Egypt. J. Chem. **Vol. 65,** No. 2 pp. 539 - 550 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Antioxidant, Anti-Cancer and Anti-Arthritic Activities of Acetogenin-Rich Extract of Avocado Pulp

Mervat Hassan[a], Samir Abdel Moneim[a], Ebtesam A. Mahmoud[a], Doha A. Mohamed[b*]

[a] *Biochemistry Department, Faculty of Agriculture, Cairo University, Cairo, Egypt*

[b] *Food Sciences and Nutrition Department, National Research Centre, Dokki, Cairo, Egypt*

Abstract

Avocado is an oil-rich fruit pleasant organoleptic characteristic, high nutritional value and health benefits due to its content of bioactive molecules. The objective of the current study was assessment of the antioxidant, anti-cancer and anti-arthritic activities of acetogenin-rich extract of avocado pulp. Total phenolic, total flavonoids, fatty acids profile, phytosterols and hydrocarbons of the extract were determined. Acetogenin was detected using GC-MS. The antioxidant activity of the extract was determined using DPPH, ABTS and FRAP assay. The cytotoxicity of the extract was assessed in different cancer cell lines (liver, colorectal and breast carcinoma cells). The anti-arthritic activity of the extract was evaluated in adjuvant induced arthritis (AIA) model in rats. The results revealed that acetogenin-rich extract of avocado contains phenolic compound (58.6±0.579 mg GAE/g extract) and total flavonoids (10.8±0.449 mg QE/g extract). GC-MS analysis revealed the presence of persediene as one of acetogenin compounds by 1.1% in the extract. The extract possessed antioxidant activity in all the studied methods and the maximum activity was against ABTS free radical. The IC50 of the extract against hepatocellular carcinoma cells (HEPG2), breast adenocarcinoma cells (MCF7) and colorectal cancer cells (HT29) was 8.1, 52.1 and 11.3 µg/ml, respectively. The extract showed significant anti-arthritic activity in rats through reduction of inflammation volume, improvement in the antioxidant status of arthritic rats as observed by reduction of plasma levels of malondiadehyde and elevation of the antioxidant enzymes (SOD, catalase and glutathione peroxidase) in association with reduction of the inflammatory marker TNF-α. The extract showed significant improvement in the histopatological studies of the joint tissue of arthritic rats. **In conclusion:** Acetogenin-rich extract of avocado pulp can be used as alternative treatment for prevention or treatment of cancer and rheumatoid arthritis due to its antioxidant and anti-inflammatory activities.

Keywords: Avocado pulp, Antioxidant, Anti-cancer, Anti-arthritic, Acetogenin-rich extract.

1. Introduction

Chronic diseases that comprise Alzheimer's disease, rheumatoid arthritis (RA), cancer, cardiovascular diseases (CVD) and diabetes persists the main reason of mortality and disability globally and accounting for 60% of all deaths [1]. Oxidative stress is the main root of various chronic diseases, which arises due to the disturbance in balance between body's oxidants and antioxidants [2]. Inflammation, acute and chronic, also plays an important role in the development of chronic diseases [3]. Numerous inflammatory markers are secreted in chronic diseases such as NF-κB and inflammatory cytokines (TNF-α, IL-6) [4]. Oxidative stress (free radicals) is the main effectors of inflammatory response and both of them enhance the development of chronic diseases especially cancer and RA [5]. RA is an autoimmune disease characterized by the production of inflammatory cytokines, which lead to joint destruction [1]. Till now all drug used for treatment of chronic diseases are very expensive and leads to adverse side effects [6]. So, searching for safe, highly efficacious natural sources for protection and management of chronic diseases is a very important goal. Plant kingdom is the richest source of bioactive compounds exhibited different health benefits towards chronic diseases [1].

Avocado (*Persea americana Mill.* Family Lauraceae) is an oil-rich fruit. The fruit is pleasant organoleptic characteristic, high nutritional value and health benefits due to its content of bioactive molecules [7]. Avocado pulp is very rich in unsaturated fatty acids (60% oil, mainly

*Corresponding author e-mail: dohamohamed@yahoo.com.; (dohamohamed).

Receive Date: 11 August 2021, Revise Date: 09 November 2021, Accept Date: 01 September 2021

DOI: 10.21608/EJCHEM.2021.90308.4309

monounsaturated fatty acids), vitamin B, C, and E, and several nutrients such as potassium and dietary fibers [8]. Avocado oil has sparked a growing interest in human nutrition, food industry, and cosmetics [9]. Major bioactive compounds present in avocado include phenolic compounds (such as hydroxycinnamic acids, flavonoids and proanthocyanins), acetogenins, phytosterols, carotenoids and alkaloids [10, 11]. The bioactive compounds separated from avocado have a role in scavenging free radicals [12]. Acetogenins, present in avocado leaves and fruits, are fatty acid derivatives contain an odd-carbon aliphatic chain (17, 19 or 21) and an acetoxy group that contributes two additional carbons [13]. The objective of the current study was assessment of the antioxidant, anti-cancer and anti-arthritic activities of acetogenin-rich extract of avocado pulp. To fulfil the aim total phenolic, total flavonoids, fatty acids profile, phytosterols and hydrocarbons of the extract were determined. Acetogenin was detected using GC-MS. The antioxidant activity of the extract was determined. The cytotoxicity of the extract was evaluated in different cancer cell lines. The effect of acetogenin-rich extract of avocado pulp against adjuvant induced arthritis was assessed.

2. Materials and Methods

2.1. Preparation of acetogenin-rich extract of avocado pulp

Avocado fruits were purchased from local markets in Egypt. Avocado fruits were washed by tap water, peeled, sliced into thin slices and freeze-dried. The freeze-dried sample of avocado fruits pulp was extracted using acetone for preparation of acetogenin-rich extract of avocado fruits pulp [13]. The solvent was completely removed by evaporation under reduced pressure at a temperature not exceeding 40 °C. Extract were kept in deep-freeze till used.

2.2. Determination of phenolic compounds and total flavonoids in acetogenin-rich extract of avocado pulp

Total phenolics content was determined colorimetrically in the acetogenin-rich extract of avocado pulp using Folin-Ciocalteu reagent [14]. Total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g sample. Total flavonoids content of acetogenin-rich extract of avocado pulp was determined by the aluminium chloride colorimetric method [15]. The total flavonoids content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per g (QE). The results were expressed as Mean±SD for three replicates.

2.3. Assessment of hydrocarbons and phytosterols contents of acetogenin-rich extract of avocado pulp

The unsaponifiable fraction of acetogenin-rich extract of avocado pulp was prepared according to AOAC [16] to be subjected to GLC analysis of hydrocarbons and phytosterols.

Hydrocarbons and phytosterols were analyzed by GLC adopting the following conditions: Column: 10% OV-101 packed column; Stationary phase: Chromosorb W-HP; Detector temperature: 290°C; Injector temperature, 28°C; Carrier gas N_2; flow-rate 30 ml/min; air flow-rate: 300ml/min; H_2 Flow-rate 30ml/min; Detector FID; Chart speed: 0.5 cm/min; Oven program: Initial temperature, 70°C; Final temperature, 270°C; programmed 4°C/min. For 35min at 270°C, total time, 85 min. Identification of hydrocarbons and phytosterols contents of the unsaponifiable matter was carried out by comparison of their retention times with co-injected authentic reference compounds. Quantization was based on peak area integration.

2.4. Assessment of fatty acids of acetogenin-rich extract of avocado pulp

Fatty acid methyl esters of acetogenin-rich extract of avocado pulp was prepared according to AOAC [16] to be subjected to GLC analysis of fatty acids. Assessment of the methyl ester was carried out by injecting 2µl into a Hewlett Packard HP-system 6890 gas chromatograph equipped with FID. HP-5 capillary column (30 m x 0.32 mm i.d.; 0.25 um film thickness) was used to separate the different methyl esters. The chromatographic analysis conditions were: initial temperature 70 °C with a hold for 1 min, then rose to 120 °C at a rate of 40 °C /min with 2 min hold then the temperature was finally raised to 220 °C at a rate of 4 °C /min with another 20 min hold. The injector and detector temperatures were 250°C and 280 °C respectively. Identification of the fatty acid methyl esters were carried out by direct comparison of retention times of each of the separated compounds with standards of the fatty acid methyl esters analysed under the same conditions. Quantization was based on peak area integration.

2.5. Gas chromatography–mass spectrometry (GC-MS) analysis of acetogenin-rich extract of avocado pulp

Acetogenin-rich extract of avocado pulp was subjected to GC-MS. The GC-MS analysis were carried out using gas chromatography-mass spectrometry instrument stands with the following specifications, Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass

Spectrometer).The GC-MS system was equipped with a TR-5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 60 C for 1 min; rising at 4.0 C/min to 240 C and held for 1min. The injector and detector were held at 210oC. Diluted samples (1:10 hexane, v/v) of 1µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. The identification of the chemical constituents of acetogenin rich extract of avocado pulp was de-convoluted using AMDIS software (www.amdis.net) and identified by its retention indices (relative to n-alkanes C8-C22), mass spectrum matching to (authentic standards (when available)., Wiley spectral library collection and NSIT library database)**.**

2.6. Determination of antioxidant activity of acetogenin-rich extract of avocado pulp

Antioxidant activity of acetogenin-rich extract of avocado pulp was assessed on the basis of the scavenging activity of the stable radicals 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [17] and 2, 2-azinobis (2-ethyl-benzolhiaxoline-6-sulfonic acid) (ABTS) [18]. Also the ferric reducing ability assay (FRAP) was carried out according to the method of Benzi *et al*. [19], with minor modifications to be carried out in microplates, briefly; A freshly prepared tripyridyltriazine (TPTZ) reagent (300 mM Acetate Buffer (PH=3.6), 10 mM TPTZ in 40mM HCl, and 20 mM $FeCl_3$, in a ratio of 10:1:1 v/v/v, respectively). 190 µL from the freshly prepared TPTZ reagent were mixed with 10 µL of the sample in 96 wells plate (n=3), the reaction was incubated at room temperature for 30 min in dark. At the end of incubation time the resulting blue color was measured at 593 nm. Data are represented as means ± SD of three replicates.

The inhibition % in all the studied methods was calculated according to the following equation

Inhibition %= [A0 - A1 / A0] × 100.

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample.

2.7. Anticancer activity of acetogenin-rich extract of avocado pulp

Anticancer activity of acetogenin-rich extract of avocado pulp was tested using the cell line technique according to Cordero *et al*. [20]. Cells of HEPG2 (liver carcinoma cell), MCF7 (breast cancer cell) and HT29 (Colorectal cancer cell) were plated in 96-multiwell plate (10^4 cells/well) for 24h before treatment to be attached to the wall of plate. The extract was dissolved in dimethyl sulfoxide (DMSO) at 10 mM as a stock solution. Dilutions with culture media were prepared just prior to addition to test plates. Different concentration of the extract (0, 0.03, 0.3, 3, 30 and 300 µg/ml) were added to the cell monolayer, triplicate wells were prepared for each individual dose of each plant powder. Plates were incubated for 48h at 37 °C and in atmosphere of 5% CO_2. After 48h, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain, then excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction of cancer cell and plants powder dose was plotted. IC_{50} (concentration which reduces survival of the exposed cancer cells to 50%) was obtained from the curves.

2.8. Animals

Male Wister rats of 147.3 ± 4.258g as mean ± SD were obtained from the animal house of the National Research Centre, Cairo, Egypt. Animals were kept individually in stainless steel cages under standard laboratory conditions (23–25 °C, 12 h light/dark cycle) and with free access to diet and water. All animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Cairo, Egypt, and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.9. Animals' diet

Balanced diet (12% casein as a protein source, 10% corn oil, 10% sucrose, 58.5% maize starch, 5% fiber, 3.5% salt mixture, and 1% vitamin mixture), salt and vitamin mixtures were prepared in accordance with AIN-93 [21].

2.10. Evaluation of anti-arthritic effect of acetogenin-rich extract of avocado pulp

Twenty-four rats were divided into 4 groups each comprised six rats. Group one was served as normal control. Group two were served as arthritic control. Group three and four were given oral dose of acetogenin-rich extract of avocado pulp as 150 and 300 mg/kg rat body weight for three weeks. All rats groups were feed on balanced diet all over the study period. At the second day of study all rats groups were injected with Freund's complete adjuvant into the subplantar region of the right hind-foot paw [22] except group one which served as normal control. To increase the severity of arthritis 0.1 ml of Freund's complete adjuvant were injected in the paw at day five from the first injection in all rats except normal control [23]. The inflamed paws of all arthritic rats were measured before induction of arthritis and at the end of study using vernier calipers. Inflammation volume was calculated for all arthritic rats by subtracting the foot volume in the final of the experiment from its volume in the starting of the induction. Food intake and body weight of all rats were recorded weekly. Blood

samples were collected from all rats at the end of the study after overnight fasting for determination of plasma tumor necrosis factor-α (TNF-α) (ELISA kit, Catalogue # SL0722Ra, Sunlong®) an inflammatory marker and malondialdehyde (MDA) [24] as indicator of lipid peroxidation. Catalase activity [25], superoxide dismutase (SOD) (ELISA kit, Catalogue # SL1341Ra, Sunlong®) and glutathione peroxisdase (GPx) (ELISA kit, Catalogue # SL1033Ra, Sunlong®) were evaluated as indicator of antioxidant status. Plasma level of creatinine [26], uric acid [27] and urea [28] were determined as indicator of kidney function, while the activity of transaminases [29] aspartate transaminase (AST) and alanine transaminase (ALT) were determined as indicator of liver function.

2.11. Histopathology studies

Samples were obtained from the rat paw at the end of the study. Joint tissue was fixed in neutral-buffered formalin for 48 h at room temperature, decalcification, embedded in paraffin and then sections were routinely deparaffinized by normal method, then cut into 4μm –slice and stained with hematoxylin and eosin (HE) and evaluated for morphological changes and cellular infiltration [30].

2.12. Statistical analysis. The results of animal experiments were expressed as Mean±SE and they were analyzed statistically using one-way analysis of variance ANOVA followed by Duncan's test.

2. Results and Discussion

3.

3.1. Total phenolic content and total flavonoids of acetogenin-rich extract of avocado pulp

The total phenolic content and total flavonoids of acetogenin-rich extract of avocado pulp are presented in Table 1. Acetogenin-rich extract of avocado pulp contains 58.6±0.579 total phenolic compounds as mg GAE/g extract and 10.8±0.449 total flavonoids as mg QE/g extract. Avocado fruits contain 200 mg GAE/100g, this level is a moderate level of phenolic content according to Dreher and Davenport [31].

Table (1): Total phenolic and total flavonoids content of acetogenin rich extract of avocado pulp.

Parameters	Acetogenin-rich extract of avocado pulp
Total phenolic content (mg GAE/g extract)	**58.6±0.579**
Total flavonoids (mg QE/ g extract)	**10.8±0.449**

3.2. Fatty acids profile, hydrocarbon andphytosterols of acetogenin-rich extract of avocado pulp

Table 2 showed hydrocarbons and phytosterols of acetogenin-rich extract of avocado pulp. Tetracosane (C24) was the highest hydrocarbons (37.93%) present in the extract, while dodecane (C12) was the lowest hydrcarbon (0.21%) present in the extract. Total hydrocarbons were present in the extract by 79. 06%. Total phytosterols were present in the extract by 8.63%, whereas stigmasterol was the highest one (6.92%) followed by β-sitosterol (1.71%). Phytosterols are one of the most important classes of unsaponifiable matter components [32]. Nuts and oily seeds are the main source of phytosterols [33]. Avocados are the richest known fruit source of phytosterols [34] with about 87 mg/100g [31]. The present results are in agreement with the results of Law and Alkhalaf *et al*. [8, 35]. These authors reported the presence of β-sitosterol and stigmasterol in the unsaponifible matter of the avocado fruit oil.

Table 3 showed the fatty acids present in acetogenin-rich extract of avocado pulp. The results revealed that oleic acid was the major unsaturated fatty acid it was present by 67.42%, while linolenic acid (ω-3) was the lowest unsaturated fatty acid (0.92%) present in the extract. Palmitic acid was the only saturated fatty acid present in the extract by 13.31%. Total unsaturated fatty acids were present in the extract by 81.91%. It was reported previously by Alkhalaf *et al*. [8] that oleic acid was the main fatty acid present in avocado oil. A typical fatty acid profile composed of palmitic (C16:0) 10-25%, oleic (C18:1) 60-80%, linoleic (C18:2) 7-20%, and linolenic (C18:3) 0.2-1% acids of avocado oil was observed by some authors [36, 37].

3.3. Gas chromatography–mass spectrometry (GC-MS) analysis of acetogenin rich extract of avocado pulp

The results of GC-MS revealed that acetogenin rich extract of avocado pulp contain persediene ($C_{21}H_{36}O_4$) by 1.1% and it's appeared at Rt 29.35. Persediene is one of acetogenin compound which reported previously in avocado fruit [13].

3.4. Antioxidant activity of acetogenin-rich extract of avocado pulp

In the present study antioxidant activity of acetogenin-rich extract of avocado pulp was assessed using three different methods; DPPH,

ABTS and FRAP assay (Table 4). The extract possesses antioxidant activity in all the studied methods. The extract was highly effective in scavenging ABTS free radical followed by FRAP assay. The lowest activity of the extract was against DPPH free radical. The present results are in agreement with many previous studied that reported the highest antioxidant activity of avocado oil against free radicals [8, 9, 38, 39]. Avocado fruit has a total antioxidant capacity of 1350 µmol Trolox Equilvalent (TE) per one-half fruit [31].

Table (2): GLC analysis of hydrocarbons and phytosterols of acetogenin-rich extract of avocado pulp

Hydrocarbon & Phytosterols	Acetogenin-rich extract of avocado pulp
Hydrocarbon:	
Dodecane (C10)	-
Undecane (C11)	0.68*
Dodecane (C12)	0.21
Tridecane (C13)	-
Tetradecane (C14)	-
Pentadecane (C15)	0.24
Hexadecane (C16)	0.98
Heptadecane (C17)	-
Octadecane (C18)	1.21
Nonadecane (C19)	2.00
Eicosane (C20)	13.35
Heneicosane (C21)	13.03
Docosane (C22)	4.11
Tetracosane (C24)	37.93
Hexacosane (C26)	0.81
Heptacosane (C27)	-
Octacosane (C28)	4.51
Nonacosane (C29)	-
Total hydrocarbon	79.06
Phytosterols:	
Stigmasterol	6.92
β-Sitosterol	1.71
Total phytosterols	8.63

*: Values are expressed as relative area percentage of total hydrocarbons and phytosterols.

Table (3): Fatty acids contents of acetogenin-rich extract of avocado pulp.

Fatty acids	*Acetogenin-rich extract of avocado pulp
Plamitic acid: C16 (0)	13.31
Oleic acid: C18 (1)	67.42
Linoleic acid: C18(2)	13.57
Linolenic acid:C18 (3)	0.92
Total identified saturated fatty acids	13.31
Total identified monounsaturated fatty acids	67.42
Total identified polyunsaturated fatty acids	14.49
Total identified polyunsaturated fatty acids	81.91

*:Values are expressed as relative area percentage of total fatty acids.

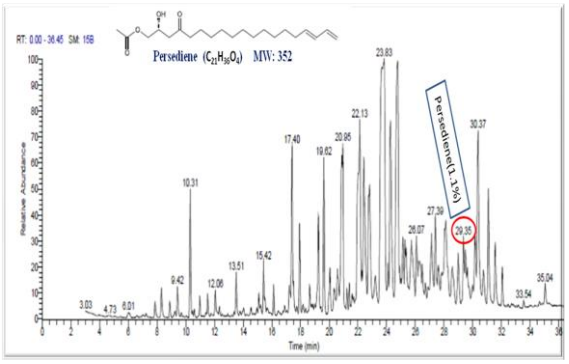

Figure (1): GC-MS chart of acetogenin-rich extract of avocado pulp

Table (4): Antioxidant activity of acetogenin rich extract of avocado pulp.

Parameters	Acetogenin-rich extract of avocado pulp (Mean ± SD)
DPPH IC50 (µg/ml)	1150±4.691
ABTS (µM TE /equivalent)	67.13±11.15
FRAP (µM TE /equivalent)	82.86±6.64

3.5. Anticancer activity of acetogenin-rich extract of avocado pulp

Table 5 showed the IC50 dose of acetogenin-rich extract of avocado pulp in different types of cancer cells. The results revealed that acetogenin-rich extract of avocado pulp showed anti-cancer activity against liver carcinoma cells (HEPG2), breast cancer cells (MCF7) and colorectal cancer cells (HT29). The IC50 of acetogenin-rich extract of avocado pulp against HEPG2 cells, MCF7 cells and HT29 cells was 8.1, 52.1 and 11.3 µg/ml, respectively. Cancer represents a serious health worldwide problem and its incidence is increased in Egypt and may reach three times starting from 2013 to 2050 [40]. Searching for new cytotoxic compounds is necessary, especially when come from natural sources. The cytotoxic effect of acetogenin-rich extract of avocado pulp may be attributed to the presence of phenolic compounds, flvonoids, fatty acids and acetogenin (persediene C21). It was reported previously that oleic acid and linoleic acid, which present in the studied extract possess apoptosis in human colon cancer cells HT-29 and Caco-2 [41]. Acetogenin showed cytotoxic activity against different types of cancer cell lines such as breast, colon, prostate and pancreas [42]. Persin as one of acetogenin compounds inhibited the proliferation of breast cancer cell lines [43]. An aliphatic acetogenin (C17) inhibited the proliferation of oral cancer cells 83-01-82CA [44]. Phenolic compounds are established to exhibit potent anti-cancer activities as well as combat various diseases associated with oxidative stress. The anti-cancer activity of phenolic compounds is due to its activity as antioxidant through modulation

of free radicals, inhibition of oncogenic signalling cascades, angiogenesis and apoptosis [45].

Table (5): IC50 of acetogenin rich extract of avocado pulp in different types of cancer cells.

Cancer cells	IC50 of acetogenin rich extract of avocado pulp (µg/ml)
Hepatocellular carcinoma cells HEPG2	**8.1**
Breast Adenocarcinoma cells MCF7	**52.1**
Colorectal cancer cells HT29	**11.3**

3.6. Anti-arthritic activity of acetogenin-rich extract of avocado pulp

In the present study the anti-arthritic activity of acetogenin-rich extract of avocado pulp was assessed in adjuvant induced arthritis in rats, which is a model mimic to rheumatoid arthritis in human. Arthritis was induced in rats by injection of Freund's Complete Adjuvant (FCA). In many previous studied a single dose of FCA was injected in the right hind paw of rats [22, 46-48], in the present study for increasing the severity of rheumatoid arthritis two doses of FCA were injected in the right hind paw of rats in the second and the six day from the beginning of the study [23]. Nutritional and biochemical parameters of the different experimental groups are presented in table 6. Induction of arthritis in rats produced significant reduction in final body weight, body weight gain in all the arthritic rats compared with normal group. Administration of acetogenin-rich extract of avocado pulp in the low and high dose improved the reduction observed in the final body weight and body weight gain in comparison to arthritic control (Table 6). It was reported previously that AIA lead to decrease in body weight gain and final body weight in the arthritic rats [22, 47,48]. Reduction in body weight of arthritic rats has been attributed to rheumatoid cachexia that appeared due to the increase of pro-inflammatory cytokines levels such as TNF-α and IL-1β [49]. Spleen weight and spleen % elevate significantly in arthritic rats compared with normal rats. Low and high dose of acetogenin-rich extract of avocado pulp showed significant decrease in spleen weight and spleen % compared with arthritic control but still significantly higher than normal control. The present results are in agreement with the results of Chen *et al*. [50]. Spleen is important organ for immune response and regulation, which acts to produce immune cells and filters the dead cells [50, 51]. Increase in the organ index of spleen in arthritic rats could be explained with the filtration of immune cells along with dead RBCs and WBCs (Chen *et al*., 2020).

In the present study injection of rats with Freund's complete adjuvant leads to significant elevation of the activity of liver enzymes (AST & ALT) and kidney function indicators (uric acid, urea and creatinine), respectively compared with normal rats (Table 6). As shown in table 6 FCA injection enhances inflammation as shown by elevation of TNF-α as an inflammatory marker. The antioxidant status of arthritic rats was significantly disturbed through elevation of formation of MDA as lipid peroxidation marker in association with reduction of plasma levels of antioxidant enzymes (SOD, Gpx and catalase) compared with normal rats (Table 6). The results revealed that arthritic rats were under oxidative stress due to FCA injection. The administration of high and low dose of acetogenin-rich extract of avocado pulp significantly reduced the increase in the liver enzyme, kidney function indicators. Also significantly improved the inflammation and antioxidant status of arthritic rats compared with arthritic control but still higher than normal control. The high dose of the extract was superior in all the studied biochemical parameters.

Disturbance in oxidative stress is considered as major risk in arthritis. Prevention of the information of oxidative stress has been reported to improve arthritis in human and animal models [22, 52, 53]. Hou *et al*. [54] reported that FCA injection produces free radical, oxygen and hydrogen peroxide, which leads to inflammatory cells such as granulocytes and macrophages. Inflammation and oxidative stress in the ankle joints leads to formation of lipid peroxidation [54] as shown by elevation of MDA which produce extreme injury in tissues. Inflammation of the cells leads to release of inflammatory cytockines [55] as observed in the present study by elevation of TNF-α. In the present study the reduction in the activities of antioxidant enzymes may be attributed to degradation of SOD, inactivation of catalase and consuming of GPx by hydrogen peroxide and other free radicals produce during inflammation [56, 57].

In the present research injection of FCA in rat's hind paws showed significant increase the volume of paws of arthritic control (Table 7) compared with rats given oral administration of the low and high dose of acetogenin-rich extract of avocado pulp. The measurement of paw swelling is clearly easy, critical, and fast process for determine the degree of inflammation and assessing curative effects of different treatments.

The injected paws of all arthritic rats were swelling, redness and erythema compared with the paw not injected in the same rat. The present research revealed that both dosses of acetogenin-rich extract

of avocado pulp reduced the inflammation noticed by changes in inflammatory parameters of inflammation volume and paw volume. The present results are in accordance with many previous studies results [22, 23, 48], which reported significant elevation of injected rats paws volume.

Table (6): Nutritional and biochemical parameters of different experimental groups.

Parameters	Normal control	Arthritic control	Avocado pulp extract low dose	Avocado pulp extract high dose
Initial body weight (g)	147.2^{a} ±2.242	147.3^{a} ±0.954	147.5^{a} ±1.707	147.2^{a} ±2.242
Final body weight (g)	182.2^{b} ±3.102	173.8^{a} ±1.869	181.5^{b} ±2.029	181.3^{b} ±2.246
Body weight gain (g)	35.0^{b} ±1.879	26.5^{a} ±1.176	34.0^{b} ±1.591	34.2^{b} ±1.701
Spleen weight (g)	1.08^{a} ±0.031	1.34^{c} ±0.043	1.25^{b} ±0.071	1.21^{b} ±0.071
Spleen %	0.597^{a} ±0.015	0.771^{c} ±0.026	0.692^{c} ±0.042	0.669^{b} ±0.037
ALT (U/l)	8.7^{a} ±1.229	14.5^{c} ±1.118	9.3^{b} ±0.667	9.0^{b} ±0.683
AST (U/l)	56.8^{a} ±2.468	73.0^{a} ±1.897	59.8^{a} ±1.536	58.0^{a} ±2.279
Creatinine (mg/dl)	0.654^{a} ±0.025	0.792^{c} ±0.005	0.682^{b} ±0.016	0.675^{b} ±0.014
Urea (mg/dl)	38.1^{a} ±0.60	42.9^{c} ±0.961	39.1^{b} ±0.546	38.3^{a} ±0.791
Uric acid (mg/dl)	2.25^{a} ±0.041	2.4^{b} ±0.045	2.3^{a} ±0.033	2.28^{a} ±0.038
MDA (nmol/ml)	5.89^{a} ±0.319	10.0^{e} ±0.373	7.55^{c} ±0.388	6.97^{b} ±0.365
Catalase (U/L)	876.0^{d} ±6.097	605.8^{a} ±8.084	761.7^{b} ±11.375	825.8^{c} ±11.134
GPx (IU/ml)	48.8^{b} ±1.424	37.5^{a} ±1.668	43.8^{b} ±1.327	48.8^{b} ±1.469
SOD (/ml)	11.3^{d} ±0.367	2.25^{a} ±0.112	8.77^{b} ±0.272	9.5^{c} ±0.428
TNF-α (pg/ml)	17.8^{d} ±1.434	40.5^{a} ±1.335	24.2^{b} ±0.477	22.7^{c} ±0.558

In each row same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability.

Table (7): The thickness of the injected foot before and after 21 days of adjuvant arthritis induction of arthritic rats given acetogenin-rich extract of avocado pulp.

Parameters	Arthritic control	Acetogenin-rich extract of avocado pulp low dose	Acetogenin-rich extract of avocado pulp high dose
Rat paw initial	**0.333^{a} ±0.011**	**0.317^{a} ±0.011**	**0.317^{a} ±0.011**
Rat paw volume (mm)	**0.808^{c} ±0.024**	**0.508^{b} ±0.008**	**0.433^{a} ±0.011**
Inflammation volume (mm)	**0.475^{c} ±0.017**	**0.192^{b} ±0.015**	**0.117^{a} ±0.011**
Inhibition %	**-**	**59.6**	**75.4**

In each row same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability.

3.7. Histopathological studies

The results of photomicrographs of rat's paws section from different experimental groups are presented in figures 2 and 3. The results of rat paw sections from normal control (Figure 2 A, B & C) showed normal skin (arrow), normal subcutaneous tissue (arrow head), normal metatarsal joint (Fig. 2 A), thin epiderm (arrow), normal subcutaneous tissue (arrow head), normal metatarsal joint (*) (Fig. 2 B), normal joint with normal synovial membrane (Sm) of thin synovial cells and normal bone marrow (Bm) with no leucocytic infiltration or bone erosions sub (Fig. 2 C). From the photomicrographs of rat paw sections of arthritic control (Figure 2 D, E & F) we can noted the keratinized thick epidermis (*) with underlying loose subepidermal zone infiltrated by chronic inflammatory cells (arrow) (Fig. 2 D), the cartilage damage (c), bone erosion (*), inflammatory cells (arrow), and bone marrow degeneration (Bm) (Fig. 2 E), sever inflammation with congested blood vessel (arrows) and leucocytic cells infiltration (*) (Fig. 2 F). Paws sections of rats given low oral dose (150 mg/Kg RBW) of acetogenin-rich extract of avocado pulp (Figure 3 G, H & I) revealed regression in the thick epidermis to a normal thin layer (arrow) (Fig. 3 G), moderate cartilage regeneration (c), minimal bone erosion (*), focal

inflammatory cells (arrow), bone marrow regeneration (Bm) (Fig. 3 H), regression in subepidermal inflammatory reaction to focal mononuclear inflammatory cells infiltration (*) (Fig. 3 I). The paws sections of rats administered with high dose (300 mg/Kg RBW) of acetogenin-rich extract of avocado pulp (Figure 3 J, K & L) showed normal thin epidermis (arrow) and skin appendages (*) (Fig. 3 J), normal skin layers with absence of subepidermal inflammatory reaction (*) (Fig. 3 K), marked reduction in the inflammatory cells infiltration, preserved articular cartilage (C), regular bone marrow (Bm), and reduced synovial hyperplasia (Fig. 3 L). The present results are in accordance with the results of many previous studies [49, 58-60].

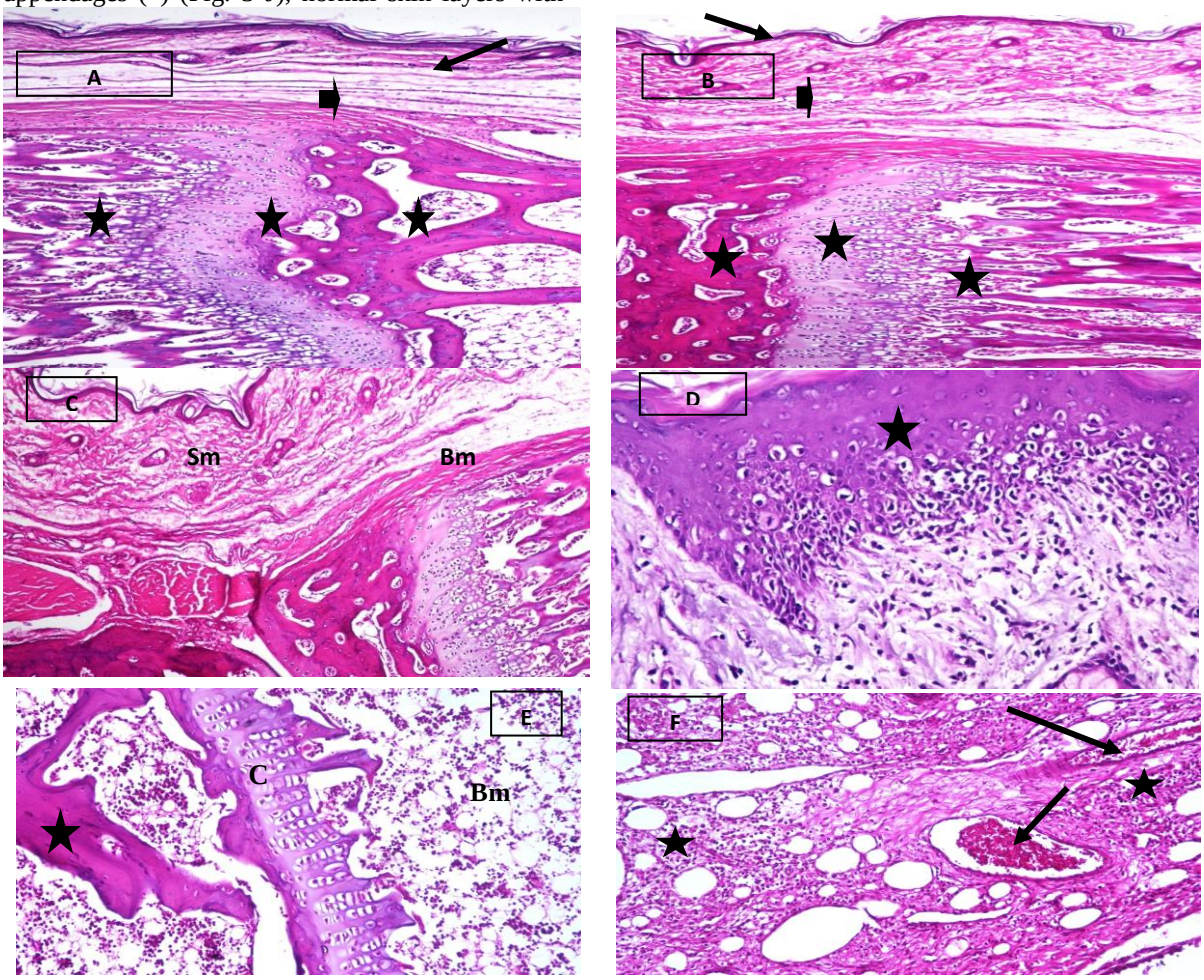

Figure (2): Photomicrographs for rat paw section from normal and arthritic control groups.
Figure (2 A): Photomicrographs for rat paw section from normal control group showed normal skin (arrow), normal subcutaneous tissue (arrow head) and normal metatarsal joint (*), (H&E X200).
Figure (2 B): Photomicrographs for rat paw section from normal control group showed thin epiderm (arrow), normal subcutaneous tissue (arrow head) and normal metatarsal joint (*), (H&E X200).
Figure (2 C): Photomicrographs for rat paw section from normal control group showed normal joint with normal synovial membrane (Sm) of thin synovial cells, normal bone marrow (Bm) with no leucocytic infiltration or bone erosions sub, (H&E X200).
Figure (2 D): Photomicrographs for rat paw section from arthritic control group showed keratinized thick epidermis (*) with underlying loose subepidermal zone infiltrated by chronic inflammatory cells (arrow), (H&E X200).
Figure (2 E): Photomicrographs for rat paw section from arthritic control group showed cartilage damage (c), Bone erosion (*), inflammatory cells (arrow), and bone marrow degeneration (Bm), (H&E X200).
Figure (2 F): Photomicrographs for rats paw section from arthritic control group showed sever inflammation with congested blood vessel (arrows) and leucocytic cells infiltration (*). (H&E X200).

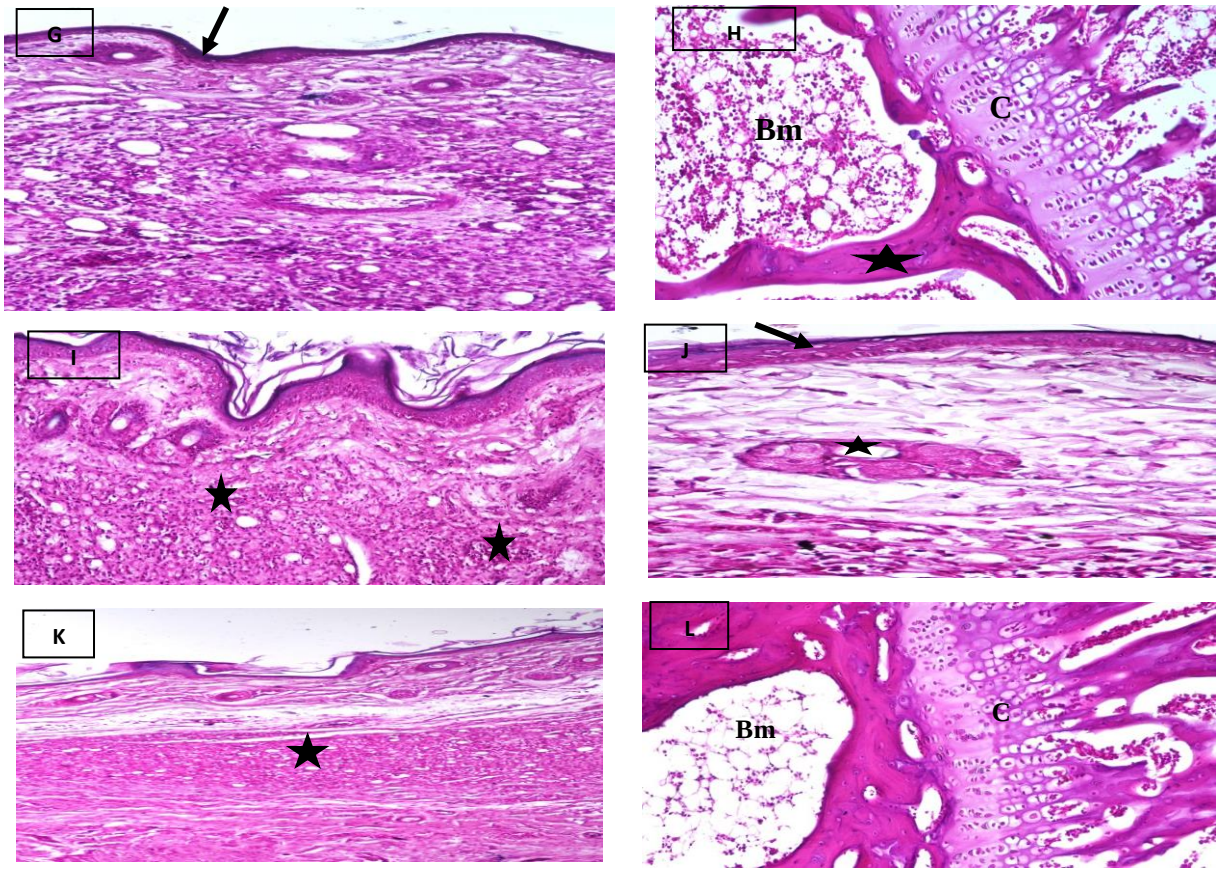

Figure (3): Photomicrographs for rat paw section from rats given low and high dose of acetogenin-rich extract of avocado pulp.
Figure (3 G): Photomicrographs for rat paw section from rats group given low dose of acetogenin-rich extract of avocado pulp showed regression in the thick epidermis to a normal thin layer (arrow), (H&E X200).
Figure (3 H): Photomicrographs for rat paw section from rats group given low dose of acetogenin-rich extract of avocado pulp showed moderate cartilage regeneration (c), minimal Bone erosion (*), focal inflammatory cells (arrow), and bone marrow regeneration (Bm), (H&E X200).
Figure (3 I): Photomicrographs for rat paw section from rats group given low dose of acetogenin-rich extract of avocado pulp showed regression in subepidermal inflammatory reaction to focal mononuclear inflammatory cells infiltration (*), (H&E X200).
Figure (3 J): Photomicrographs for rat paw section from high dose of acetogenin-rich extract of avocado pulp showed normal thin epidermis (arrow) and skin appendages (*), (H&E X200).
Figure (3 K): Photomicrographs for rat paw section from high dose of acetogenin-rich extract of avocado pulp showed normal skin layers with absence of subepidermal inflammatory reaction (*), (H&E X200).
Figure (3 L): Photomicrographs for rat paw section from high dose of acetogenin-rich extract of avocado pulp showed marked reduction in the inflammatory cells infiltration, preserved articular cartilage (C), regular bone marrow (Bm), and reduced synovial hyperplasia, (H&E X200)

4. Conclusion

The present research revealed that acetogenin-rich extract of avocado pulp has antioxidant, anti-cancer and anti-arthritic potential and can be used as anti-cancer and anti-arthritic treatment. These activities are attributed to presence of many phytochemicals such as phenolic compounds, flavonoids, acetogenin, phytosterols and fatty acids.

5. Conflicts of interest

The authors declare no conflicts of interests.

6. References

1. Kunnumakkara A., Sailo B, Banik K., Harsha C., Prasad S., Gupta S., Bharti A., Aggarwal B. Chronic diseases, inflammation, and spices: how are they linked? J Transl Med. 16(1):14 (2018).
2. Fonseca L., Nunes-Souza V., Goulart M., Rabelo L. Oxidative Stress in Rheumatoid Arthritis: What the Future Might Hold regarding Novel Biomarkers and Add-On Therapies. Oxid Med Cell Longev. 2019: 7536805 (2019).
3. Nasef NA., Mehta S., Ferguson LR. Susceptibility to chronic inflammation: an update. Arch Toxicol. 91(3):1131–41 (2017).
4. Laveti D., Kumar M., Hemalatha R., Sistla R., Naidu VG., Talla V., Verma V., Kaur N., Nagpal R. Anti-inflammatory treatments for chronic diseases: a review. Inflamm Allergy Drug Targets. 12(5): 349–61 (2013).
5. Colotta F., Allavena P., Sica A., Garlanda C., Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic

instability. Carcinogenesis. 30(7):1073–81 (2009).
6. Prasad S., Sung B., Aggarwal BB. Age-associated chronic diseases require age-old medicine: role of chronic inflammation. Prev Med. 54(Suppl):S29–37 (2012).
7. Berasategi I., Barriuso B., Ansorena D., Astiasarán I. Stability of avocado oil during heating: Comparative study to olive oil. Food Chem. 1: 439–446 (2012).
8. Alkhalaf M., Alansari W., Ibrahim E., ELhalwagy M. Anti-oxidant, anti-inflammatory and anti-cancer activities of avocado (*Persea americana*) fruit and seed extract. Journal of King Saud University – Science. 31:1358–1362(2019).
9. Flores M., Saravia C., Vergara C., Avila F., Val dés H., Ortiz-Viedma J. Avocado Oil: Characteristics, Properties, and Applications. Molecules. 24 (11): 2172 (2019).
10. D'Ambrosio SM., Han C., Pan L., Kinghorn AD., Ding H. Aliphatic acetogenin constituents of avocado fruits inhibit humanoral cancer cell proliferation by targeting the EGFR/RAS/RAF/MEK/ERK1/2 pathway. Biochem Biophys Res Commun 409:465–469 (2011).
11. Salazar-López N., Domínguez-Avila A., Yahia E., Belmonte-Herrera B., Wall-Medrano A., Montalvo-González E., González-Aguilar G. Avocado fruit and by-products as potential sources of bioactive compounds. Food Res Int. 138(Pt A): 109774 (2020).
12. Bhuyan J., Alsherbiny A., Perera S., Low M., Basu A., Devi A., Barooah S., Li G., Papoutsis K. The odyssey of bioactive compounds in Avocado (*Persea americana*) and their health benefits. Antioxidants 8: 426 (2019).
13. Rodriguez-Sanchez D. G., Flores-García M., Silva-Platas C., Rizzo S., Torre-Amione G., De la Peña-Diaz A., Hernández-Brenes C., García-Rivas G. Isolation and chemical identification of lipid derivatives from avocado (*Persea americana*) pulp with antiplatelet and antithrombotic activities**.** Food Funct. 6(1):193-203 (2015).
14. Singleton L., Orthofer R., Lamuela-Raventó´s M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Method Enzymol. 299: 152–178 (1999).
15. Chang CC, Yang MH, Wen HM and Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 10:178-182 (2002).
16. AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists, 19th ed., Washington D.C., USA. (2012).
17. Shekhar T.C., Anju G. Antioxidant Activity by DPPH Radical Scavenging Method of *Ageratum conyzoides* Linn. Leaves. American Journal of Ethnomedicine. 1 (4): 244-249 (2014).
18. Arnao B., Cano A., Acosta M. The hydrophilic and lipophilic contribution of total antioxidant activity. Food chemistry, 73: 239-244 (2001).
19. Benzie F., Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analytical Biochemistry. 239: 70-76 (1996).
20. Cordero P., Morantes J., Páez A., Rincón J., Aristizábal A. Cytotoxicity of withanolides isolated from *Acnistusar borescens*. Fitoterapia. 80: 364-368 (2009).
21. Reeves G., Nielsen H., Fahey C. AIN-93 purified diets for laboratory rodents: Final report of the American institute of nutrition Ad hoc writing committee on the reformulation of the AIN-76A rodent diet. The Journal of Nutrition 123:1939-51 (1993).
22. Mohamed DA., Mohamed RS., Fouda K. Anti-inflammatory potential of chia seeds oil and mucilage against adjuvant induced arthritis in obese and non-obese rats. Journal of Basic and Clinical Physiology and Pharmacology. 31 (4): 20190236 (2020).
23. Snekhalatha U., Anburajan M., Venkatraman B., Menaka M. Evaluation of complete Freund's adjuvant-induced arthritis in a Wistar rat model. Comparison of thermography and histopathology. Z Rheumatol. 72(4): 375-82 (2013).
24. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta 20: 37-43 (1978).
25. Aebi H. Catalase in vitro. Methods Enzymol. 105: 121-6 (1984).
26. Bartles H., Bohmer M., Heierli C. Serum creatinine determination without protein precipitation. Clinica Chimica Acta. 37: 193-197 (1972).
27. Watts E. Determination of uric acid in blood and urine. Ann. Clin. Biochem. 11: 103-111 (1974).
28. Fawcett K., Scott E. A rapid and precise method for the determination of urea. J. Clin. Pathol. 13 (2): 156-159(1960).
29. Reitman S., Frankel S. Colorimetric methods for aspartate and alanine aminotransferase. Am. J. Clin. Pathology. 28 (1): 56-63 (1957).
30. Bancroft JD., Suvarna K., Layton C. Bancroft's Theory and Practice of Histological Techniques.

7th ed.Churchill Livingstone, Elsevier, London, p. ix. 2012 E book ISBN: 978-0-7020-5032–9 (2012).
31. Dreher M., Davenport A. Hass Avocado Composition and Potential Health Effects. Critical Reviews in Food Science and Nutrition. 53:738–750 (2013).
32. Gómez-Coca RB., Pérez-Camino MC., Moreda W. Analysis of Neutral Lipids: Unsaponifiable. In Nollet L, Toldrá F, (Eds) *Handbook of Food Analysis, 3rd ed*. CRC Press: Boca Raton, 459–491 (2015).
33. Hicks KB., Moreau RA. Phytosterols and phytostanols: functional food cholesterol busters. Food technology 55 (1): 63–67 (2001).
34. Duester K C. Avocado fruit is a rich source of beta-sitosterol. J Am Diet Assoc. 101(4): 404-5 (2001).
35. Law M. Plant sterol and stanol margarines and health. BMJ Br. Med. J. 320 (7238): 861 (2000).
36. Woolf A., Wong M., Eyres L., McGhie T., Lund C., Olsson S., Wang Y., Bulley C., Wang M., Friel E., Requejo-Jackman C. Avocado oil. From cosmetic to culinary oil. in Moreau R, Kamal-Eldin A (Eds.) *Gourmet and Health-Promoting Specialty Oils, 1st ed*. AOCS: Urbana, 73–125 (2009).
37. Fernandes GD., Gómez-Coca RB., Pérez-Camino MC., Moreda W., Barrera-Arellano D. Chemical characterization of commercial and single-variety avocado oils. Grasas y aceites. 69 (2): e256 (2018).
38. Zavala-Guerrero B., Hernández-García A., Torres-Martínez R., Meléndez-Herrera E., Ríos-Chávez P., Ochoa-Zarzosa A., López-Meza JE., Saavedra-Molina A., Salgado-Garciglia R. Antioxidant and anti-inflammatory activities of methanolic fractionfrom native Mexican avocado seed oil. FASEB J 34(S1):1–1(2020).
39. Ochoa-Zarzosa A., Báez-Magaña M., Guzmán-Rodríguez J., Flores-Alvarez L., Márquez M., Zavala-Guerrero B., Salgado-Garciglia R., López-Gómez R., López-Meza J. Bioactive Molecules From Native Mexican Avocado Fruit (Persea americana var. drymifolia): A Review. Plant Foods Hum Nutr. 76(2):133-142 (2021).
40. AbdelSalam I., Ashmawy A., Hilal A., Eldahshan O., Ashour M. Chemical Composition of Aqueous Ethanol Extract of *Luffa cylindrica* Leaves and Its Effect on Representation of Caspase-8, Caspase-3, and the Proliferation Marker Ki67 in Intrinsic Molecular Subtypes of Breast Cancer in Vitro. Chem Biodivers. 15(8):e1800045 (2018).
41. Llor X., Pons E., Roca A., Alvarez M., Mañé J., Fernández-Bañares F.,Gassull MA. The effects of fish oil, olive oil, oleic acid and linoleic acid on colorectal neoplastic processes. Clin Nutr. 22(1):71–79 (2003).
42. Oberlies NH., Rogers LL., Martin JM., McLaughlin JL. Cytotoxic and insecticidal constituents of the unripe fruit of *Persea americana*. J Nat Prod. 61:781–785 (1998).
43. Butt AJ., Roberts CG., Seawright AA., Oelrichs PB., Macleod JK., Liaw TY., Kavallaris M., Somers-Edgar TJ., Lehrbach GM., Watts CK., Sutherland RL. A novel plant toxin, persin, with in-vivo activity in the mammary gland, induces Bim-dependent apoptosis in human breast cancer cells. Mol Cancer Ther. 5:2300–2309 (2006).
44. D'Ambrosio SM., Han C., Pan L., Kinghorn AD., Ding H. Aliphatic acetogenin constituents of avocado fruits inhibit human oral cancer cell proliferation by targeting the EGFR/RAS/RAF/MEK/ERK1/2 pathway. Biochem. Biophys. Res. Commun. 409 (3), 465–469 (2011).
45. Anantharaju PG., Gowda PC., Vimalambike MG., Madhunapantula SV. An overview on the role of dietary phenolics for the treatment of cancers. Nutrition Journal 15:99 (2016).
46. Al-Okbi SY., Mohamed DA., Donya SM., Abd El Khalek AB. Role of *Bifidobacterium bifidum* and Plant Food Extracts in Improving Microflora and Biochemical and Cytogenetic Parameters in Adjuvant Arthritis. Grasas y aceites, 62 (3): 308-320 (2011).
47. Abdel-Moein NM., Abdel-Moniem EA., Mohamed DA., Hanfy EA. Evaluation of the Anti-inflammatory and Anti-arthritic Effects of Some Plants Extracts. Grasas y aceites. 62 (3), 365-374 (2011).
48. Mohamed DA., Hanfy EA., Fouda K. Evaluation of Antioxidant, Anti-inflammatory and Anti-arthritic Activities of Yarrow (*Achillea millefolium*). J. Biol. Sci. 18 (7): 317-328 (2018).
49. Kumar R., Singh S., Saksena A., Pal R., Jaiswal R., Kumar R. Effect of Boswellia Serrata Extract on Acute Inflammatory Parameters and Tumor Necrosis Factor-α in Complete Freund's Adjuvant-Induced Animal Model of Rheumatoid Arthritis**.** Int J Appl Basic Med Res. 9(2):100-106 (2019).
50. Chen G., Song Y., Ma F., Ma Y. Anti-arthritic activity of D-carvone against complete Freund's adjuvant-induced arthritis in rats through modulation of inflammatory cytokines. Korean J Physiol Pharmacol. 24(6): 453-462 (2020).
51. Chen Y., Xue R., Jin X., Tan X. Antiarthritic activity of diallyl disulfide against Freund's adjuvant-induced arthritic rat model. J Environ Pathol Toxicol Oncol. 37:291-303 (2018).
52. Al-Okbi SY., Ammar NM., Sorour Kh., Mohamed DA. Impact of natural oils

supplements on disease activity and antioxidant state of Egyptian patients with rheumatoid arthritis. Medical Journal of Islamic Academy of Sciences. 13 (4): 161-171(2000).
53. Huang X., Xi Y., Pan Q., Mao Z., Zhang R., Ma X., You H. Caffeic acid protects against IL-1β-induced inflammatory responses and cartilage degradation in articular chondrocytes. Biomed Pharmacother. 107:433-439 (2018).
54. Hou SM., Hou CH., Liu JF. CX3CL1 promotes MMP-3 production via the CX3CR1, c-Raf, MEK, ERK, and NF-κB signalling pathway in osteoarthritis synovial fibroblasts. Arthritis Res Ther. 19:282 (2017).
55. Jiang L., Li L., Geng C., Gong D., Jiang L., Ishikawa N., Kajima K., Zhong L. Monosodium iodoacetate induces apoptosis via the mitochondrial pathway involving ROS production and caspase activa tion in rat chondrocytes in vitro. J Orthop Res. 31: 364-369 (2013).
56. Veselinovic M., Barudzic N., Vuletic M., Zivkovic V., Tomic-Lucic A., Djuric D. Oxidative stress in rheumatoid arthritis patients: relationship to diseases activity. Mol Cell Biochem. 391: 225–232 (2014).
57. Mateen S., Moin S., Khan A., Zafar A., Naureen F. Increased Reactive Oxygen Species Formation and Oxidative Stress in Rheumatoid Arthritis. PLoS One. 11(4): e0152925 (2016).
58. Patel S., Shah P. Evaluation of anti-inflammatory potential of the multidrug herbomineral formulation in male Wistar rats against rheumatoid arthritis**.** J Ayurveda Integr Med. 4(2): 86-93 (2013).
59. Zhang Z., Zhang S., Jin B., Wu Y., Yang X., Yu B., Xie Q. Ciclamilast ameliorates adjuvant-induced arthritis in a rat model. Biomed Res Int. 2015: 786104 (2015).
60. Pita LM., Spadella MA., Montenote MC., Oliveira PB., Chies AB. Repercussions of adjuvant-induced arthritis on body composition, soleus muscle, and heart muscle of rats**.** Braz J Med Biol Res. 53(3):e8969 (2020).