European Journal of Integrative Medicine 48 (2021) 101400

Contents lists available at ScienceDirect

European Journal of Integrative Medicine

journal homepage: www.sciencedirect.com/journal/european-journal-of-integrative-medicine

Research paper

Hexane extract of *Curcuma longa* L. inhibits the activities of key enzymes and pro- inflammatory adipokines linked to obesity

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ARTICLE INFO

Keywords:
Curcuminoids
Inflammation
Human adipose tissue
Curcuma longa L.
obesity

ABSTRACT

Introduction: Obesity is associated with chronic activation of low-grade inflammation produced mainly from adipose tissue, which is implicated in the pathogenesis of several diseases. Consequently, there is a need to screen for new anti-obesity medicines. Clinical evidence regarding the anti-obesity properties of *Curcuma longa* L. (*C. longa*) is inconclusive. Therefore, we aimed to investigate for the first time the influence of curcuminoids and hexane extract derived from *C. longa* 1) On the release of pro-inflammatory adipokines from human abdominal subcutaneous adipose tissue (ASAT) and induced-mononuclear cells (iMC) and 2) On the activities of α-amylase, α-glucosidase, and lipase enzymes.

Methods: ASAT explants and lipopolysaccharide-iMC were treated with either curcuminoids or hexane extract of *C. longa*. Protein concentration, anti-lipase, anti-amylase and anti-glucosidase activities were evaluated employing colorimetric methods.

Results: Treatment of ASAT with curcuminoids or hexane extract inhibited the secretion of leptin, CCL5 and Il-1β. Treatment of iMC cells with curcuminoids or hexane extract inhibited the secretion of TNF-α, CCL5 and Il-1β and leptin was not detected. Curcuminoids possessed a significant inhibitory activity against lipase, α-amylase and α-glucosidase in a dose-dependent manner.

Conclusion: We demonstrate for the first time that curcuminoids and *C. longa* exert anti-inflammatory properties on human ASAT and iMC and inhibit the activities of lipase, α-amylase and α-glucosidase enzymes. This suggests that *C. longa* and curcuminoids not only may ameliorate obesity-associated comorbidities such as metabolic syndrome but may be used as a preventive approach against obesity. However, this requires *in vivo* validation.

1. Introduction

Obesity has reached epidemic proportions and is still escalating at an alarming rate worldwide [1]. According to the World Health Organization (WHO), cases of obesity have approximately tripled since 1975. In 2016 > 1.9 billion adults (~39%) were overweight, of these over 650 million were obese (~13%) [2]. Obesity prevalence in the United States of America has increased to over 42% among adults in 2017–2018 [3]. In the Middle East and North Africa, 58% of men and more than 65% of women suffer from either overweight or obesity [4]. Palestine is not an exception, as the prevalence of obesity has been shown to be approximately four times among women (49%) and two times among men (30%) higher than the prevalence worldwide [4,5].

Obesity increases the risk of chronic diseases such as metabolic syndrome, type-2 diabetes mellitus, cardiovascular diseases, and even some cancers [6], which account for more than 70% of early deaths [7]. Although the etiology of obesity and its associated chronic diseases is complex, and accumulating evidence suggests that chronic low-grade inflammation [8] plays a major role [9,10]. Adipose tissue is the largest endocrine tissue and contains multiple cell types, including adipocytes and macrophages, which both play a major role in the inflammatory response throughout the obesity course and are considered

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https://doi.org/10.1016/j.eujim.2021.101400
Received 13 August 2021; Received in revised form 17 October 2021; Accepted 19 October 2021
Available online 2 November 2021

the major source of low-grade inflammation [11–15]. Leptin is a hormone produced by adipose tissue that links metabolism and immune homeostasis. Leptin is a pro-inflammatory factor that is exclusively produced by adipocytes and not from the macrophages of adipose tissue [16]. Tumor necrosis factor-alpha (TNF-α) and interleukin 1 beta (Il-1β) are pro-inflammatory adipokine produced by adipose tissue and implicated in the inflammatory and catabolic effects of macrophages on adipocytes. TNF-α signaling pathway plays a role in chronic low-grade inflammation originating from adipose tissue [17,18]. C—C Motif Chemokine Ligand 5 (CCL5) is a pro-inflammatory chemokines produced by adipose tissue, including both adipocytes and immune cells. Adipose tissue released CCL5 is a chemoattractant that recruits immune cells including macrophages [19]. This highlights the emergence of obesity and its associated diseases as major contributors to the burden of ill health and its costs in developed countries such as the USA, which is estimated to be 20.6% of U.S. national health expenditure [20]. Therefore, prevention should be a global public health priority to reduce and prevent obesity and its associated diseases.

At the present time, many conventional drugs are being used for the management of obesity, because weight-loss interventions aimed at reducing calorie intake and increasing energy expenditure are frequently not successful in the long term [7]. However, synthetic medicines are expensive and possess potentially dangerous side effects, which limit their utilization [21]. Therefore, there is a critical need to screen for and develop safe, effective, and cheap alternatives. Plants are a potential source of medicines against obesity. *C. longa* (turmeric) is a distinctive yellow spice, which is a part of the ginger family (Zingiberaceae). It has long been recognized for its medicinal properties. Curcuminoids are the primary polyphenol in *C. longa* and it is the compound for which most of the biological effects of *C. longa* have been attributed [22,23]. *C. longa* research suggests curcuminoids are safe in both animal studies (up to 15 mg/ml) and human clinical trials (up to 2500 mg per day). However, few clinical trials have been conducted to investigate the influence of *C. longa* and curcuminoids supplementation on BMI, inflammation, glucose and lipids; and results have been inconclusive [24–36]. This is most probably due to scarcity of clinical studies, complexity in the setup of clinical studies and inter-individual differences. This is supported by the notion that the studies used more bioavailable forms of curcuminoids or longer intervention duration succeeded to show anti-obesity properties. To avoid such complexity, we investigated for the first time organic extract derived from *C. longa* and the pure curcuminoids on an *ex vivo* model, human abdominal subcutaneous adipose tissue and induced monocytes since they are the major sources of inflammation-induced during obesity. In addition, we aimed to investigate their effect on the activities of α-amylase, α-glucosidase and lipase enzymes and consequently on the inhibition of glucose and lipids release into the blood.

2. Materials and methods

2.1. Chemicals and reagents

Gentamicin, glucose, curcuminoids, LPS, PBS and bovine serum albumin (BSA), PNPB, PNPG, porcine pancreatic lipase type II, α-amylase, α-glucosidase, acarbose, DMSO, orlistat and tris-HCl were purchased from Sigma and M199, L-glutamine, penicillin-streptomycin antibiotics and RPMI media from Invitrogen (USA). Lymphoprep was purchased from “stem cell” technologies (Norway) and DNSA from Alfa Aesar (UK). Human leptin, TNFα, Il-1β and CCL5 DuoSet ELISA kit and Substrate Solution were purchased from R and D systems (USA) and ELISA plates were purchased from Greiner Bio-One (Germany). Tween 20 was purchased from Sun Pharm LTD (Palestine). Hexane, Na_2CO_3 and acetonitrile were bought from S.D Fine Chemicals (India).

2.2. Preparation of C. longa

The dried rhizomes of C. longa were obtained from a local herbal shop in Nablus, Palestine, originally imported from India. The plant was characterized in the Pharmacy department at An-Najah National University and kept under the voucher specimen code (Pharm-PCT-2709). They were shredded into small threads then ground using a mechanical blender and kept in special glass jars for further use.

2.3. Extraction procedure

Hexane extract was prepared as described earlier [37]. Briefly, approximately 100 g of the ground plant were soaked in 1 liter of hexane and incubated in a shaker device at 200 rounds per minute for 72 h at room temperature and stored in a refrigerator for 4 days. The extracts were then filtered using filter papers and concentrated under a vacuum on a rotator evaporator. The crude extract was stored at 4 °C for further use.

2.4. Patient recruitment

A total of 26 subjects participated in our study was 26 subjects. Blood was collected from four subjects who were randomized students recruited by AnNajah National University (Table 2) to isolate mononuclear cells. ASAT was received from the remaining 22 patients who were randomly recruited from Rafidia and AnNajah National University Hospitals in Nablus district. These patients were enrolled for various surgical procedures including hernia repair, cholecystectomy, splenectomy, tumor resection, and colon, stomach, and esophagus resections at various levels (Table 1). The study was approved by the Institutional Review Board of the AnNajah National University of Nablus, Palestine (January 19, 2017). All patients gave their informed and signed consent to participate in the study.

2.5. Human abdominal subcutaneous adipose tissue culture

Human ASAT explants were obtained from 22 patients. The patients were randomly divided into 2 groups to study the effects of C. longa and curcuminoids. The ASAT of the first 10 patients was used to evaluate C. longa. The remaining 14 patients were used to study the effects of curcuminoids. In some cases, we received large amounts of ASAT sufficient to study the effects of both C. longa and curcuminoids. ASAT explants were derived from patients who underwent surgeries for the reasons described in Table 1. BMI, gender, age and other criteria of these patients were described in Table 1. The study was approved by An-Najah National University Institutional Review Board (IRB) with an archived number 65/Aug/2015. All methods regarding patients were performed in accordance with An-Najah National University IRB guidelines and regulations, which is based on the Declaration of Helsinki. Patients were invited to participate in our study and samples were collected after they signed a consent form. ASAT culture was performed as described previously [38–40], but with few modifications. After the last washing step, ASAT explants were either treated with hexane extract derived from *C. longa* or curcuminoids. ASAT explants in triplicate obtained from 10 patients were incubated for 24 h with 0, 7.5, 75 or 750 μg/ml of *C. longa* and cultured at 37 °C and 5% CO_2. ASAT explants in triplicate obtained from 14 patients were incubated for 24 h with 0, 10, 100, 500 and 1000 μM of curcuminoids (>94% curcuminoids) and cultured at 37 °C and 5% CO_2. Subsequently, the medium was collected and stored in a freezer at −20C before ELISA measurements.

2.6. Human mononuclear cells culture

Four healthy volunteers were invited to participate in our study and blood was collected after they signed a consent form. 5 ml of peripheral blood was drawn into EDTA tubes from each volunteer (Table 2).

Table 1
Criteria of the subjects from whom ASAT explants were derived.

Patient ID	Age	Gender	BMI	Cause of Admission	Chronic Diseases	Medication
1	72	F	34	Laproscopic cholecystectomy	Free	Free
2	82	F	33	Subtotal gastrectomy	HTN, DM and Stroke	Enalapril, Furosemide and Propranolol
3	56	M	24	Colostomy closure and ileostomy creation	BPH, HTN and IHD	Candesartan and Hydrochlorothiazide
4	33	M	24	Left inguinal hernia	Free	Free
5	52	F	33.3	Incisional abdominal hernia	Free	Free
6	71	F	38	Abdominal wall mass for incisional biopsy	HTN and IHD	Enalapril, Aspirin and Metformin
7	40	M	27	Inguinal hernia repair	Free	Free
8	66	M	24.4	Cystojejunostomy and rollen-ex anastomosis	Free	Free
9	65	F	29	Hiatal hernia and cholecystitis	HTN and Osteoporosis	Enalapril, Furosemide and Calcium Supplements
10	63	M	31.1	Left inguinal hernia	HTN and DM	Enalapril and Metformin
11	52	M	26	Colonic segmental resection	Free	Free
12	58	M	26	Incisional abdominal hernia	Free and Cholecystectomy	Free
13	70	M	24.2	Low anterior colonic resection	DM and HTN	Dexamethason, Esomeprazole, Enalapril and Acetylcysteine
14	36	F	29.8	Hysterectomy	Free	Free
15	47	M	27.3	Splenectomy	Lymphoma	Enoxaparin and Esomeprazole
16	54	F	27.5	Right inguinal hernia	Free	Free
17	54	M	28.5	Hartmann's procedure	Free	Free
18	59	M	27.7	Trachoabdominal esophagectomy	DM, HTN,IHD and CVA	Chlorpromazine and Multivitamin
19	61	M	27.1	Open cholecystectomy	DM and HTN	Metoprolol, Losartan and Metformin
20	67	F	30	Retroperitoneal mass resection and incisional hernia repair	HTN and Hypothyroidism	Acetaminophen,Enoxaparin, Esomeprazole, Losartan, Metoclopramide and Metronidazole
21	65	F	29.5	Right hemicolectomy	HTN and DM	Enoxaparin, Esomeprazole and Lisinopril
22	32	M	26.6	Open cholecystectomy	Free	Tramadol

Abbreviations: HTN: Hypertension; DM: Diabetes Mellitus; BPH: Benign Prostatic Hyperplasia; IHD: Ischemic Heart Disease; CVA: Cerebrovascular accident.

Table 2
Criteria of subjects from whom mononuclear cells were collected.

Patient ID	Age	Gender	BMI	Past medical history	Drug history
23	24	M	26	Free	Free
24	24	M	25	Free	Free
25	21	M	23	Free	Free
26	22	M	22.5	Free	Free

Mononuclear cells were isolated using the Lymphoprep method according to the manufacturer guidelines (StemCell Technologies, Norway). Buffy coat layer was removed and mixed with RPMI 1640 medium. Then cells were seeded in 12-well plates and after 1 h incubation at 37 °C and 5% CO_2, the medium was withdrawn from the wells and they were washed by PBS to remove all non-adherent cells. At this stage, the well plate was examined under the microscope and most of the adherent cells were identified as mononuclear cells. Mononuclear cells were co-treated with both LPS (10 μg/ml) and curcuminoids or hexane extract derived from *C. longa*. The following concentrations of curcuminoids were used, 0uM (control), 10 μM, 100 μM, 500 μM or 1000 μM. concentrations of hexane extract derived from *C. longa* used in this study were 75, 750 and 3750 μg/ml. Co-treated mononuclear cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U of penicillin/ml and 100ug of streptomycin/ml for 24 h at 37 °C and 5% CO_2. The medium was collected and stored at −20 C^o prior to ELISA measurements.

2.7. ELISA

Secreted leptin, TNF-α, Il-1β, and CCL5 quantities were measured in the culture media by DuoSet ELISA kit according to the manufacturer's instructions (R and D Systems, USA).

2.8. Pancreatic lipase inhibitory activity assay

The porcine pancreatic lipase inhibitory method was performed as described earlier [41], but with slight modifications. Porcine pancreatic lipase (1 mg/mL) was mixed with 0.0, 0.37 (1 uM), 3.7 (10 uM), 37 (100 uM), 185 (500 uM) or 370 (1000 uM) μg/mL of curcuminoids and incubated at 37 °C for 15 min. Thereafter, the substrate, p-nitrophenyl butyrate (PNPB) was added to the mixture and was incubated for 30 min at 37 °C. The pancreatic lipase activity was determined by measuring the hydrolysis of PNPB into p-nitrophenolate ions at 410 nm using a spectrophotometer. The same procedure was repeated for the positive control sample (orlistat) (Sigma-Aldrich, Germany). The inhibitory percentage of the anti-lipase activity was calculated using the following equation:

Lipase inhibition% = (Ab-As)/Ab × 100%, where Ab is the recorded absorbance of the blank solution and as is the recorded absorbance of the tested sample solution.

2.9. Pancreatic α-amylase inhibitory activity assay

The α-amylase inhibitory activity of curcuminoids and acarbose (positive control) was assessed according to the standard method reported by Nyambe-Silavwe et al. with minor modifications [41]. Porcine pancreatic α-amylase enzyme solution (Sigma-Aldrich, USA) was mixed with 0.0, 0.37 (1 uM), 3.7 (10 uM), 37 (100 uM), 185 (500 uM) or 370 (1000 uM) μg/mL of curcuminoids and incubated at 30 °C for 10 min. Thereafter, freshly prepared starch solution (1%) was added and the mixture was incubated for at least 3 min. The reaction was stopped by the addition of dinitrosalicylic acid (DNSA) (Alfa-Aesar, UK), then the mixture was diluted with distilled water and heated in a water bath at 90 °C for 10 min. The mixture was left to cool down to room temperature and the absorbance was measured at 540 nm. The α-amylase inhibitory activity was calculated using the following equation:

% of α-amylase inhibition = (Ab –As)/Ab *100%, where Ab is the absorbance of the blank and As is the absorbance of the tested sample or control.

2.10. Pancreatic α-glucosidase inhibitory activity assay

The α-glucosidase inhibitory activity of curcuminoids and acarbose (positive control) was performed according to the earlier described standard protocol, however with slight modifications [42]. Porcine

pancreatic α- glucosidase enzyme solution (Sigma-Aldrich, USA) was mixed with 0.0, 0.37 (1 uM), 3.7 (10 uM), 37 (100 uM), 185 (500 uM) or 370 (1000 uM) μg/mL of curcuminoids and incubated at 30 °C for 15 min. 4-Nitrophenyl β-D-glucuronide (PNPG) (Sigma-Aldrich, USA) was added as a substrate to the reaction mixture and incubated at 37 °C for additional 20 min. The reaction was terminated by adding Na_2CO_3. The absorbance released from the substrate was measured by a spectrophotometer at 405 nm. The inhibition percentage was calculated using the following equation:

% of α- glucosidase inhibition = (Ab –As)/Ab *100%, where Ab is the absorbance of the blank and As is the absorbance of the tested sample or control.

2.11. Statistics

GraphPad Prism software version 6.01 was used to perform the statistical analysis as we described earlier [43,44], but with slight modifications. To compare between 3 groups or more belong to one factor, one-way ANOVA followed by Bonferonni's post hoc test was employed. A nonlinear regression test was used to determine the IC_{50} and comparison between multiple groups belong to two factors was analyzed via two-way ANOVA followed by Tukey's post hoc test. Results were considered to be statistically significant at $P < 0.05$.

3. Results

3.1. Details on study participants

The number of subjects who participated in our study was 26 subjects, 65.4% of them were males. Their ages ranged from 21 to 82 (mean=51.8 ± 17.5 years) years old and their BMI ranged from 22.5 to 38 (mean=27.9 ± 3.7) Kg/m^2. To isolate mononuclear cells, blood was collected from four subjects, who were students recruited from An-Najah National University (Table 2). The remaining 22 patients were admitted to both Rafidia and An-Najah National University Hospitals in Nablus district for various surgical operations, including hernia repair, cholecystectomy, splenectomy, tumor resection as well as various levels of colonic, gastric and esophageal resections (Table 1). Almost 57.7% of the patients were free from chronic diseases, while the rest were suffering from several chronic diseases such as type II diabetes mellitus, cardiovascular diseases, benign prostatic hyperplasia, osteoporosis, hypothyroidism and cancer. Approximately 46.2% of the patients were under medications that included esomeprazole, furosemide, propranolol, metoclopramide, metoprolol, candesartan, enoxaparin, losartan, chlorpromazine, tramadol, hydrochlorothiazide, spironolactone, atorvastatin, insulin, enalapril, metformin and aspirin.

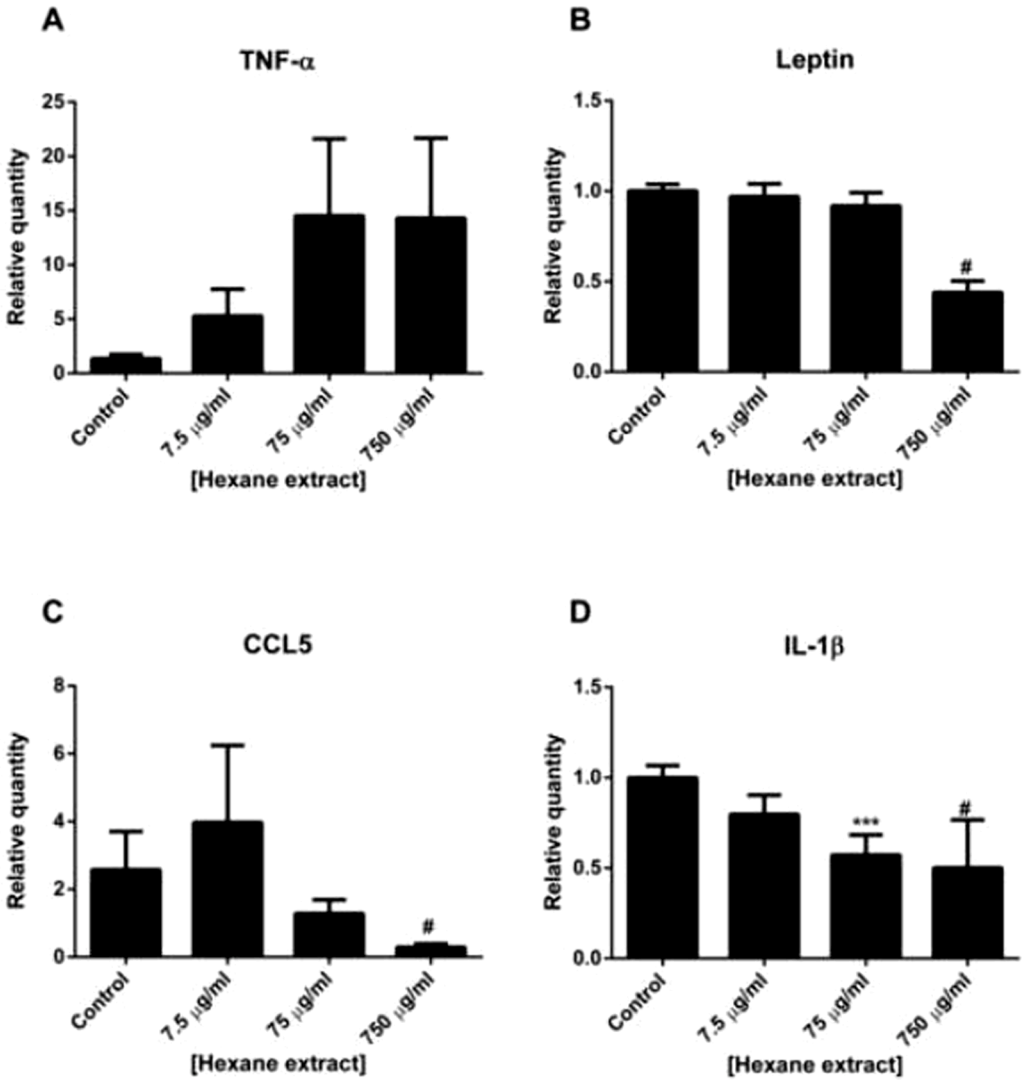

Fig. 1. Effect of hexane extract derived from *C. longa* on the release of inflammatory adipokines from human abdominal subcutaneous adipose tissue. ASAT explants (in triplicates) of each patient were incubated with or without 07.5, 75, 750 μg/ml of hexane extract for 24 h. Secreted quantities of (A) TNF-α, B) Leptin, (C) CCL5 and (D) IL-1β in the media were determined by ELISA. Results were depicted as relative quantities (RQ) compared to the control (without extract; control). *#P < 0.0001*, ****P < 0.001* and **P <* 0.05, vs. control. Error bars, SEM.

3.2. Organic extract derived from C. longa inhibits the release of pro-inflammatory adipokines from human abdominal subcutaneous adipose tissue and induced mononuclear cells

ASAT explants (in triplicate) were derived from 10 patients and incubated for 24 h with or without hexane extract derived from *C. longa* (7.5, 75, and 750 μg/ml**)**. Secreted quantities of TNF-α, Leptin, CCL5, and Il-1β into media were quantified employing the ELISA technique. As shown in Fig. 1A–D, 750 μg/ml of hexane extract significantly ($p < 0.0001$) reduced the release of leptin, CCL5 and Il-1β from ASAT by approximately 60%, 72%, and 50%, respectively, while TNF-α was not influenced significantly. 75 μg/ml of hexane extract significantly ($p < 0.001$) reduced the release of Il-1β from ASAT by approximately 60%, while the remaining tested adipokines showed no significant effect.

Organic extract derived from *C. longa* inhibited the release of inflammatory markers from human adipose tissue and as adipose tissue is composed of macrophages, which are derived mainly from circulating mononuclear cells [45]. Consequently, we investigated the influence of the extract on human induced-mononuclear cells. Mononuclear cells were isolated from 4 patients and were co-treated with LPS (10 μg/ml) and 0, 75, 750, or 3750 μg/ml of *C. longa* organic extract and incubated for 24 h in duplicates. Protein quantities were tested by ELISA. As shown in Fig. 2A and B, LPS-induced mononuclear cells treated with 75, 750, and 3750 μg/ml of C. *longa* organic extract for 24 significantly inhibited ($p < 0.0001$) the release of TNF-α and Il-1β by approximately > 90%. Leptin was not detected in the media of induced mononuclear cells.

3.3. Curcuminoid molecules inhibit the release of inflammatory adipokines from human abdominal subcutaneous adipose tissue and human mononuclear cells

As the most abundant molecules of the organic extract of *C. longa* are curcuminoids, we investigated whether curcuminoids are responsible for the observed effects. To achieve this ASAT explants (in triplicates) were obtained from 14 patients and treated for 24 h with 0, 10, 100, 500, or 1000 μM of curcuminoids. Secreted quantities of TNF-α, Leptin, CCL5, and Il-1β into media were quantified employing the ELISA technique. As demonstrated by Fig. 3A, 10, 100, 500 or 1000 μM of curcuminoids significantly inhibited the release of TNF-α by approximately 20% ($P < 0.01$), 56% ($P < 0.0001$), 83% ($P < 0.0001$) and 90% ($P < 0.0001$), respectively. Secreted quantity of leptin was inhibited significantly by approximately 68% ($P < 0.001$) and 83% ($P < 0.0001$) after treating adipose tissue by 500 and 1000 μM of curcuminoids, respectively (Fig. 3B). Secreted quantity of CCL5 was inhibited significantly by approximately 80% ($P < 0.0001$) after treating adipose tissue by 500 and 1000 μM of curcuminoids (Fig. 3C). As shown by Fig. 3D, only 500 and 1000 μM of curcuminoids inhibited significantly the release of Il-1β by approximately 50% ($P < 0.01$) and 80% ($P < 0.0001$), respectively.

We also investigated the influence of curcuminoids on human induced-mononuclear cells. Mononuclear cells were isolated from 4 patients and were co-treated by LPS treatment 0, 10, 100, 500, or 1000 μM of curcuminoids for 24 h in duplicates. Protein quantities were tested by ELISA. As shown in Fig. 4A LPS-induced mononuclear cells treated with 10, 100, 500 and 1000 μM of curcuminoids for 24 h lead to a significant inhibition in the release of TNF-α by approximately 56% ($P < 0.001$), 85% ($P < 0.0001$), 84% ($P < 0.0001$) and 76% ($P < 0.0001$), respectively. 500 and 1000 μM of curcuminoids significantly ($P < 0.001$) inhibited the secretion of CCL5 by approximately 30% (Fig. 4B). released quantity of Il-1β was inhibited significantly ($P < 0.0001$) by approximately 70% and 98% after treating adipose tissue by 10 μM and the rest of examined concentrations of curcuminoids, respectively (Fig. 4C). Leptin was not detected in the media of induced mononuclear cells.

3.4. Curcuminoids inhibitory activity against pancreatic lipase, α-glucosidase, and α-amylase enzymes

As demonstrated in Fig. 5, curcuminoids possessed a significant *in vitro* inhibitory activity against porcine pancreatic lipase activity at all tested concentrations (0.37, 3.7, 37, 185 and 370 μg/mL) in a concentration-dependent manner. IC_{50} values of curcuminoids and orlistat (reference compound for anti-lipase) were 9 and 26 μg/mL, respectively (Fig. 5). As illustrated in Fig. 6, Curcuminoids inhibited the activity of the α-amylase enzyme in a dose-dependent manner. IC_{50} values of both curcuminoids and acarbose (reference compound for anti-α-amylase) were 69 and 3.5 μg/mL, respectively. The α-glucosidase activity was also inhibited significantly by all tested concentrations of curcuminoids in a dose-dependent manner. As shown in Fig. 7, IC_{50} values of curcuminoids and acarbose (reference compound for anti-α-glucosidase) were 50 and 5 μg/mL, respectively.

4. Discussion

Few clinical trials have been conducted to investigate the influence of *C. longa* and curcuminoids supplementation on obesity and its related disorders; however, the results were contradictory. For example, BMI has been shown to be not influenced in few studies [24–28] while reduced in others [29–31]. Although the preclinical anti-inflammatory effect of curcuminoids has been well established [46,47], the clinical evidence on the influence of curcuminoids supplementation on inflammatory parameters associated with obesity and its related diseases is scarce and inconclusive. In one trial, curcuminoids have been shown to

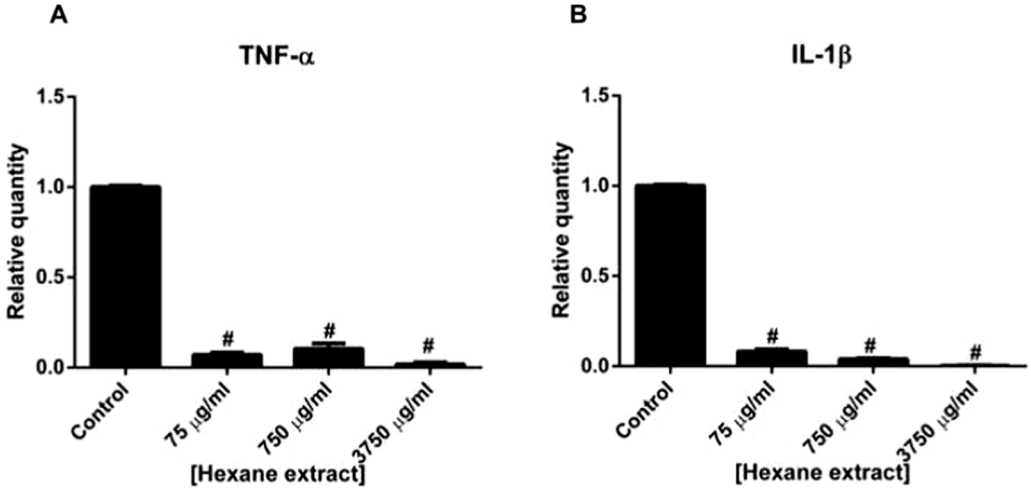

Fig. 2. Effect of hexane extract derived from *C. longa* on the Release of inflammatory adipokines from human induced-mononuclear cells. Mononuclear cells were co-treated with both LPS (10 μg/ml) and hexane extract derived from *C. longa* for 24 h. Various concentrations of 0 (control), 75, 750, 3750 μg/ml were used. Inflammatory adipokines (A) TNF- α and (B) IL-1β were measured by ELISA. Results are depicted as relative quantities (RQ) compared to the control (LPS treatment without extract; control). *#P < 0.0001* vs. control. Error bars, SEM.

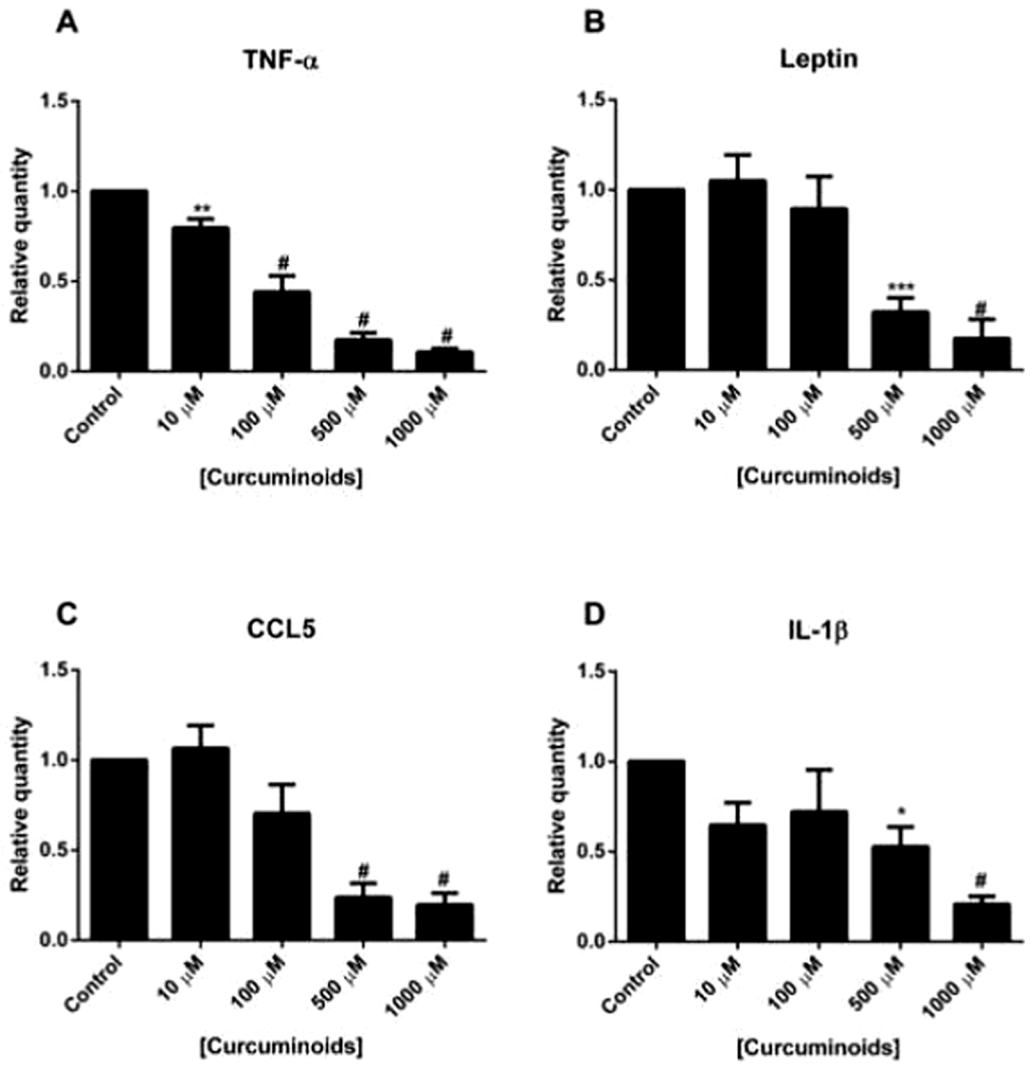

Fig. 3. Effect of curcuminoids molecule on the release of inflammatory adipokines from human abdominal subcutaneous adipose tissue. ASAT explants (in triplicates) of each patient were incubated with or without 10, 100, 500, 1000 μM of curcuminoids for 24 h. Secreted quantities of (A) TNF-α, B) Leptin, (C) CCL5 and (D) IL-1β in the media were determined by ELISA. Results were depicted as relative quantities (RQ) compared to the control (without curcuminoids; control). *#P < 0.0001*, ***P < 0.001, **P < 0.01 and *P < 0.05, vs. control. Error bars, SEM.

decrease pro-inflammatory factor IL-1 beta and anti-inflammatory IL-4 at the same time and have no effects on other cytokines such as IL-1, IL-2, IL-6, IL-8, IL-10, interferon-gamma, epidermal growth factor, monocyte chemoattractant protein 1, and TNF-a levels [35]. In other studies, IL-6 and TNF-alpha and MCP-1 have been reduced [33,36]. Very recently leptin release, another pro-inflammatory factor, shown to decrease in one clinical trial [48] while in another trial it was slightly, but insignificantly increased [49]. In the current study, for the first time, we demonstrate that curcuminoids and *C. longa* extract exerted anti-inflammatory properties on human adipose tissue and induced mononuclear cells.

The primary source of pro-inflammatory parameters that are associated with obesity and its related diseases is adipose tissue. As clinical studies are scarce and inconclusive due to complex setup and inter-individual differences, one could assume that adipose tissue is a potential and reliable *ex vivo* model to determine and confirm the effect of curcuminoids on pro-inflammatory adipokines induced by obesity and its related diseases. Therefore, this is the first time to investigate the influence of organic extract derived from *C. longa* and pure curcuminoids molecules on the release of adipokines from human abdominal subcutaneous adipose tissue. We have found that organic extract derived from *C. longa* inhibited the secretion of the tested pro-inflammatory parameters from human adipose tissue. Curcuminoids are the main natural polyphenol found in the rhizome of *C. longa* (turmeric), which contributes to most of the biological activities related to C. *longa*. Therefore, we investigated whether the curcuminoids molecule is responsible for the observed anti-inflammatory properties and indeed we found that curcuminoids possess anti-inflammatory properties. This suggests that the anti-inflammatory property of *C. longa* is most probably mediated by the curcuminoids molecule. However, we cannot exclude other components of *C. longa* that may have also anti-inflammatory activities. Indeed in our earlier study, we have shown that the polar extract of *C. longa* inhibits leptin secretion from human adipose tissue [39], while it is known that curcuminoids are present in the organic extract rather than in the polar extract.

Obesity is characterized by a chronic low-grade inflammation that is implicated in the development of obesity-related disorders such as insulin resistance, metabolic syndrome, type II diabetes mellitus cardiovascular diseases, and even certain cancers. With progressive obesity, adipose tissue macrophages, recruited mainly from blood mononuclear cells, play a major role in the adipose tissue induced-inflammation and consequently associated diseases [45]. To further dissect the role of curcuminoids on adipose tissue inflammation, we investigated the effect of *C. longa* and curcuminoids on human mononuclear cells. It appeared that both *C. longa* and curcuminoids inhibited the pro-inflammatory parameters except for leptin, which was not detected. Leptin is a pro-inflammatory factor that is exclusively produced by adipocytes and therefore it is not detected in the secretome of the mononuclear cells. Our data suggest a crosstalk between adipocytes and macrophages in mediating the anti-inflammatory properties of *C. longa* and

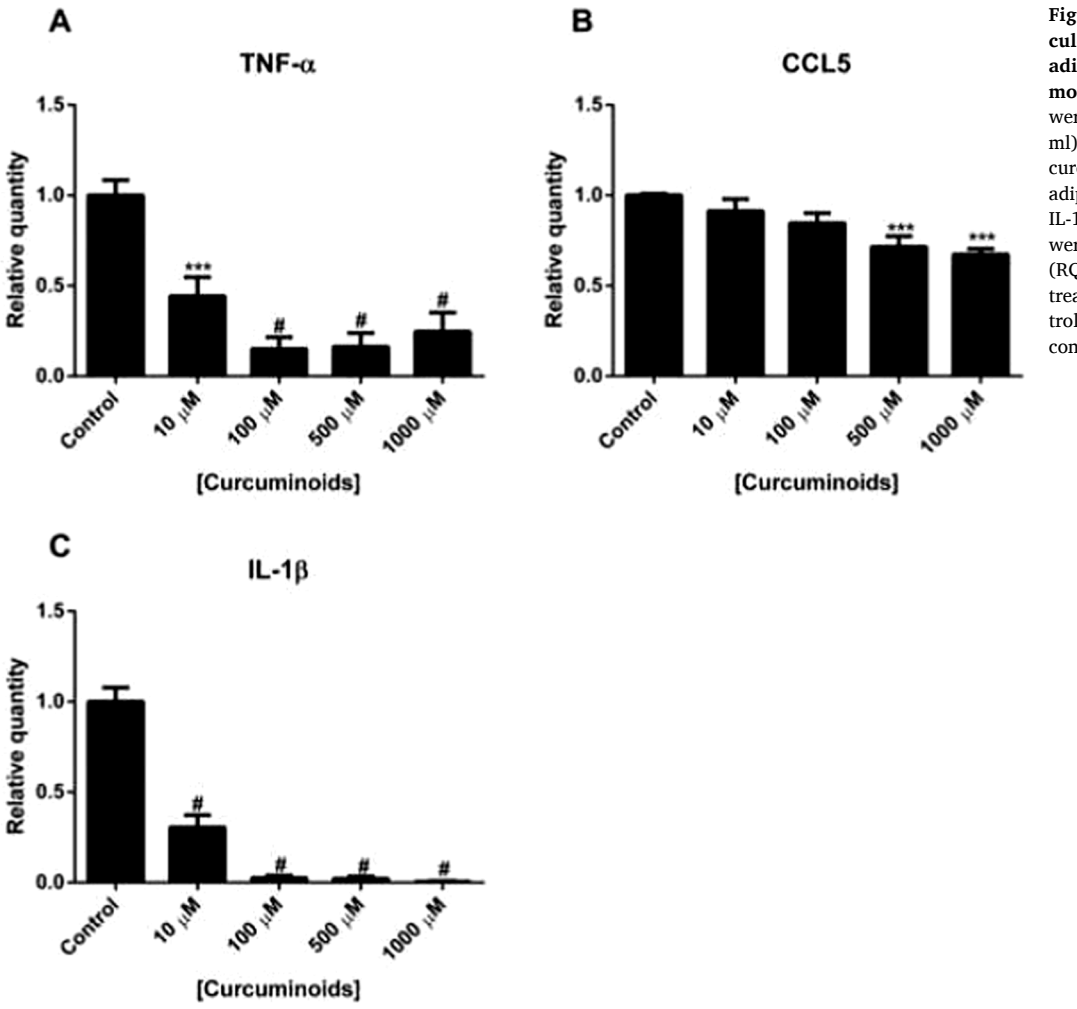

Fig. 4. Effect of curcuminoid molecules on the release of inflammatory adipokines from human induced-mononuclear cells. Mononuclear cells were co-treated with both LPS (10 μg/ml) and 0, 10, 100, 500, 1000 μM of curcuminoids for 24 h. Inflammatory adipokines (A) TNF-α, (B) CCL5 and (C) IL-1β were measured by ELISA. Results were depicted as relative quantities (RQ) compared to the control (LPS treatment without curcuminoids; control). $\#P < 0.0001$ and $***P < 0.001$ *vs.* control. Error bars, SEM.

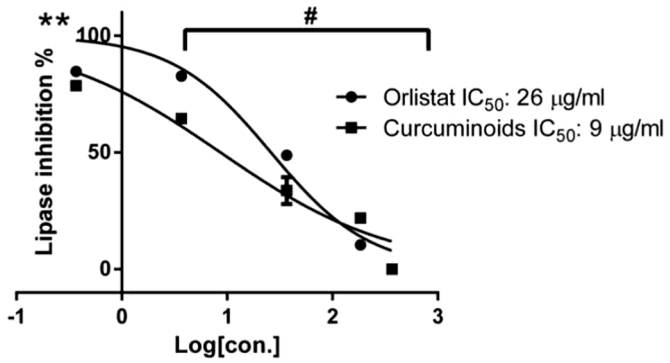

Fig. 5. Dose-response inhibition of porcine pancreatic lipase by curcuminoids. Porcine pancreatic lipase (1 mg/mL) was mixed with various concentrations of curcuminoids (0.37, 3.7, 37, 185 and 370 μg/mL) and incubated at 37 °C for 15 min. Thereafter, substrate solution was added and incubated for 30 min at 37 °C. The absorbance released from the substrate was measured by a spectrophotometer at 410 nm and results were depicted as percentage of lipase inhibition, which was calculated as described in the methods. $\#P<0.0001$ curcuminoids vs. orlistat.

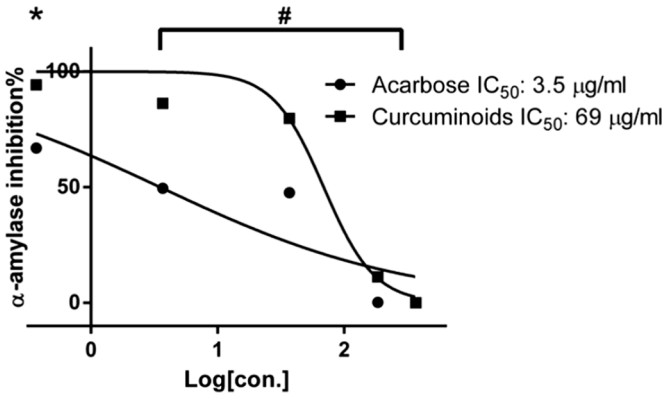

Fig. 6. Dose-response inhibition of porcine pancreatic α-amylase by curcuminoids. Porcine pancreatic α-amylase was mixed with various concentrations of curcuminoids (0.37, 3.7, 37, 185 and 370 μg/mL) and incubated at 37 °C for 10 min. Thereafter, substrate solution was added and incubated for 3 min at 37 °C and the reaction was stopped by DNSA treatment. The absorbance released from the substrate was measured by a spectrophotometer at 540 nm and results were depicted as a percentage of α-amylase inhibition, which was calculated as described in the methods. $\#P < 0.0001$, curcuminoids vs. Acarbose.

curcuminoids on human adipose tissue. Our results are in accordance with animal studies, both *in vitro* and *in vivo* studies, suggesting that most probably curcuminoids have a similar beneficial effect on humans. However, due scarcity and complexity of setting up human studies, this finding has not yet been confirmed. This is supported by the notion that the studies succeeded in showing significant results on anthropometric measures have used the more bioavailable form of curcuminoids or have maintained a longer intervention duration [50].

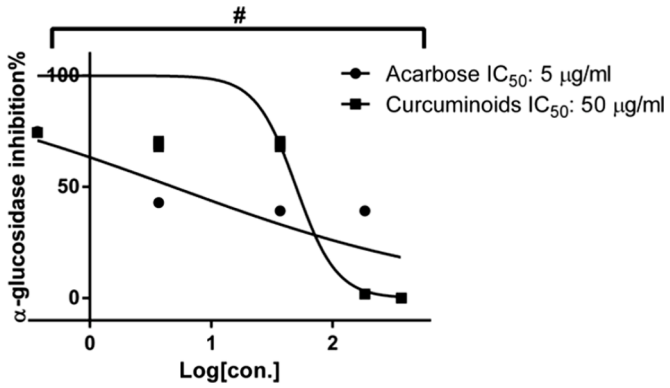

Fig. 7. Dose-response inhibition of porcine pancreatic α-glucosidase by curcuminoids. Porcine pancreatic α- glucosidase was mixed with various concentrations of curcuminoids (0.37, 3.7, 37, 185 and 370 μg/mL) and incubated at 37 °C for 15 min. Thereafter, substrate solution was added and incubated for 20 min at 37 °C and the reaction was terminated by Na_2CO_3 treatment. The absorbance released from the substrate was measured by a spectrophotometer at 405 nm and results were depicted as a percentage of α-glucosidase inhibition, which was calculated as described in the methods. *#P* < 0.0001, curcuminoids vs. acarbose.

Therapies against obesity and its related disorders such as insulin resistance, metabolic syndrome, type II diabetes mellitus, and cardiovascular diseases involve the use of drugs as inhibitors of related enzymes to inhibit the hydrolysis and absorption of dietary carbohydrates and lipids. However, these synthesized drugs are expensive and possess potentially dangerous side effects. For example, ephedra, fenfluramine, phenylpropanolamine, and sibutramine possess cardiovascular toxicities [21] and therefore they were banned from the market. In addition, anti-obesity drugs approved for long-term use by FDA such as orlistat, have shown limited weight loss, approximately 3% for orlistat [51]. Therefore, there is a need to screen for new natural products that inhibit enzymes like α-amylase, α-glucosidase, and lipase and consequently inhibit the release of glucose and lipids into the blood. This approach is preferable for curcuminoids since it avoids the poor solubility and consequently poor bioavailability of curcuminoids in blood, which has hindered its use so far. Our data indicate that curcuminoids has a stronger effect than orlistat medicine as an anti-lipase inhibitor and has a moderate effect against amylase and glucosidase. Although the observed inhibitory activities of curcuminoids against α-amylase and α-glucosidase are moderate, it is a duplicated effect, since α-glucosidase is necessary to degrade disaccharides produced by α-amylase into glucose. Our finding is in agreement with a very recent study where they have demonstrated that curcuminoids inhibit α-glucosidase and α-amylase enzymes *in vitro* and possess hypoglycemic effect in cell assay and in oral glucose tolerance test in animals. However, in human the story is inconclusive. In few studies curcuminoids administration has been shown to improve the level of lipids [25,28,32] and to decrease glucose level [28,32], while in other studies, it has been shown to have no effect on lipids and glucose levels [29,33,34].

In our study, we demonstrate for the first time that *C. longa* and curcuminoids molecules exert anti-inflammatory properties on human abdominal subcutaneous tissue most probably on human immuno-cells. Our data suggest that C. *longa* and curcuminoids molecule can inhibit obesity-induced low-grade inflammation and therefore it may treat or prevent the consequences and comorbidities associated with obesity such as insulin resistance, metabolic syndrome, type II diabetes mellitus and cardiovascular diseases. However, this needs further *in vivo* validation in the future. In addition, our study shows a strong inhibitory effect of *C. Longa* and curcuminoids on α-lipase, α-amylase and α-glucosidase enzymes. This is a preventive approach that acts on enzymes present in the small intestine and does not need to be absorbed and therefore avoids the poor solubility and consequently the poor bioavailability of curcuminoids. Due to the agreement of our study with animal studies we believe that curcuminoids have similar effects on humans *in vivo*, even though earlier human studies are inconclusive. This needs to be further validated in human studies in the future; however, with better-designed studies using more bioavailable forms of curcuminoids and/or longer intervention duration.

Financial support

None.

CRediT authorship contribution statement

Sa'ad Al-Lahham: Conceptualization, Visualization, Writing – original draft, Writing – review & editing, Investigation, Formal analysis, Methodology. **Nidal Jaradat:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Abdallah Hamayel:** Investigation, Data curation, Formal analysis, Methodology, Writing – review & editing, Writing – original draft. **Abdalrahman Assaassa:** Investigation, Data curation, Formal analysis, Methodology, Writing – review & editing, Writing – original draft. **Faris Hammad:** Investigation, Data curation, Formal analysis, Methodology, Writing – review & editing, Writing – original draft. **Ahmed Mosa:** Investigation, Data curation, Formal analysis, Methodology, Writing – review & editing, Writing – original draft. **Fouad Nafaa:** Data curation, Methodology, Writing – review & editing, Writing – original draft. **Mustafa Ghanim:** Data curation, Methodology, Writing – review & editing, Writing – original draft. **Majdi Dwikat:** Data curation, Methodology, Writing – review & editing, Writing – original draft. **Malik AlQub:** Data curation, Methodology, Writing – review & editing, Writing – original draft. **Ahmad Abdal Rahim:** Investigation, Data curation, Formal analysis, Methodology, Writing – review & editing, Writing – original draft. **Abdelkarem Barqawi:** Data curation, Methodology, Writing – review & editing, Writing – original draft.

Declaration of Competing Interest

No conflict of interest was declared by the authors. Dr. Nidal Jaradat is an associate editor of the European Journal of Integrative Medicine, which is worth noting.

Acknowledgement

We are grateful to An-Najah National University for providing financial assistance for this work, which was made feasible via an undergraduate project. We also thank Rafidia and An-Najah National University Hospital for supplying us with human adipose tissue samples.

Data availabilty

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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