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Anti-Obesity Actions of Two Separated Aqueous Extracts From Arbutus (*Arbutus unedo*) and Hawthorn (*Crataegus monogyna*) Fruits Against High-Fat Diet in Rats via Potent Antioxidant Target

Soumaya Wahabi[1], Kais Rtibi[1], Amal Atouani[2], and Hichem Sebai[1,3]

Abstract

Arbutus unedo and *Crataegus monogyna* are widely distributed throughout the Mediterranean basin and commonly used in folk medicine against a wide range of diseases. Therefore, the present study has been designed to evaluate the anti-obesity potential of two aqueous extracts of the fruits of *A. unedo* (AUAE) and *C. monogyna* (CMAE). Male *Wistar* rats were supplied with a standard diet (SD), high-fat diet (HFD), HFD with the two separated extracts at the same dose (300 mg/kg, BW, p. o.), or HFD with atorvastatin-(ATOR) (2.1 mg/kg, BW, p. o.) for 12 weeks. Lipid profile and the liver and kidney linked-markers were assessed. Besides, obesity-related disorders' biomarkers were measured. AUAE, CMAE, and ATOR were observed to reduce significantly total body and organ weights following HFD-induced obese rat models. Likewise, epididymal and abdominal adipose tissue weights were noticeably decreased in HFD rats treated with both extracts and ATOR. Added to that, biochemical and metabolic changes were normalized by significant attenuation of lipid peroxidation accompanied with an increase of thiol-group concentrations and antioxidant status. More importantly, a modulation in trace element levels was revealed when compared with HFD group. Altogether, current study concluded that AUAE and CMAE could be potential candidates for the prevention and treatment of obesity and related disturbs induced by HFD.

Keywords

Arbutus unedo, *Crataegus monogyna*, obesity, oxidative stress, high-fat diet, liver and kidney biomarkers

Introduction

Obesity has emerged as a major global health issue, particularly obesity-related cardiovascular disease, non-alcoholic fatty liver disease, and type 2 diabetes. Thus, prevention and treatment of obesity have attracted intense research worldwide.[1,2] New researches reveal that 39% of the adult population is overweight and 13% is obese. A pooling of statistical data analysis shows that a total of 2.16 billion individuals will become overweight and 1.12 billion people will be obese in 2030.[3,4] However, the COVID-19 pandemic status aggravates it, following the strict restrictions on movement and the number of obese patients will augment all over the world.[5]

[1] Laboratory of Functional Physiology and Valorization of Bio-Ressources-Higher Institute of Biotechnology of Beja, University of Jendouba, Beja, Tunisia
[2] Clinical Biology Laboratory, Beja Regional Hospital, Beja, Tunisia
[3] University of Jendouba, Jendouba, Tunisia

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Corresponding Author:
Kais Rtibi, Laboratory of Functional Physiology and Valorization of Bio-Ressources-Higher Institute of Biotechnology of Beja, University of Jendouba, B.P. 382-9000 Beja, Tunisia.
Email: rtibikais@yahoo.fr

As a result, obesity can shorten one's life by lowering one's quality of life. Thus, limiting the prevalence of obesity could reduce not only the risk of mortality and morbidity, but also the risk of obesity-related diseases.[6,7] Although diet and exercise can effectively reduce obesity, treatment that includes therapeutic agents in addition to exercise and a controlled diet makes this process more effective. Currently, several therapeutic agents for the treatment of obesity are available, including orlistat, sibutramine, diethylpropion, and phentermine.[8]

However, due to the unfavorable effects of these drugs, such as cardiovascular disease, overstimulation, and abuse, alternative therapies such as dietary supplements and herbal products are required. As a result, there has recently been an increase in interest in the use of new natural antioxidants, primarily of plant origin.[9] It is well understood that phenolic compounds derived from plants have numerous pharmacological properties, particularly antioxidant and anti-hyperlipidemic effects.[10,11] For thousands of years, medicinal plants have been recognized as a rich source of therapeutic agents to prevent and cure a variety of ailments. For this, natural products are considered safe and have the potential to treat different medical issues. Phenolic compounds need more attention because some may be used as an alternative for the development of anti-obesity approaches.[12]

The strawberry tree (*Arbutus unedo L., Ericaceae*) grows well in the Iberian Peninsula, the Mediterranean basin, and other areas with hot summers and mild rainy winters. The spherical fruits with a diameter of about 2–3 cm are red and tasty only when fully ripe. The fruits are edible but usually processed before consumption.[13] *A. unedo* fruits are a source of health, namely, of vitamin C and dietary fibers, while also rated as a source of bioactive compounds for dietary supplements or functional foods.[14] It contains phenolic acids and flavonols,[15] as well as fatty acids,[16,17] vitamins,[18] organic acids,[14,15] sugar,[19,20] and minerals.[21]

Crataegus monogyna (Rocacea) is one of the species that is highly recommended in traditional medicine and the "berries" are usually consumed by shepherds, hunters, and children, because they are considered to be "healthy" and nutritious for human and animals.[22] *C. monogyna* has been used traditionally to cure diarrhea, gall bladder disease, and insomnia,[23] as well as it has been applied for respiratory problems including coughs, flu, bronchitis, and asthma.[24] In Europe, it has been used in the treatment of heart problems due to their antispasmodic, cardiotonic, hypotensive, and anti-atherosclerotic effects.[25] Moreover, in Traditional Chinese Medicine, hawthorn fruits are used to improve circulation, remove blood stasis, to treat indigestion, abdominal pain, hyperlipidemia, and hypertension.[26]

The present research aimed to investigate two separated aqueous extracts from *A. unedo* and *C. monogyna* fruits potentials to manage obesity and its adverse sequelae. This object was achieved by determining hematological parameters, lipid profiles, oxidative stress biomarkers, and liver and kidney functions as well as histological changes of liver and white adipose tissues in male *Wistar* rats.

Materials and Methods

AUAE and CMAE Preparation

A.unedo and *C. monogyna* fruit were collected from the area of Béja, northwest of Tunisia, during Octobor and November 2021 and identified by the botanic coordinator, Institute of Biotechnology of Beja, University of Jendouba. Thereafter, the fruit material (all the fruit for *A. unedo* plant and the pericarp for the *C. monogyna* plant) was dried at 40°C for a duration of 5 days with air circulation and later rigorously crushed in a traditional metallic mortar and then with an electric blender. AUAE and CMAE were prepared by adding the powder to boiled bi-distilled water (70°C), and the mixture was stirred for 1h. The aqueous extracts were filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrates were quickly frozen at −40°C and dried for 48 h using a freeze dryer (Refrigerated Vapor Trap RVT450).

Polyphenol and Flavonoid Contents Determination

The overall phenolic content was quantified by the colorimetric method, the Folin–Ciocalteu method.[27] Briefly, 500 µL of the extract was added to 10 mL of water and 0.5 mL of Folin–Ciocalteu reagent. After 5 min, 8 mL of 7.5% sodium of carbonate solution was added. The reaction was kept in the dark for 2 h and was measured at 765 nm using a UV-visible detector spectrophotometer. Gallic acid was applied as a standard, and the results were expressed in milligram gallic acid equivalent per gram dry matter (mg GAE/g DM).

The total flavonoid content was detected by the $AlCl_3$ colorimetric method.[28] In a brief step, 1 mL of the sample was mixed with 1 mL of 2% $AlCl_3$ solution. After 15 min incubation at room temperature, the optical density of their action mixture was evaluated at 430 nm. Quercetin was used as a reference standard and the total flavonoid content was expressed as milligram quercetin equivalent per gram dry matter (mg QE/g DM).

ABTS/DPPH Free Radical-Scavenging Assays

The antioxidant capacity of the AUAE and CMAE were realized with the help of DPPH.[29] In brief, various concentrations of both extracts (100, 200, 300, 400, 500, and 600 µg/mL) were added to 1 mL of .1 mM methanol solution of DPPH and incubated at 27°C for 30 min. The optical density of the sample was measured at 517 nm. DPPH radical scavenging (RSA), expressed as a percentage, was estimated using the following formula:

$$\mathrm{RSA}\,(\%) = \left[\left(\mathrm{A_{DPPH}}\right) - \left(\mathrm{A_{sample}} - \mathrm{A_{control}}\right)\right] / \mathrm{A_{DPPH}} \times 100$$

Using ABTS, 1 mL of diluted extract was mixed with 3 mL of 7 mM ABTS radical solution ($ABTS^{+}$) and was kept in the dark at room temperature for 60 min. Absorbance was determined at 734 nm.[30] The scanning capability was calculated as

$$([1 - A_b/A_0] \times 100)$$

A_b and A_0 are the absorbance of samples as well as the $ABTS^+$ solution at 734 nm. Ascorbic acid was used as the reference molecule in the same concentration as the extract tested. All analyses were carried out in triplicate. Efficacy 50 was determined as the value of concentration (µg/mL) of the compound needed to recover 50% of the both radicals.

Anti-Hyperlipidemic Activity

Preparation of High-Fat Diet

The high-fat diet was prepared according to the protocol described by Smine.[31] The HFD consisting of the usual food that has been previously saturated with melted lamb fat. This fat was melted by heating to 100°C, and then the plugs were soaked for 15 min in the melted fat. The HFD food (Table 1) was administered after drying at room temperature.

Animals and Experimental Protocol Design

Male *Wistar* rats weighing 170–180 g were purchased from the Central Society of Pharmaceutical Industries of Tunisia (SIPHAT, Ben-Arous, Tunisia) and kept under standard pet shop conditions (22 ± .5°C, and 12 h/12 h light/dark cycle), with free access to food and water. After 2 weeks of acclimatization, the rats were divided into five equal groups randomly; each group consisted of 6 rats and treated as follows:

Group-1 (SD): Fed with standard laboratory diet and drinking water *ad libitum* and served as a control.

Group-2 (HFD): Fed with high-fat diet to generate hyperlipidemia for 12 weeks

Group-3 (HFD + AUAE): Fed with HFD and AUAE (300 mg per kg body weight-(BW) per day).

Group-4 (HFD + CMAE): Fed with HFD and CMAE (300 mg per kg BW per day).

Group-5 (HFD + ATOR): Fed with HFD and ATOR at a dose of 2.1 mg per kg BW per day.

The AEAU, AECM, and ATOR were administered by oral route for 12 consecutive weeks. The BW and food intake were recorded weekly. The animals were treated according to the Tunisian code of practice [Comite d'Ethique Bio-medicale (CEBM) (JORT472004)] for the Care and Use of Animals for Scientific Purposes.

Table 1. Composition of the Experimental Diet (Smine, 2017).

	Control Diet	High Fat Diet
Fat	3.00%	28.00%
Carbohydrates	40.00%	32.00%
Proteins	14.50%	11.60%
Iron	50 mg/kg	40 mg/kg
Calcium	1.45 mg/kg	1.16 mg/kg
Energy density	11.40 kj/g	18.05 kj/g

For the dosage determination, based on the ethnobotanical survey and personal communications with traditional medicine, practitioners revealed that 300 mg/kg BW was adopted as an appropriate dosage to determine the acclaimed anti-obesity potential of both extracts. Moreover, the preliminary experiment indicated that both extracts at this dose (300 mg/kg, BW, p. o.) give significant actions. In addition, the experiments also showed that the selected extracts did not cause any sign of toxicity during the 10 days of treatment (data not shown). Test substances were newly prepared immediately before administration, and each administration was performed based on the most recently recorded body weight. The test fruit extracts was administered through gastric intubation at 9 o'clock, a fixed time every day.

Collection of Blood, Adipose Tissues, Liver, and Kidney

By the end of the experimental periods, all the rats were fasted for 12 h overnight, and then sacrificed by decapitation. Blood was collected and immediately centrifuged at 3000 rpm for 10 min at 4°C. Then, the separated plasma was kept at −80°C until needed. The liver and the kidney were removed immediately, rinsed with cold saline solution, and properly weighed. The white adipose tissues including epididymal adipose tissues (EAT) and abdominal adipose tissues (AAT) were collected carefully and weighed.

Preparation of Liver Extracts

.1 g of the liver was homogenized in 1 mL of Tris-buffered saline (pH 7.4) using a T-18 digital Ultra-Turrax homogenizer. The homogenate was then centrifuged during 15 min at 4°C. Supernatants were stored at −80°C until use.

Plasma Analysis of Various Obesity-Related Biomarkers

The protective effects of the fruit aqueous extract on HFD-induced obesity were determined by measuring the plasma levels of liver function enzymes (AST, ALP, ALT, BD, BT, and GGT) and lipids, including low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), triglycerides (TG), and total cholesterol (TC). The level of kidney function biomarkers like urea, creatinin and uric acid, and iron (Fe), calcium (Ca), and phosphorus (P) levels were evaluated.

Plasma biochemical analysis were quantified using a SELECTRA PRO XL automatic biochemical analyzer with the corresponding commercial kits (ELI Tech Group Clinical system SAS, Tunisia).

- Atherogenic index (AI) and coronary risk index (CRI) calculation

AI and CRI were calculated by the following formula.[32]

- AI = TC − HDL-C/HDL − C
- CRI = TC/HDL − C

Histological Study of Liver and White Adipose Tissues

Liver histological analysis has been performed to determine the effects of AUAE and CMAE on hepatocytes. For this purpose, 10% of buffered formalin was used for embedding the small portion of liver tissues. A small portion (of abdominal and epididymal adipose tissues) was collected from all rats. Adipose tissues were fixed in 10% buffered formalin and then processed with hematoxylin and eosin to assess the morphology of adipocytes.

Protein Concentrations

Protein concentrations were determined according to the method of Bradford[33] by using bovine serum albumin as a standard.

Antioxidant Enzyme Activities of Liver Homogenates

The SOD activity was obtained using modified epinephrine assays.[34] At alkaline pH, the superoxide anion (O_2^-) causes the auto-oxidation of epinephrine to adrenochrome, while competing with this reaction; SOD decreased the formation of adrenochrome. An SOD unit is the quantity of the extract that inhibits the rate of adrenochrome formation by 50%. The enzyme extract was added to 2 mL reaction mixture containing 10 µL bovine catalase (.4 U/µL), 20 µL epinephrine (5 mg/mL), and 62.5 mM sodium carbonate/bicarbonate buffer pH 10.2. Absorbance changes were observed at 480 nm.

The CAT activity was evaluated by measuring the initial rate of hydrogen peroxide (H_2O_2) disappearance at 240 nm.[35] The reaction mixture contained 33 mM H_2O_2 in 50 mM phosphate buffer at pH 7.0 and the activity of CAT was calculated using the extinction coefficient of 40 mM^{-1} cm^{-1} for H_2O_2. The GPx activity was quantified by the Flohé and Gunzler method.[36] In brief, 1 mL of reaction mixture containing .2 mL liver homogenates supernatant, .2 mL (.1 M) phosphate buffer pH 7.4, .2 mL GSH (4 mM), and .4 mL H_2O_2 (5 mM) was incubated at 37°C for 1 min and the reaction was stopped by addition of .5 mL TCA (5%, w/v). After centrifugation at 1500*g* for 5 min, aliquot (.2 mL) of the supernatant was combined with .5 mL of .1 M phosphate buffer pH 7.4 and .5 mL of DTNB (10 mM) and the absorbance was read at 412 nm. The GPx activity was expressed in nanomolar of GSH consumed per minute per milligram of protein.

Determination of the Concentration of the Total Thiol Groups

Liver homogenates were added to .25 M Base/Tris and 20 mM ethylene diamine tetraacetic acid, pH = 8.2.[37] The mixture was vortexed and its absorbance was determined at 412 nm. The first value was A1. After that, 10 mM 5,5-dithiobis (2 nitrobenzoic acid) (DTNB) was added. After incubation for 15 min, a new value A2 was determined. The white tube of DTNB contained only DTNB and buffer, its absorbance value was noted as B. We calculated the concentration of thiol groups per tube by using this expression: (A2-A1-B) x 1.57 mM.

Lipid Peroxidation Measurement of Liver Homogenates

The liver lipids peroxidation was determined by measuring MDA using the double heating method.[38] In brief, aliquots of liver homogenates were mixed with a BHT-TCA solution that contained 1% BHT (w/v) dissolved in 20% TCA (w/v) and centrifuged at 1000 g for 5 min at 4°C. The supernatant was mixed with .5 N HCl and 120 mM TBA within 26 mM Tris and heated at 80°C for duration of 10 min. The absorbance of the resulting chromophore was determined at 532 nm after cooling. The levels of MDA were determined using an extinction coefficient for the MDA-TBA complex of 1.56×10^5 M^{-1} cm^{-1}.

Statistical Analysis

All the values in the results were expressed as mean ± SEM (standard error of the mean). Statistical analysis was done by one-way analysis of variance (ANOVA) using the Graph Pad Prism statistical software, Version 8.01 (Graph Pad Software Inc., La Jolla, CA, USA) with the comparison between groups. P-values less than .05 were considered significant.

Results

Secondary Metabolites Quantities

According to the findings of a colorimetric examination of the chemical components of AUAE, the mean total polyphenols and flavonoids contents were equivalent to 31.66 ± .75 mg GAE/g DM and 23.97 ± 1.70 mg QE/g DM, respectively. In contrast, CMAE indicates that these constituents were equivalent to 23.92 ± 1.2 mg GAE/g DM and only 05.5 ± .68 mg QE/g DM, respectively. Therefore, AUAE and CMAE are very rich in secondary metabolite compounds (Table 2).

Antioxidant Activity of AUAE and CMAE

DPPH and ABTS assays were used to estimate the ability of AUAE and CMAE to scavenge free radicals. The radical-scavenging capacity shows that the two extracts exhibited very strong antioxidant activity when compared to ascorbic acid, an essential diet-derived antioxidant (Figure 1). The effective concentrations 50 (EC_{50}) calculated from the graphs are, respectively, 189.86 and 201.54 µg/mL for DPPH and 160.85 and 225.97 mg/mL for ABTS scavenging activities. These values are not very far from those of ascorbic acid

Table 2. Determination of the Crucial Phytochemical Compounds Contents in the Both Fruit Aqueous Extracts, AUAE and CMAE.

	AUAE	CMAE
Total polyphenols	31.66 ± .75 mg GAE/g.DM	23.92 ± 1.2 mg GAE/g.DM
Total flavonoids	23.97 ± 1.70 mg QE/g.DM	05.5 ± .68 mg QE/g.DM

Data are expressed as mean ± SEM of duplicate determinations.

(EC_{50} = 71.72 and 64.37 mg/mL, respectively) used as reference molecule (Table 3). Our research shows that the two fruit aqueous extracts are powerful antioxidants, particularly AUAE which has a lower EC_{50} than CMAE.

Effect of AUAE, CMAE, and ATOR on Body Weight, Gain Weight, and Food Intake

Regular following of the evolution of BW for 12 weeks, in the 5 groups of rats, allowed us to draw representative curves of the evolution of BW as well as to evaluate the absolute weight gain during this treatment period. Our results show that the HFD diet induces a significant increase in the BW of the animals between the first and the last day (Figure 2A). Indeed, BW increased from 175.91 ± 5.73 g to 284.68 ± 3.20 g in control rats and from 185.11 ± 1.66 g to 375.96 ± 5.45 g in rats receiving HFD diet.

Treatment with AUAE, CMAE, and ATOR significantly reduced the BW increase compared to the HFD control group. Indeed, BW changed from 175 ± 2.81 g to 324.2 ± 11.52 g in AUAE-treated rats and from 170 ± 1.90 to 313.5 ± 8.23 in CMAE-treated rats compared to HFD group. The values of the absolute gain in BW moved in the same direction. Indeed, this gain, which consists of the difference between the final weight and the initial weight, is respectively of the order of 108.76 ± 4.92 g, 190.84 ± 6.26, 149.2 ± 9.58, 143.5 ± 6.44 and 119.3 ± 14.30 for SD, HFD, HFD + AUAE, HFD + CMAE, and HFD + ATOR rats (Figure 2B). Indeed, we witnessed that the difference is not significant between the groups treated with both extracts (AUAE and CMAE) and ATOR, the lipid-lowering reference molecule.

The normal diet group consumed more food than the other groups (Figure 2C). This demonstrates that normal food consumption has no negative effects on animal health, whereas continuous consumption of high-fat food leads to obesity and obesity-related complications. The BW is not related to the amount of food consumed per day rather these are related to the composition of the food.

Weight of Liver, Kidney, and White Adipose Tissue (Epididymal Adipose Tissue and Abdominal Adipose Tissue)

At the end of the experiment, on the day of sacrifice, the weights of liver, kidney, epididymal white adipose tissue (EAT), and abdominal white adipose tissue (AAT) were investigated. The weights of these organs and tissues were significantly higher in the HFD group rats than the control group rats. The treatment of the animals by both extracts and ATOR allows a significant reduction in various weights compared to the HFD group, but these values remain also significant compared to the control group. However, there is a significant change in the weight of abdominal adipose tissue when the comparison concerns the two extracts. Thus, CMAE successfully reduced the increase in abdominal fat weight induced by the HFD more effectively than AUAE (Table 4).

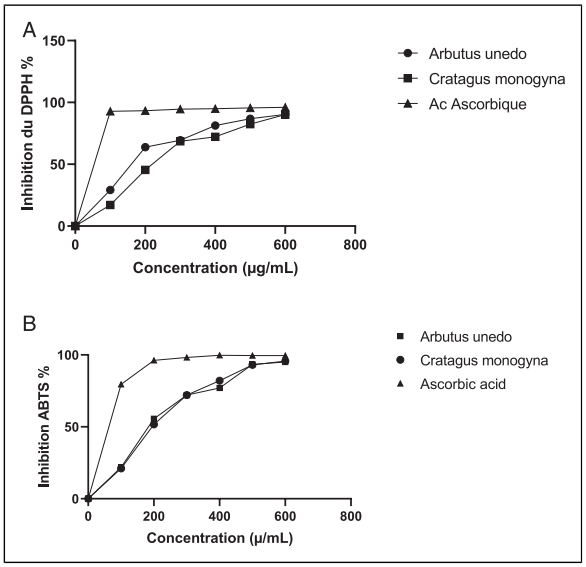

Figure 1. Dose response of the antioxidant capacity of EAAU, EACM, and ascorbic acid against the DPPH radical (A) and the ABTS radical (B).

Table 3. Determination of the Inhibitory Concentration 50 (IC_{50}) in the AUAE, CMAE, and Ascorbic Acid.

	IC_{50} (µg/mL)		
Test	AUAE	CMAE	Ascorbic Acid
ABTS	189.86	201.54	71.72
DPPH	160.85	225.97	64.37

Effects of AEAU, CMAE, and ATOR on Blood Glucose Level

The SD group blood glucose level was 6.14 mmol/L, and the HFD blood glucose level was 7.82 mmol/L, which was

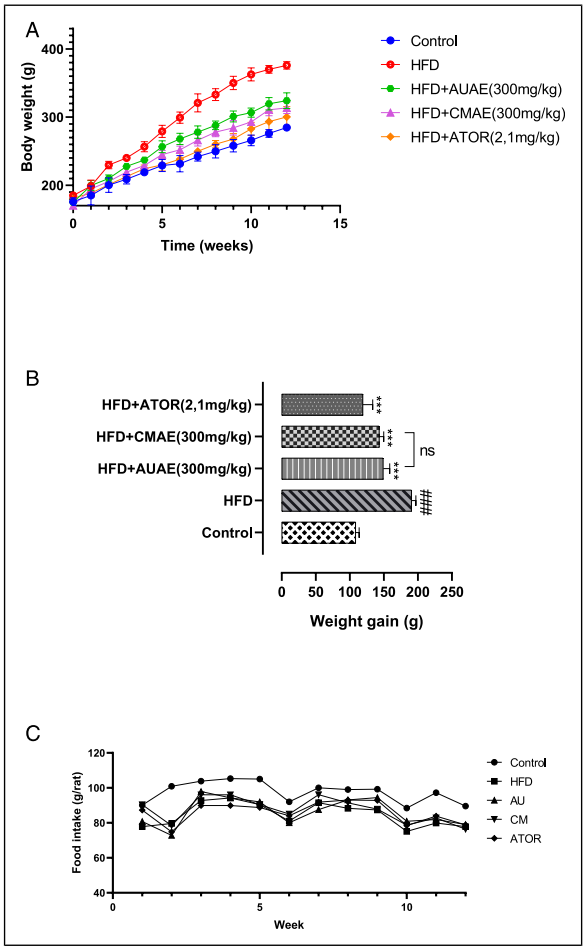

Figure 2. Effects of AUAE, CMAE, and ATOR on body weights (A), total weight gain (B), and food intake (C) in HFD-fed rats after 12 weeks (n = 6 per group).

21.48% higher than the normal group, showing the prediabetes condition of rats. According to research of Wang et al,[39] the normal blood glucose upper limit range for rats is 6.2 mmol/L or 111.6 mg/dL, which may vary in different breeds, but it is also comparable with humans. Here, the results show (Table 5) that the treatment group's blood glucose was at a normal level. The AUAE-supplemented HFD showed a blood glucose level of 6.67 mmol/L, which was close to the standard diet group. The CMAE-supplemented HFD and ATOR-supplemented HFD blood glucose levels were 6.19 mmol/L and 6.13 mmol/L, respectively. These values are the same as the SD group. Blood plasma glucose levels were significantly decreased in treatment-fed rats compared with HFD-fed rats.

Effects of AEAU, CMAE, and ATOR on Lipid Profile

The main factor causing the change in the lipid profile is the rise in body weight. An increase in LDL and TG associated to a decrease in HDL level are serious outcomes of obesity. Additionally, the risk of heart disease will also grow when total cholesterol levels rise.[40] Obesity and TG levels are closely related. Figure 3 shows the effect of HFD, AEAU, CMAE, and ATOR on the plasma lipid profile.

Feeding of HFD caused a significant ($P < .001$) increase of TC (Figure 3A), TG (Figure 3B), LDL-C (Figure 3C), AI (Figure 4A), and CRI (Figure 4B) in comparison to standard laboratory diet (SD) fed group. On the other hand, the plasma level of HDL-C was reduced significantly ($P < .001$) due to the feeding of HFD. Oral administration of AUAE and CMAE (300 mg/kg b. w) to rats fed with HFD significantly reduced ($P < .001$) the plasma levels of these parameters and the level of lipids is the same as that of normal rats. Similarly, AEAU and CMAE treatment significantly lowered the AI and CRI in HFD-fed obese rats (Figure 4). The same results were observed in the ATOR-treated group.

Comparing the effect of the two extracts, a non-significant difference between the two groups, HFD-AUAE and HFD-CMAE, was observed in the level of TC and LDL. But a significant difference was observed in the level of TG and HDL-C accompanied with a significant difference ($P < .001$) in the level of AI and CRI. Therefore, CMAE decreases the lipid level in the plasma and protects the animal against the risk of heart disease more than AUAE.

Effects of AEAU, CMAE, and ATOR on Liver and Renal Function Biomarker in HFD Rats

High-fat diet treatment induced severe liver and kidney damage evidenced. This action was shown by a significant increase ($P < .001$) of plasma AST, ALP, ALT, BD, BT, GGT, urea, creatinin, and uric acid levels (Table 5). When high-fat diet-treated rats were also treated with AUAE, CMAE, and ATOR, all these biomarkers were significantly restored to almost normal values. CMAE is significantly more effective than AUAE in reducing AST ($P < .001$), TB ($P < .05$), and ALP ($P < .01$) levels.

In Vivo Antioxidant Effects of AUAE, CMAE, and ATOR

HFD consumption significantly ($P < .001$) increased plasma and hepatic MDA (Figure 5A) levels in rats, whereas AUAE and CMAE treatment significantly reduced this lipid peroxidation, plasma, and hepatic thiol-group (Figure 5B) concentrations, as well as SOD (Figure 5C), CAT (Figure 5D), and GPx (Figure 5E) activities were significantly lower in HFD groups compared to SD groups. Oral administration of AUAE, CMAE, and ATOR significantly increased not only thiol-group concentrations but also antioxidant enzyme activities (SOD, CAT, and GPx).

A non-significant difference between the effect of the AUAE and CMAE was observed in CAT and GPx activities and MDA level in plasma and liver. CMAE significantly

Table 4. Effect of AUAE and CMAE on Organ Weights of Rats Fed SD and Rats Fed HFD Treated or Not With the Two Aqueous Fruit Extracts of Arbutus or Hawthorn.

	Liver	Kidney	AAT	EAT
Control	7.11 ± .12	1.67 ± .57	.84 ± 0.4	1.87 ± .37
HFD	9.55 ± .25$^{\#\#\#}$	2.25 ± .35	6.44 ± .38$^{\#\#\#}$	3.94 ± .71$^{\#\#\#}$
HFD + AU	7.8 ± .55***	1.77 ± .62	2.7 ± .12***	2.12 ± 015***
HFD + CM	7.56 ± .51***	1.93 ± .09	1.9 ± .73***	2.04 ± .21***
HFD + ATOR	7.45 ± .35***	1.80 ± .06	1.59 ± .46***	2.07 ± .22***

Values are expressed as mean ± SEM (n = 6). $^{\#}P < .05$, $^{\#\#}P < 0\ .01$, and $^{\#\#\#}P < .001$ vs Control group. $^{*}P < .05$, $^{**}P < 0.\ 01$ and $^{***}P < .001$ vs HFD group.

Table 5. Effects of AEAU, CMAE, and ATOR on Plasma Metabolic Parameters.

	Rats Groups				
	Control	HFD	HFD + AUAE (300 mg/kg)	HFD + CMAE (300 mg/kg)	HFD + ATOR (2.1 mg/kg)
AST (UI/l)	205 ± 1	314 ± 4.04$^{\#\#\#}$	236 ± 1***	216 ± 1.52***	213 ± 1.52***
ALT (UI/l)	174.33 ± 2.08	187 ± 3.46$^{\#\#}$	178.5 ± 3.21**	175.33 ± 1.53**	175.33 ± 3.21**
DB (µmol/l)	.12 ± .01	.43 ± .01$^{\#\#\#}$	.22 ± .02***	.21 ± .02***	.19 ± .01***
TB (µmol/l)	1.18 ± .03	1.36 ± .04$^{\#\#\#}$	1.26 ± .02***	1.21 ± .02***	1.20 ± .01***
GGT (UI/l)	1.01 ± .01	1.15 ± .131	1.03 ± .02	1.02 ± .01	1.01 ± .02
ALP (UI/l)	320 ± 11	540 ± 3$^{\#\#\#}$	442.50 ± 4.93***	422 ± 2.65***	403.33 ± 4.16***
Glucose (mmol/l)	6.14 ± .09	7.82 ± .14$^{\#\#\#}$	6.67 ± .06***	6.19 ± .23***	6.13 ± .12***
Creatinin (µmol/l)	37 ± 1	32.01 ± 1$^{\#}$	34.50 ± 2.08*	36 ± 2*	37 ± 1*
Urea (mmol/l)	11.48 ± .45	24.68 ± 3.5$^{\#\#\#}$	18.93 ± .78**	17.06 ± .04***	16.82 ± .47***
Uricacid (µmol/l)	5.38 ± 01	8.03 ± .29$^{\#\#\#}$	8.05 ± .25	7.57 ± .25*	7.17 ± .15**
Calcium (mmol/l)	2.26 ± 0.04	2.24 ± 0.01	2.36 ± 0.03	2.45 ± 0.06	2.41 ± 0.22
Phosphore (mmol/l)	2.31 ± 0.22	2.06 ± 0.08	2.32 ± 0.29	2.20 ± 0.34	2.23 ± 0.28
Fer (mmol/l)	30.71 ± 1.22	45.11 ± 1,93$^{\#\#\#}$	38 ± 0,95***	33.38 ± 0,92***	31.17 ± 1,26***

Values are expressed as mean ± SEM (n = 6). $^{\#}P < .05$, $^{\#\#}P < 0\ .01$, and $^{\#\#\#}P < .001$ vs Control group. $^{*}P < .05$, $^{**}P < 0.\ 01$ and $^{***}P < .001$ vs HFD group.SD, standard diet group; HFD, high-fat diet group; AUAE, *Arbutus unedo* aqueous extract group; CMAE,*Crataegus monogyna* aqueous extract group; ATOR, Atorvastatin group; AST, aspartate transaminase; ALT, alanine transaminase; DB, Direct bilirubin; TB, Total bilirubin; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; SEM, standard error of the mean. TG triglyceride, HDL high-density lipoprotein, LDL low-density lipoprotein.

increases ($P < .01$) hepatic SOD activity and plasma/hepatic thiol-group levels ($P < .05$) compared to AUAE.

Effects of AEAU, CMAE, and ATOR on Mineral Levels in Plasma

According to (Table 5), HFD caused a decrease in P and Ca and a significant increase in Fe levels in plasma. However, administration of AUAE, CMAE, and ATOR attenuated these changes and normalized these parameters under these conditions. A non-significant difference between the effect of the AUAE and CMAE was observed in these parameters.

Effects of AUAE, CMAE, and ATOR on Hepatocytes and Adipocytes

Figure 6 summarizes the histopathological changes in the liver tissues of rats associated with HFD feeding and HFD accompanied by treatments (AUAE, CMAE, and ATOR). The accumulation of lipid droplets was increased in the HFD group, whereas it was decreased in the AUAE-HFD, CMAE-HFD, and ATOR-HFD groups. The 12 weeks feeding of HFD generated the accumulation of fat droplets in the hepatic tissues of rats. An increase in the accumulation of fat droplets was observed in the livers of the HFD-fed rats compared to the SD-fed rats. Rats fed a standard diet reveal the normal structure of the liver. Figure 6 shows the effects of our fruit extracts on adipose tissues as well as the size of epididymal and abdominal adipocytes. The size of adipocytes was significantly increased in HFD-fed rats compared to the SD group. AUAE, and especially CMAE and ATOR-supplemented HFD, on the other hand, reduced the size of adipocytes in the previously mentioned fat tissues of rats.

Discussion

Obesity pathogenesis is an essential risk agent in the metabolic disorders including type 2 diabetes. It has been showed that it is a medical status with fat overproduction associated with a usual oxidative stress and low intensity inflammation that lead

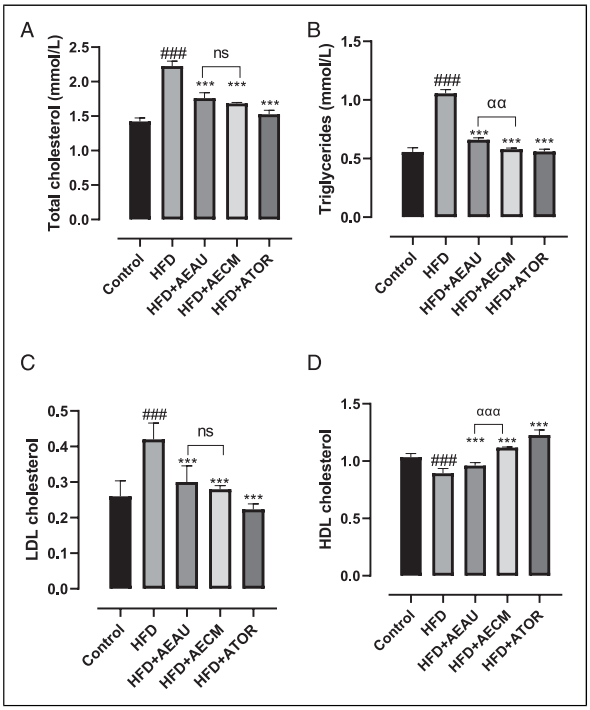

Figure 3. Effect of the AUAE, CMAE, and ATOR on serum total cholesterol (A), triglycerides (B), LDL-cholesterol (C), and HDL-cholesterol (D) in rats exposed to HFD. Data are mean ± SEM, n = 6. ###$P \leq .001$ vs Control.*$P < .05$, **$P < .01$, ***$P < .001$ vs HFD group. $^{\alpha}$ $P < .05$, $^{\alpha\alpha}$ $P < .01$, $^{\alpha\alpha\alpha}$ $P < .001$ vs CMAE group.

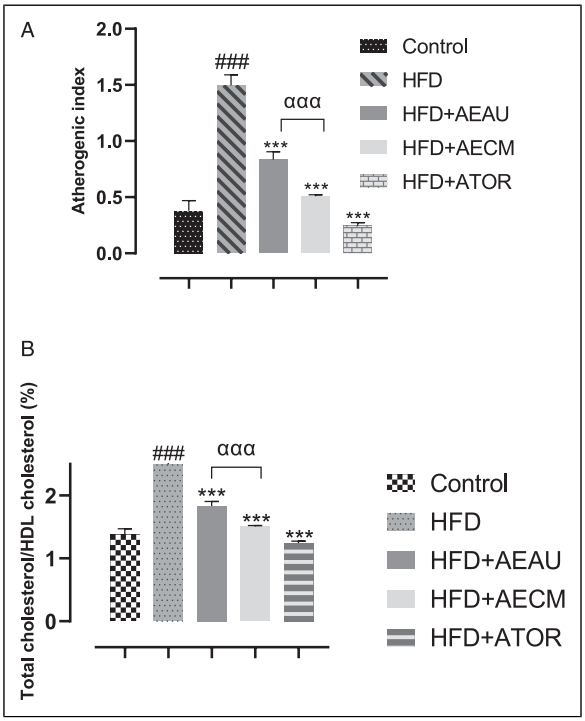

Figure 4. Effect of the AUAE, CMAE, and ATOR on the AI (A) and Coronary risk index (B) in rat exposed to HFD. Data are mean ± SEM, n = 6. ###$P \leq .001$ vs Control. *$P < .05$, **$P < .01$, ***$P < .001$ vs HFD group. $^{\alpha}$ $P < .05$, $^{\alpha\alpha}$ $P < .01$, $^{\alpha\alpha\alpha}$ $P < .001$ vs CMAE group.

to systemic alterations in the body.[41,42] For this, it was important to figure out the antioxidant activity in the obesity-related study because of the strong relationship between obesity and oxidative stress. In this context, the in vitro radical-scavenging capabilities show that AUAE and CMAE exhibit potent antioxidant activities. The imbalance prooxidants/antioxidants can be a major risk factor for metabolic disorders such as obesity, hypertension, insulin resistance, dyslipidemia, and diabetes mellitus.[43,44] Several studies have found a strong link between a high-fat diet and oxidative stress in both humans and animals.[45] Obesity caused by a high-fat diet, for example, has been linked to oxidative stress, which leads to an increase in the production of oxidized LDL cholesterol, which is involved in the pathogenesis of dyslipidemia, atherosclerosis, and other cardiovascular diseases.[46,47]

The prevalence and severity of obesity are growing worldwide.[2] Anti-obesity drugs are commercially available for use in weight control and weight reduction, but undesirable side effects have made it difficult for obese patients to achieve long-term maintenance of weight loss.[48,49] Therefore, safe and efficacious agents are needed urgently to inhibit the occurrence and development of obesity on a long-term basis. Recently, several natural photochemical compounds derived from fruits and vegetables have been demonstrated to suppress obesity and obesity-related metabolic syndrome.[48,50-52]

In this study, we found that the AUAE and specifically the CMAE could provide protection against HFD-induced obesity in rats. The same results were observed in the ATOR treatment group. ATOR is a component known as statins.[53] It is a standard anti-hyperlipidemic drug widely used for the treatment of dyslipidemia and the prevention of cardiovasular disease by reducing the level of cholesterol. It inhibits 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA), the enzyme that catalyzes the rate-limiting step of cholesterol synthesis.[54] Furthermore, it was shown that ATOR reduced fat tissue weight and adipocytes size; this was accompanied by an overall body weight loss tendency.[55] Also, this decrease in body weight may partially be mediated via the inhibition of pancreatic lipase activity and via the activation of thermogenesis through the stimulation of the β-adrenergic receptors.[56]

Many phytochemical compounds have been reported in the AUAE, including vitamins, carotenoids, flavonoids, polyphenols, and tannins, and their derivatives are the most prevalent.[57-61] CMAE contains high levels of numerous valuable secondary metabolites especially flavonoid, vitamin C, glycoside, anthocyanin, saponin, tannin, and phenolic compounds.[62-65]

According to the phytochemical compound findings, the results concerning the *A. unedo* polyphenolic contents are higher than another study which found it between 9.51 and 19.73 mg GAE/g, DW).[66] Also, this concentration is higher

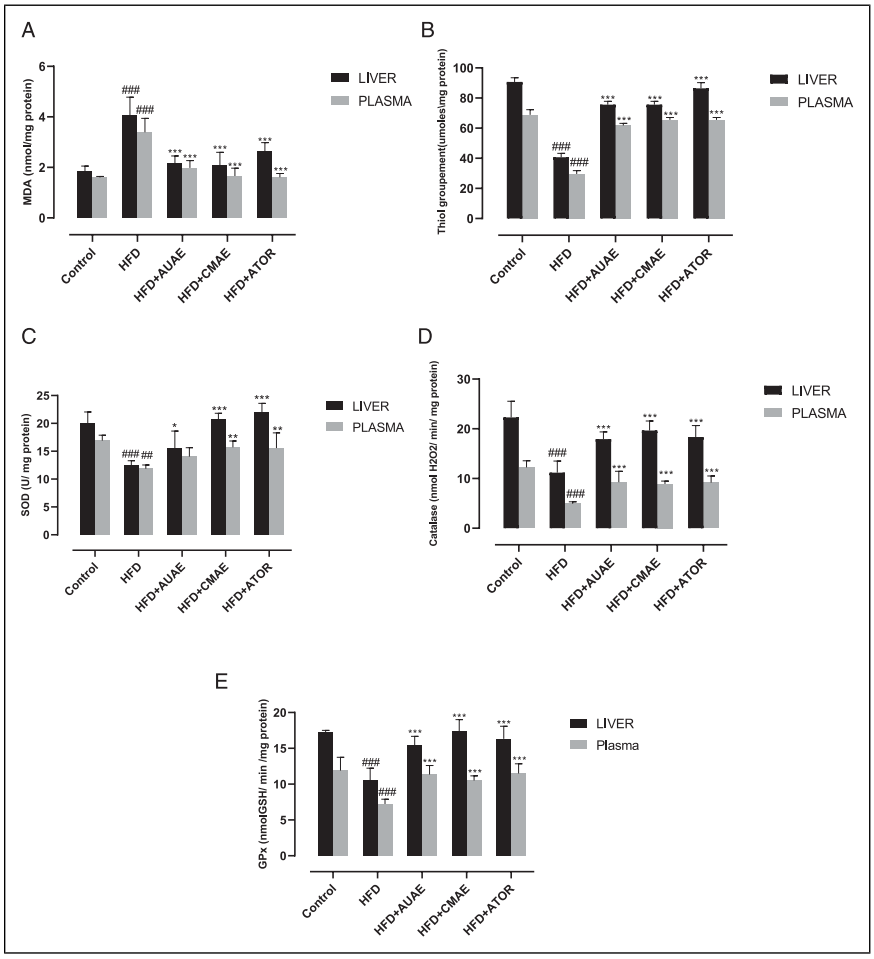

Figure 5. Effect of HFD, AUAE, CMAE and ATOR on MDA (A) and thiol group (SH) levels (B), SOD (C), CAT (D), and GPx (E) activities in serum and liver tissues. Values are expressed as mean ± SEM (n = 6). **P* < .05, ***P* < .01, and ****P* < .001 vs HFD group; $^{\#}P < .05$, $^{\#\#}P < .01$, and $^{\#\#\#}P < .001$ vs Control group. HFD, high-fat diet group; AUAE, *A. unedo* fruit aqueous extract treatment group; CMAE, *C. monogyna* fruit aqueous extract treatment group; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde.

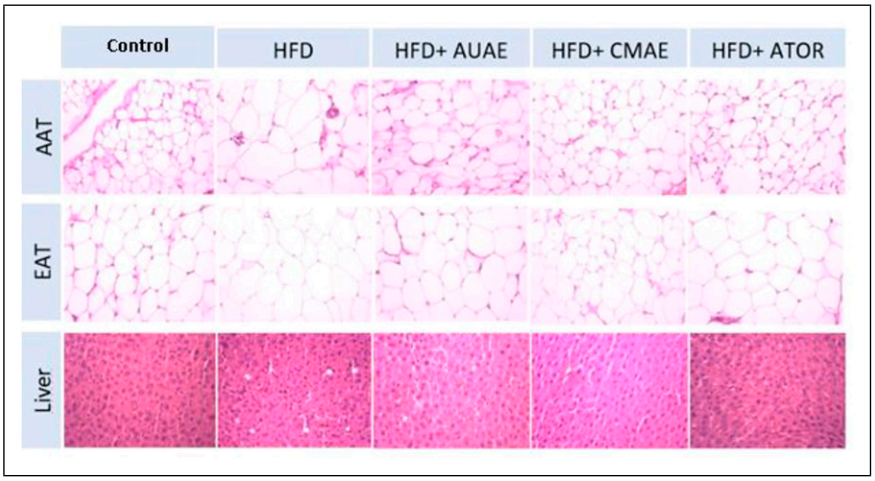

Figure 6. The effects of AUAE, CMAE, and ATOR on adipocyte accumulation in hepatic tissue, epididymal (EAT) and abdominal (AAT) adipose tissues (hematoxylin and eosin staining, 40× magnification). HFD, high-fat diet group; AUAE, *A. unedo* fruit aqueous extract treatment group; CMAE, *C. monogyna* fruit aqueous extract treatment group; EAT, Epididymal adipose tissue; AAT, Abdominal adipose tissue.

than those obtained in the fruits collected from Portugal (16.7 ± .4 mg GAE/g, DW).[67] But, it was lower than others found by Barros et al[68] (126.83 ± 6.66 mg GAE/g, DW) and 34.99 ± 1.55 mg CE/g, DW) and El Cadi et al.[69] Moreover, for *C. monogyna* fruit extract, the phenolic and flavonoid constituent amounts are superior to the ones found by Cosmulescu et al[70] (203.01 ± 9.56 mg GAE/100 g fresh weight and 31.37 ± 1.67 mg QE/100 g fresh weight).

After continuous treatment for 12 weeks, we observed a significant anti-obesity impact of AUAE and specifically CMAE at doses of 300 mg/kg/day in HFD-induced obese rats. When compared to HFD-fed rats that did not receive fruit aqueous extract supplementation, rats receiving AUAE, CMAE, and ATOR administration showed significant reductions in BW as well as liver, kidney, abdominal, and epididymal fat weights. The rise in overall BW significantly affects visceral organs, especially the liver, due to the accumulation of fat.[71]

We also investigated the lipid profile (HDL, LDL, TG, and TC) that have been changed linked to an increase of the AI/CRI. According to numerous researches, blood lipids and obesity are tightly associated.[72] Lipids are a crucial substance involved in a variety of cellular processes and homeostasis. The lipid levels in the body are disturbed by obesity, and these irregularities reveal hepatic and cardiovascular problems. Lipid metabolism, synthesis, and transport are all crucial processes that the liver is involved in. An abnormal lipid profile is the key indicator of liver dysfunction.

Similarly, elevated LDL levels and lowered HDL levels are important risk factors for coronary heart disease.[73,74] In this recent study, AUAE and CMAE-supplemented HFD rats have higher HDL levels than HFD obese rats. In HFD obese rats, LDL levels are higher, while in HFD rats fed with both extracts, LDL levels are normal and TC and free fatty acid levels in plasma are significantly reduced. ATOR treatment showed similar effects as extracts. The AI is also lower in the AUAE and CMAE-supplemented HFD rats compared to the HFD rats' groups, further demonstrating that obesity increases the plasma level of the AI. Cardiovascular disorders are linked to an increase in AI levels in plasma.[75] The decrease in the AI suggests that AUAE, CMAE, and ATOR may reduce the risk of cardiovascular atherosclerosis.

Additionally, the plasma concentrations of liver biomarkers were evaluated, particularly those linked to obesity as AST, ALT, ALP, BT, BD, and GGT. The liver is the major organ of the body in charge of detoxification and protein production, and a slew of enzymes play a role in this process. The levels of enzymes and proteins in the blood that are produced by the liver can be used to analyze liver health. Similarly, elevated levels of AST, ALT, ALP, BT, BD, and GGT indicate liver injury.[76] Another study shows the increased level of liver enzymes, especially ALP and GGT, is an indicator of non-alcoholic fatty liver disease and liver dysfunction and relates to type 2 diabetes.[77] In this recent study, the finding showed elevated levels of AST, ALP, ALT, BT, BD, and GGT in HFD obese rats. Nevertheless, oral administration of AUAE, CMAE, or the reference drug indicated relatively reduced enzyme levels. Furthermore, diet and lifestyle choices affect the development of kidney disease.[78] HFD results in increased plasma concentrations of urea, creatinin, and uric acid, which are important indicators of renal dysfunction.

As the catalytic centers of many enzymes, important trace elements, particularly Fe, Zn, Cu, and Mn, are well known to play a significant role in a variety of biological redox processes. Both a deficiency of these micronutrients and an excess of them can cause oxidative stress in cells and tissues as well as disrupt the antioxidant balance.[79] In this study, we demonstrated that HFD feeding is accompanied by a lack of phosphorus and calcium concentrations. The oxidative stress causes influx into the cytoplasm from the extracellular environment and from the endoplasmic reticulum or sarcoplasmic reticulum (ER/SR) through the cell membrane and the ER/SR channels, respectively.[80] Rising Ca^{2+} concentration in the cytoplasm causes Ca^{2+}-influx into mitochondria and nuclei. In mitochondria, Ca^{2+} accelerates and disrupts normal metabolism leading to cell death.[78]

Additionally, it was discovered in this study that HFD rats had much higher concentrations of Fe, an essential element for survival, than SD groups. Furthermore, many animal studies have demonstrated that Fe excess or deficiency can have a significant impact on the development of atherosclerosis.[81] Many epidemiological studies have revealed a favorable correlation between body Fe reserves and the prevalence of coronary heart disease in human populations. In fact, iron is a trace element that participates in the Fenton reaction and produces ROS, which can result in oxidative stress.[82]

In agreement with in vitro antioxidant and radical-scavenging activity, AUAE and CMAE were also shown to possess strong antioxidant property in vivo. This study demonstrated that oral administration of AUAE and CMAE can reduce high-fat diet-induced elevated levels of malondialdehyde (MDA) which was accompanied by augmented thiols groupement and the activities of major antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (*GPx*), and catalase (CAT). The same results were observed in the ATOR treatment group. This antioxidant power of AUAE and CMAE might be attributed to a higher level of total polyphenols and flavonoids found in the fruits. Polyphenolic compounds can inhibit the activity of xanthine oxidase enzyme which is involved in the production of superoxide radicals and stimulation of antioxidant enzymes' activity.[83]

Polyphenolic compounds can act as antioxidants directly by interacting with both radical and non-radical-type ROS, as well as indirectly by modulating the gene expression of antioxidant proteins and enzymes.[84] The increase of lipid in the blood triggered the increase of fat accumulation in the hepatic and adipose tissues as was revealed in the photomicrographs taken after hematoxylin and eosin staining. Histopathological and metabolic changes accrue due to obesity in the liver, under

metabolic imbalance conditions, the lipid droplets become larger and can easily be observed under a light microscope. Any change in lipid transport and lipoprotein secretion can cause the enlargement of normal lipid droplets in the liver.[85] The histological study of the liver shows that the lipid droplets were increased in HFD rats but reduced in the AUAE and CMAE-supplemented with HFD rats' groups. Overweighting due to a HFD causes structural and cellular composition changes in adipose tissues. However, these modifications lead to an increase in adipocyte size (hypertrophy).[86] The histopathological study of adipose tissues shows that the HFD groups' epididymal and abdominal adipose tissues adipocyte enlarged as compared to the SD group. The adipocyte size was significantly reduced in the groups of HFD rats supplemented with AUAE and CMAE.

In conclusion, as ATOR, both extracts controlled obesity and related disorders. These actions were accompanied by various ameliorations in plasma lipid fractions, glucose, lower oxidative stress, and kidney/liver function. The underlying mechanism for the restorative modulations associated with obesity may be attributed to the antioxidant target of the AUAE/CMAE polyphenols. Improving redox balance pathways is an excellent therapeutic strategy for obesity cases and their related detrimental consequences.

Appendix

Abbreviations

AAT	Abdominaladipose tissue
ABTS	2,2-azino-bis(3-ethylbenzothiazolin)-6-sulfonic acid
AI	Atherogenic index
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AUAE	*Arbutus unedo* fruit aqueous extract
AST	Aspartate transaminase
ATOR	Atorvastatin
CAT	Catalase
CI	Coronary index
CMAE	*Crataegus monogyna* fruit aqueous extract treatment group
DPPH	2,2-diphenyl-1picryl-hydrzyl
EAT	epididymal adipose tissue
GGT	gamma-glutamyl transferase
GPx	glutathioneperoxidase
HDL	high-density lipoprotein
HFD	high-fat diet
LDL	low-density lipoprotein
SD	standard diet
SEM	standard error mean
SOD	superoxide dismutase
TC	total cholesterol
TG	triglycerides

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Author Contributions

Conceptualization: SW, KR, HS, methodology and data curation: SW, KR, AA, HS, writing-original draft preparation: SW, KR, HS, writing-review and editing: SW, KR, HS supervision and validation: SW, KR, HS. All authors have read and agreed to the published version of the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board at the University of Jendouba, Tunisia.

Ethical Consideration

All procedures on animals in this study were compiled with the National Institute of Health recommendations for the use and care of animals.

ORCID iD

Kais Rtibi https://orcid.org/0000-0002-3146-0371

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