stress and lipid peroxidation have been implicated in the pathogenesis of atherosclerosis[18], asthma[18], Parkinson's disease (PD)[18], Alzheimer's disease (AD)[17,19], cancer[17,20], diabetes[17,21], ageing and chronic inflammation[9,22]. Malondialdehyde (MDA), a by-product of lipid peroxidation, is widely utilised as a biomarker for assessing oxidative stress[10]. Moreover, other lipid peroxidation derivatives, such as epoxides and aldehydes, can initiate cascades of deleterious effects on proteins, DNA and other macromolecules, amplifying cellular damage[17].

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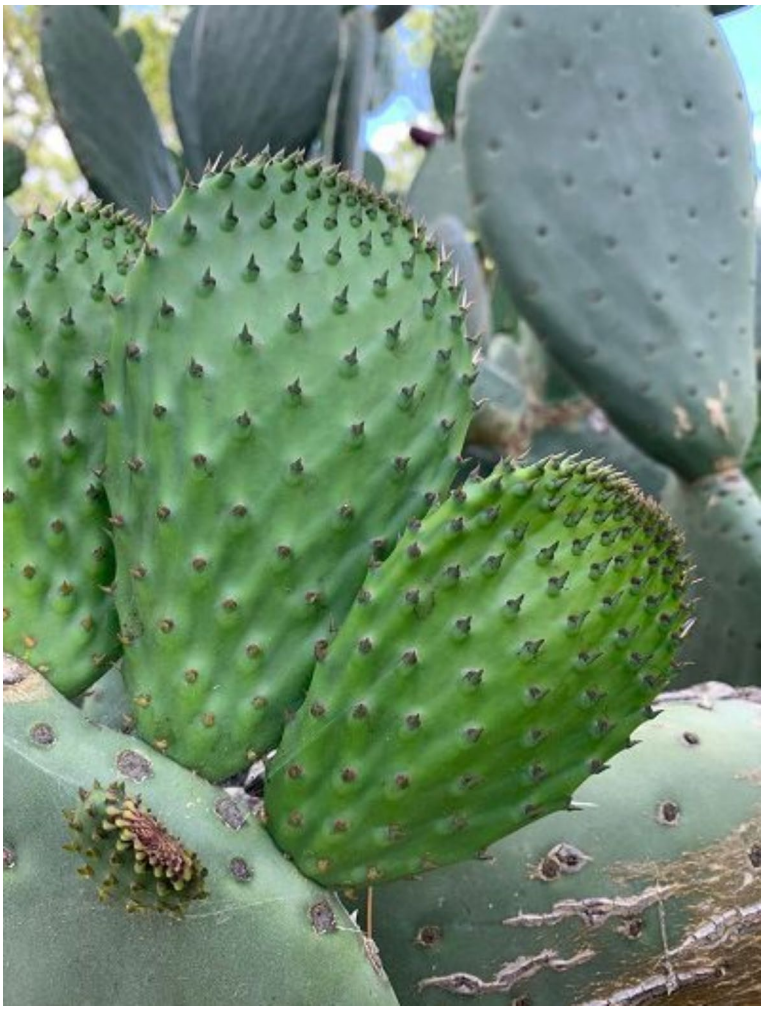

Fig. 1. The original *Opuntia ficus-indica.*

Protein oxidative damage can be evaluated by detecting 3-nitrotyrosine (3-NT), formed through the nitration of free or protein-bound tyrosine residues by peroxynitrite[23,24]. Such protein damage plays a pivotal role in ageing and the progression of age-related diseases, as the loss of native protein structure and function leads to cytoskeleton injury, potentially leading to neurodegeneration[23,24]. The accumulation of protein aggregates or fibrils in extracellular compartments, a hallmark of neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's, arises from abnormal post-translational modifications triggered by lipid peroxidation by-products[24,25]. Oxidative stress can also damage nucleic acids significantly, contributing to cellular dysfunction and disease pathogenesis[23]. One of the primary oxidative stress-induced lesions, 8-hydroxy-2′-deoxyguanosine (8-OHdG), arises from nuclear and mitochondrial DNA degradation. As a prominent biomarker, 8-OHdG is frequently associated with cancer and various degenerative diseases, reflecting the extent of oxidative damage in tissues[26].

Prevention and therapeutic management of health conditions associated with oxidative stress can be achieved by restoring the body's redox balance[22]. Both endogenous and exogenous antioxidants play a crucial role in maintaining redox homeostasis. For instance, superoxide dismutase functions as an endogenous antioxidant, while exogenous antioxidants include vitamins, minerals, polyphenols and flavonoids[27]. Most dietary antioxidant compounds are derived from plant sources[27,28], which has led to considerable interest in their potential for nutraceutical development. Several in vitro and in vivo studies have identified OFi as a plant rich in antioxidants[9,29–31]. The antioxidant properties of OFi are primarily attributed to its high contents of flavonoids, tannins, phenols and saponins[4,5]. Over 25 phenolic compounds have been identified in OFi[5], with notable concentrations of gallic acid in OFi flowers, which have been shown to mitigate DNA damage[6,7]. OFi exhibits scavenging activity against various radical species, including hydrogen peroxide (H_2O_2), hydroxyl radicals (·OH), singlet oxygen ($^1O_2$) and superoxide (O^-_2). However, the sensitivity and efficacy of OFi's antioxidant activity may vary across different species, and the extraction methods may also influence these properties to some degree[28,32,33].

The bioactive compounds in OFi contribute to its antioxidant activity through several mechanisms, including reducing lipid peroxidation, scavenger radicals and enhancing glutathione (GSH) levels[2]. In various in vitro

studies, OFi extracts have been shown to influence endogenous enzymes, such as catalase and superoxide dismutase[8,34,35].

Despite the increasing popularity and growing consumption of OFi products, the full extent of their health benefits and potential side effects remains inadequately understood due to limited clinical research. Existing studies are often characterised by short durations, insufficient follow-up periods, and small sample sizes[36]. The present study aims to evaluate the long-term health benefits of OFi supplementation in improving antioxidant levels through comprehensive clinical assessments. Specifically, this research seeks to determine the efficacy of OFi as an adjuvant therapy in managing chronic conditions associated with oxidative stress.

Results

Characteristics of participant

Fifty (50) healthy participants were recruited for this study, with no dropouts during the follow-up visits (Fig. 2). Most participants were above 40 years old (n = 28, 56%, 46.64 ± 3.72), with the cohort's mean age being 40.76 ± 8.42 years (mean ± SD). The participants consisted of 68% females (n = 34) and 32% males (n = 16). Most participants were categorised as having normal weight (n = 30, 60%), followed by overweight (n = 9, 18%), with underweight and obese participants compromising 12% and 10%, respectively. The overall mean of the participant's BMI was 23.86 ± 5.07 (Table 1). All participants' blood pressure and heart rate were monitored throughout the study, revealing no significant changes (Table 2). Both vital signs exhibited minimal variations across visits. No adverse events were reported during this study.

Changes in antioxidant status

Our results demonstrated a significant increase in total antioxidant capacity ($P < 0.001$), increasing from 2.720 ± 0.328 mmol/L during baseline to 4.028 ± 0.401 mmol/L at the final follow-up visit, reflecting a 48.1% improvement. Additionally, MDA levels were significantly reduced ($P < 0.001$), decreasing from 18.713 ± 2.051 to 13.441 ± 1.526 nmol/L, corresponding to a 28.2% reduction. Likewise, 3-NT levels significantly reduced ($P < 0.001$) from 1.477 ± 0.594 to 0.722 ± 0.263 ng/mL, corresponding to a 51.1% reduction. 8-OHdG content recorded a significant reduction ($P < 0.001$), decreasing from 0.702 ± 0.150 to 0.282 ± 0.098 ng/mL, corresponding to a 59.8% reduction (Table 3). When demographic factors were analysed as between-subject effects, no significant differences in responsiveness to the supplementation were observed.

General wellbeing

Visual analogue scale (VAS) scores improved significantly ($P < 0.001$), increasing from 6.43 ± 1.46 points to 7.72 ± 1.07 points, reflecting a 20.1% improvement. No significant differences were observed when age, gender and BMI were tested as the between-subject factors (Table 4).

Discussion

Oxidative stress is a key contributor to many age-related and chronic non-communicable diseases[37,38]. It arises when the body's antioxidant defence mechanisms cannot effectively neutralise ROS, leading to cellular damage[39]. Consequently, it has been hypothesised that restoring or maintaining redox homeostasis through dietary antioxidant supplementation could mitigate oxidative stress and its associated pathophysiological effects[40]. Natural antioxidants, particularly those derived from plant-based sources, are often preferred due to their perceived safety profile and potential health benefits[27]. However, several clinical trials have shown that many antioxidant-rich natural products achieve the anticipated therapeutic outcomes[41]. These phytochemicals are typically embedded in complex biological matrices, which may hinder their bioavailability due to low solubility or inefficiency in reaching target tissues[41]. Additionally, discrepancies between in vivo and in vitro studies may stem from the low dosages used in human trials, which might be insufficient to replicate the therapeutic effects observed in cell culture models[37]. Poor bioavailability remains a significant barrier to the clinical efficacy of many natural antioxidants, limiting their ability to reach effective concentrations at target sites[42]. Therefore, well-designed clinical trials are essential to confirm the true health benefits of antioxidant-rich supplements. In this regard, the present study evaluated the clinical efficacy of OFi, a widely recognised natural antioxidant-rich supplement. The results revealed a statistically significant improvement ($P < 0.001$) in overall health, suggesting potential benefits in maintaining oxidative balance and promoting general well-being. Nonetheless, it is essential to acknowledge that salivary biomarkers may be influenced by underlying health conditions, oral health status, medication use, and smoking habits, which could introduce potential confounding effects on the study outcomes.

The results of our study revealed a significant 48.1% increase in TAC, which is consistent with the anticipated effects of polyphenol-rich species such as OFi[43]. Polyphenols can mitigate cellular damage by downregulating the expression of specific proteins and inhibiting the transcription of mRNA, genes or signalling pathways[44–47]. Natural polyphenols can also modulate the enzymatic activity of Sirtuins (SIRT), a class III histone deacetylase crucial in regulating inflammation, metabolic disorders and ageing. The modulation of SIRT enzymatic activity highlights the therapeutic potential of polyphenol-rich products in managing oxidative damage and promoting cellular health[48,49]. SIRT1 activates manganese superoxide dismutase (MnSOD) through the forkhead box O (FOXO) pathway, enhancing the cell's antioxidant capacity to combat oxidative stress[50]. The FOXO signalling pathway is vital for various cellular physiological processes, including apoptosis, cell cycle regulation, glucose metabolism, oxidative stress resistance, and the promotion of longevity. Wang et al. (2015) reported that plant-derived polyphenols induce apoptosis in HepG2 cells by inhibiting the MEK/ERK signalling pathway, which in turn blocks the PI3K/PKB/mTOR pathway[51,52]. PI3K/PKB/mTOR pathway regulates the cell cycle, and its overactivation during cancer initiation and progression reduces apoptosis while promoting cell proliferation. This pathway has emerged as a significant therapeutic target for various malignancies. Most polyphenols exert

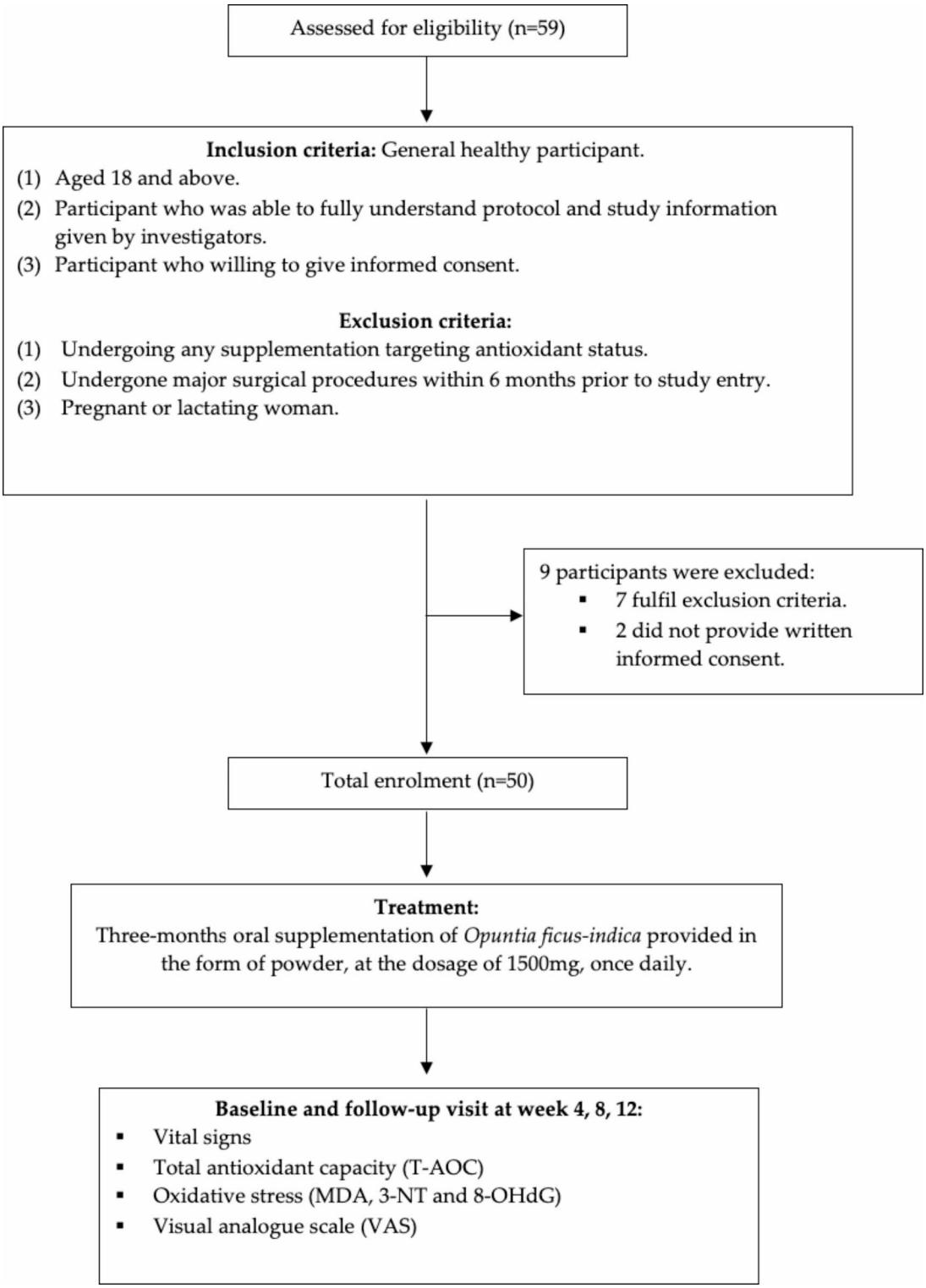

Fig. 2. Consort protocol for the study described with flow diagram.

their effects by targeting multiple pathways to reduce oxidative stress[52]. Therefore, further research is needed to elucidate the precise molecular targets and potential synergistic effects of polyphenols and non-polyphenolic compounds present in OFi.

While TAC is a valuable indicator of overall antioxidant activity in the body, it does not directly correlate with specific pathological conditions. Three additional oxidative stress biomarkers were measured for a more comprehensive assessment of oxidative stress concerning OFi supplementation: MDA, 3-NT and 8-OHdG. These markers are well-established in clinical assessments, aiding in the diagnosis, prognosis and monitoring of various

Characteristic	Frequency (n/%)	Mean ± SD
Gender		
Male	16 (32.0)	
Female	34 (68.0)	
Age (years)		
≤ 30	6 (12.0)	40.76 ± 8.42
31–40	16 (32.0)	
> 40	28 (56.0)	
Body Mass Index (BMI)		
Underweight (< 18.5)	6 (12.0)	23.86 ± 5.07
Normal Weight (18.5–24.9)	30 (60.0)	
Overweight (25–29.9)	9 (18.0)	
Obese (≥ 30)	5 (10.0)	

Table 1. Characteristics of participant.

Vital sign	Baseline	First follow-up	Second follow-up	Third follow-up	*P*-value
Blood pressure (mmHg)					
Systolic	118.62 ± 18.77	116.58 ± 13.56	118.08 ± 13.26	118.98 ± 11.92	0.298
Diastolic	79.62 ± 11.10	77.98 ± 10.95	78.50 ± 10.13	77.02 ± 9.24	0.278
Heart rate (BPM)	80.40 ± 10.93	81.30 ± 10.00	79.52 ± 8.06	77.54 ± 7.93	0.079

Table 2. Changes in vital sign of participant during study, values were expressed as means ± SD.

Parameters	Baseline	First follow-up	Second follow-up	Third follow-up	*p*-value
Antioxidant status					
TAC (mmol/L)	2.720 ± 0.328	2.925 ± 0.426	3.517 ± 0.458	4.028 ± 0.401	< 0.001*
MDA (nmol/L)	18.713 ± 2.051	16.352 ± 3.860	15.135 ± 2.248	13.441 ± 1.526	< 0.001*
3-NT (ng/mL)	1.477 ± 0.594	1.301 ± 0.436	1.076 ± 0.418	0.722 ± 0.263	< 0.001*
8-OHdG (ng/mL)	0.702 ± 0.150	0.487 ± 0.121	0.403 ± 0.115	0.282 ± 0.098	< 0.001*

Table 3. Changes in the participants' antioxidant status, values were expressed as mean ± SD. *TAC* Total Antioxidant Capacity; *MDA* Malondialdehyde; *3-NT* 3-Nitrotyrosine; *8-OHdG* 8-hydroxy-2-deoxyguanosine. Statistically significant *p* values are marked in asterisks (*). *p*-value was calculated using general linear model (GLM) for repeated measures model, with sampling time point as the within-subjects factor.

Parameters	Baseline	First follow-up	Second follow-up	Third follow-up	*P*-value
Visual analogue scale (VAS)	6.43 ± 1.46	7.00 ± 1.28	7.28 ± 1.14	7.72 ± 1.07	< 0.001*

Table 4. Changes in the participants' self-perceived general well-being, values were expressed as mean ± SD. Statistically significant *p* values are marked in asterisks (*). *p*-value was calculated using a general linear model (GLM) for repeated measures model, with sampling time point as the within-subjects factor.

conditions associated with oxidative stress[24,53]. Membrane lipids and lipoproteins are particularly susceptible to oxidative damage by free radicals, making MDA a primary end-product of lipid peroxidation[54]. MDA is widely recognised as a biomarker for oxidative stress, particularly in conditions such as chronic inflammation[55], asthma[55], diabetes mellitus[23], atherosclerosis[23] and various cancers[14,23,55]. In this study, we observed a significant 28.3% reduction in MDA levels following 3 months of OFi supplementation ($P < 0.001$). The decrease in lipid peroxidation is likely due to the synergistic effects of phenolic compounds, ascorbic acid, and betalains, which are the major bioactive compounds in OFi[56,57]. Phenolic compounds are well-documented for their potent inhibitory action against lipid peroxidation, while ascorbic acid and betalains act as radical scavengers, further suppressing oxidative damage to lipids[14,58]. Supporting these findings, Butera et al. (2002) reported that the white and red varieties of OFi fruits are highly effective in inhibiting lipid oxidation[9,33]. Additionally, a separate study demonstrated that OFi supplementation effectively reduced free radical activity induced by strenuous exercise[59]. Regular OFi supplementation reduces oxidative stress and lowers total and LDL-cholesterol levels, with minimal effects on HDL-cholesterol levels[59].

3-NT is another well-established biomarker of oxidative stress[14,60,61], which demonstrated a significant decrease of 51.5% ($P<0.001$) after 3 months of OFi supplementation in this study. This reduction in 3-NT levels correlates with the concomitant increase in TAC. Among the various amino acids comprising proteins, tyrosine is particularly vulnerable to oxidative damage, especially from peroxynitrite, leading to the formation of 3-NT[62]. Previous studies have shown that natural polyphenols can effectively suppress 3-NT formation in vitro by inhibiting peroxynitrite-dependent nitration of proteins[52,63,64]. Moreover, dose-dependent reductions in 3-NT levels have been observed in the midbrain and striatum of rats with Parkinson's disease[52,65]. In alignment with these findings, our study's significant reduction of 3-NT highlights the neuroprotective potential of OFi supplementation, particularly associated with neurodegenerative disorders, including Alzheimer's and Parkinson's diseases[24]. Additionally, Dok-Go et al. reported that quercetin, (1)-dihydroquercetin and quercetin 3-methyl ether, extracted from OFi, effectively mitigated neuronal damage caused by oxidative stress in primary cortical cell culture[66,67]. The exceptional adaptability of 3-NT as a biomarker further enhances its utility in the early diagnosis and monitoring neurodegenerative diseases[24,68]. The observed suppression of 3-NT levels following OFi supplementation suggests that it is potentially effective in early prevention and slowing down the progression of neurodegenerative conditions affecting the brain and central nervous system.

The last biomarker evaluated for oxidative stress in this study was 8-OHdG, demonstrating a significant reduction of 59.8% ($P<0.001$) following OFi supplementation. As a biomarker for DNA damage, 8-OHdG is closely associated with ageing, degenerative diseases, and the development and progression of cancer[14,60,61,69]. A previous study reported that OFi extracts exhibit concentration-dependent suppressive effects on hydrogen peroxide (H_2O_2)-induced oxidative stress, thereby mitigating toxic damage to genetic material in human peripheral lymphocytes[70]. This strong suppressive activity is hypothesised to be attributed to flavonoids and betalains in OFi[9,70]. Further evidence supports a dose-dependent anticlastogenic effect of OFi extracts against various genotoxic agents, including methyl methanesulfonate (MMS), the mycotoxin zearalenone (ZEN), aflatoxin B_1 and benzo(a)pyrene)[9]. In mouse models, OFi extracts have been shown to prevent DNA damage by inhibiting micronucleus formation and DNA fragmentation while simultaneously reducing the frequency of chromosomal aberrations[9]. Similarly, catechins derived from natural sources have been found to effectively repair damaged DNA bases at low dosages and rapid rates through a unique electron transfer mechanism targeting damaged sites on single-stranded DNA[60,61]. Additionally, polyphenols have exhibited DNA repair capabilities in in-vitro models[71–74], suggesting potential anti-cancer properties via antioxidant-mediated DNA protection.

No significant changes were observed in blood pressure or heart rate during the study period. However, participants reported a significant 20.1% improvement in self-perceived general well-being following three months of OFi supplementation (Table 4). General well-being was assessed using the Visual Analogue Scales (VAS), a psychometric instrument designed for subjective evaluation of disease-related symptom severity or general well-being. The VAS is a validated tool with high validity and excellent reliability in evaluating general well-being and quality of life[75]. The observed improvement in well-being may be attributed to increased antioxidant activity and a corresponding reduction in oxidative stress markers (Sect. 4.1). These findings align with those of Kressel et al. who reported that supplementation with OFi flower extract reduced subsyndromal fatigue in 70% of participants[76]. Dietary supplements have been shown to significantly enhance self-perceived health and well-being[36]. A balanced diet optimises physical and physiological functions, improving brain function and mental well-being[77]. Diets rich in polyphenols have been associated with improved cognitive performance and modulation of brain activity, mediated through neurogenesis and the inhibition of inflammatory processes[78–80]. Notably, oxidative stress-induced inflammation has been implicated in mental health disorders such as anxiety and depression. Individuals with these conditions often exhibit abnormalities in antioxidant enzyme levels and disruptions of redox homeostasis[81,82]. Given these mechanisms, dietary supplements enriched with antioxidants may alleviate psychiatric symptoms by counteracting oxidative stress, mitigating stress-induced inflammation and addressing mitochondrial dysfunction[82]. Thus, polyphenol-rich supplements containing non-enzymatic antioxidants hold significant potential for improving the mental health of affected individuals[83–85].

The absence of a placebo group and the open-label design represent significant limitations in this study, as they restrict the ability to fully account for potential confounding effects and biases. Participants may have reported perceived benefits influenced by their expectations of the intervention rather than its efficacy. This limitation introduces challenges in discerning whether the observed outcomes are directly attributable to the intervention or are influenced by psychological factors or other external variables. To address these limitations, salivary biomarker analysis was incorporated as an objective measure to substantiate the study outcomes. This approach helps mitigate the potential impact of subjective bias and confounding effects, providing a more reliable assessment of the intervention's efficacy without a placebo group.

Conclusions

Regular supplementation of OFi significantly improved participants' self-perceived well-being in this study. These improvements align with biochemical analyses, which revealed notable enhancements in redox homeostasis through increased antioxidant levels and reduced oxidative stress. These results underscore the potential of OFi, a polyphenol-rich dietary supplement, in managing oxidative stress and mitigating related health conditions. Nonetheless, further validation through a randomised controlled trial (RCT) is essential to confirm these findings and establish their clinical efficacy.

Methods

Study design

This open-label, single-arm, prospective study was conducted over a three-month supplementation period. The research adhered wholly to the principles of the Declaration of Helsinki and the Malaysian guidelines for

Fig. 3. The *Opuntia ficus-indica* powder form.

Good Clinical Practice[86]. Participant eligibility was confirmed according to the protocol checklist, and written informed consent was obtained from all participants before enrolment. Ethical approval was granted by the Institutional Ethics Committee of UCSI University, Malaysia, via the approval code IEC-2022-FMHS-082. This study was registered as a clinical trial with the identifier NCT06657963, registered on 24 October 2024 and first posted on 26 October 2024.

Participants selection

Participants were recruited through off-campus advertisements, and the study was conducted at UCSI University in Kuala Lumpur, Malaysia. The inclusion criteria were: (1) generally healthy individuals, (2) aged 18 years and above, (3) capable of understanding the study protocol and related information, and (4) willing to provide written informed consent. Exclusion criteria included: (1) current use of supplementation aimed at enhancing antioxidant status, (2) history of major surgical procedures within 6 months before study enrolment and (3) pregnant or lactating woman. All participants received a participant information sheet and a comprehensive explanation of the study from the investigator. Written informed consent was obtained from each participant, and potential risks, including the possibility of food allergies, were communicated to participants during the consent process.

Supplementation

Demographic data and participants' medical histories were collected during the baseline visit. Participants then began daily oral supplementation of OFi fine powder (Fig. 3) packaged in individual sachets (LifeGreen™, LifeTree Asia, Selangor, Malaysia) at the dosage of 1500 mg once daily for three months. This dosage was derived from the effective equivalent dose conversion from a preclinical animal study, which demonstrated improvements in antioxidant capacity and reductions in oxidative stress[87]. The recommended dosage and preparation instructions were communicated to participants upon enrolment. Each sachet was to be mixed with 150 mL of lukewarm water, which participants were instructed to consume before their first meal of the day. Monthly follow-up visits were conducted at months 1, 2, and 3. During each visit, data were collected using a case report form (CRF) to record vital signs, self-perceived general well-being, and saliva samples for laboratory investigations. A compliance form was issued to each participant to ensure adherence to the intervention plan. Participants were reminded during follow-up visits to avoid consuming any other supplements that might enhance antioxidant levels during the study duration. Additionally, participants were instructed to immediately inform the research team of any adverse reactions to the supplement.

Laboratory examinations

Unstimulated saliva samples were collected from participants using sterile 2.0-mL vials. Participants were instructed to refrain from eating or drinking at least 30 min before sample collection to ensure consistency. However, no specific time of day was designated for the sample collection. Participants uncapped the vial, placed

the straw into the vial, and passively drooled down the straw for 90 s. A total of 2 mL of saliva was collected from each participant and stored at − 20 °C to maintain marker stability. All samples were analysed within 30 days of collection.

The collected samples were assayed in duplicate for various parameters. TAC was measured using the Elabscience total T-AOC colourimetric assay kit (Elabscience Biotechnology Co. Ltd, Texas, United States) based on the principles of the Ferric Reducing Antioxidant Power (FRAP) assay[88]. Lipid peroxidation was assessed by measuring MDA levels using the Elabscience MDA colourimetric assay Kit (Elabscience Biotechnology Co. Ltd, Texas, United States) based on the principles of Thiobarbituric Acid (TBA) assay[89]. Oxidative stress-derived protein damage was evaluated by quantifying 3-NT levels using Elabscience 3-NT ELISA Kit (Elabscience Biotechnology Co. Ltd, Texas, United States), following previously established protocols[90]. Oxidative stress-induced DNA damage was determined by measuring 8-OHdG using Elabscience 8-OHdG ELISA Kit (Elabscience Biotechnology Co. Ltd, Texas, United States) based on established protocols[91].

Vital signs and general wellbeing

The Omron automatic blood pressure monitor (HEM 7120, Omron Healthcare, Kyoto, Japan) measured blood pressure and heart rate. Temperature was measured using a Braun forehead infrared thermometer (NTF 3000, Braun GmbH, Kronberg, Germany). The Visual Analogue Scale (VAS) was incorporated to assess participants' subjective well-being. Participants self-reported their health status on a 10-cm horizontal VAS scale, where 0 represents the least healthy condition, and 10 denotes the healthiest condition.

Statistical analysis

Demographic characteristics were presented as categorical data, expressed as frequencies and percentages. All outcomes were analysed as continuous dependent variables and are presented as mean ± standard deviation (SD) when normally distributed. Changes in vital signs, laboratory outcomes and VAS scores from baseline to the final follow-up visit were analysed using the general linear model (GLM) and repeated measures ANOVA model. The within-subjects factor was defined as the time point of assessment. Gender, age and BMI were tested as between-subject factors. Levene and Box M tests assessed the homogeneity of the variance and covariance structure of the dependent variables. The sphericity test of the residual covariance matrix was evaluated using Mauchly's sphericity test. Results were considered significant if $P < 0.05$ with a 95% confidence interval. Statistical analysis was performed using SPSS 26.0 (IBM Corp., New York, United States) for MacOS.

Data availability

The datasets generated and/or analysed during the current study are not publicly available due to reasons of sensitivity but are available from the corresponding author on reasonable request.

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Author contributions

Writing, original draft preparation—RZ; review and editing—ETSS, NAB, FA, MSS, HYB; Conceptualization, methodology, supervision, funding acquisition, project administration—TCK.

Declarations

Competing interests

The authors declare no competing interests.

Institutional review board

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of UCSI University, Malaysia (protocol code: IEC-2022-FMHS-082 and date of approval: 1/11/23).

Informed consent

Informed consent was obtained from all subjects involved in the study. Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this study" where an affirmative reply was required to enter the study. They were able to withdraw from the study at any time without giving a reason. The products tested were safe for consumption."

Additional information

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