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Obesity Prevention Effects of Avocado (*Persea americana*) Seed Powder in High-Fat Diet-Induced Obesity in Rats

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Abstract: Avocado seed contains 64% of the phenolic compounds of the whole fruit. This makes avocado seed a potential candidate for the development of treatments for different illnesses, including obesity (the major risk factor for metabolic disorders). The aim of this study was to investigate the effects of avocado seed powder on high-fat diet-induced obesity in rats. Sprague Dawley rats (16 rats) were fed a high-fat diet for 10 weeks. After 10 weeks, the rats were assigned into two groups of eight animals each and were fed either a high-fat diet (HFD; control group) or a high-fat diet containing avocado seed powder (HFD-A; treatment group) for 6 weeks. Animals were weighed weekly, and weekly weight gain was determined. Animals in the treatment (avocado seed) group showed significantly lower body weight gain (7.8 ± 9.63 g) than animals in the control group (33.9 ± 10.84 g) at the end of this study. The treatment group presented with lower triglycerides than the control, with LDL and HDL comparable to the control group. Avocado seed powder showed potential to reduce obesity in rats fed a high-fat diet. Avocado seed can therefore be investigated further as a potential anti-obesity nutraceutical.

Keywords: *Persea americana*; avocado seed; high-fat diet; obesity reversal; anti-obesity; weight gain; weight loss

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1. Introduction

Obesity may lead to metabolic syndrome, a cluster of metabolic disorders that includes insulin resistance, hyperglycemia, dyslipidemia and hypertension [1]. There is an increased risk of developing type 2 diabetes, cardiovascular diseases [2] and certain cancers [3] in obese individuals. The aforementioned diseases are major contributors to the increased morbidity and premature mortality recorded worldwide [4]. According to the World Health Organization (WHO), more than 1 billion people were obese worldwide in 2022 and the prevalence of obesity will continue to rise [5]. Urgent interventions are therefore needed to combat obesity and related disorders. Among the interventions against obesity and its co-morbidities, is the use of anti-obesity agents and nutraceuticals. Plant materials are potential sources of therapeutic agents and nutraceuticals. They contain abundant bioactive compounds that play major roles in the prevention and treatment of diseases [6]. In addition to their nutritive value, nutraceuticals possess medicinal properties and have been used in the management of different diseases such as cancers, arthritis and metabolic disorders [7]. Different nutraceuticals possessing anti-obesity effects have been reported [8]. With increasing interest towards the use of functional foods for disease management, avocado fruit and seed are potential leads to the discovery of anti-obesity nutraceuticals.

Avocado (*Persea americana* Mill) has been reported to possess medicinal properties [9–11]. It is from the Lauraceae family, and it is cultivated in tropical and sub-tropical areas in Mexico, Brazil, India and South Africa [12]. Avocado fruit has an olive-green peel with a smooth, fatty, thick and almost creamy-textured pulp. Avocado seed makes up 13% of the

fruit and contains 64% of the phenolic compounds contributing to 57% of the antioxidant activity of the whole fruit [13]. The seed contains higher antioxidant activity, and higher procyanidin content than the pulp [14].

Phytochemicals found in avocado seed include flavonoids, alkaloids, saponins, glycosides and tannins [15–17]. The phenolic compounds in avocado seed include protocatechuic acid, kaempferide, vanillic acid, chlorogenic acid, syringic acid, rutin and kaempferol [13]. Phenolic compounds reduce adipogenesis, inhibit digestive enzymes and possess anti-inflammatory and antioxidant effects [18,19]. These properties play a major role in obesity management. The seed also contains nutrients such as carbohydrates, proteins and fats [16,20] and minerals such as magnesium, potassium, sodium, zinc and chromium, in addition to vitamin C [12,16,21]. These trace elements affect different pathways of glucose and lipid metabolism [22].

Avocado seed extracts have been found to have anti-hypertensive properties, lower total cholesterol levels and possess hypoglycemic activities [12,15,23]. Even though the seeds contain beneficial phytochemicals, they are usually disposed during processing and consumption, thus contributing to environmental waste. The use of avocado seed may contribute towards the management of obesity and its comorbidities, while also reducing organic waste burden. This study therefore aimed to investigate the obesity prevention effects of avocado seed powder on obesity induced by a high-fat diet in rats.

2. Materials and Methods

Hass avocado (*Persea americana Mill*) fruits were purchased from a local market in Makhado, Limpopo province, South Africa. Sprague Dawley rats were sourced from South African Vaccines Producers, Johannesburg, while standard animal pellets were bought from Epol, South Africa. Palm stearin was obtained from commercial food provider Ingreto in Johannesburg, South Africa.

2.1. Animal Feed Preparation

The avocado seeds were cut into small pieces with a knife and dried in an oven at 30 °C for 72 h. Dried seeds were milled into powder and stored in the fridge at 4 °C until further use. Standard commercial Epol pellets were ground to a powder together with molasses (constituting 8% of the final feed) as a binding agent. Palm stearin (20% of the total final feeds) was added to the chow to make a high-fat diet (HFD). Powdered avocado seed (20% of the total feed) was included in the HFD mix to make a high-fat diet with avocado seed powder for treatment. The ingredients were all mixed and pelleted using a pelleting machine. The formulated pellets were HFD and HFD with avocado seeds (HFD-A). The pellets were spread on an open surface for approximately 1 h to air cool after production. They were then weighed, packaged in 5 kg plastic bags, sealed, labeled and stored in a refrigerator (−4 °C) until use.

2.1.1. Nutritional Analysis of Food Pellets

Nutritional analysis of the formulated pellets was conducted at the South African Grain Laboratory (SAGL) using validated methods. The pellets were analyzed for protein content, fat content, fiber content, moisture content and ashes [24]. Protein content was determined using an AACC international approved method of analysis (the combustion method). Fat was extracted from the samples with petroleum ether using Soxhlet extraction apparatus, based on AACC method 30-25.01. To determine the moisture content, the feed sample weighing 2 g was dried in an oven at 105 °C for 5 h and the dried feed was reweighed. The moisture content was calculated as the difference in weights.

2.1.2. Elemental Analysis of Food Pellets

Elemental analysis was performed using the method by [25]. The pellets were crushed into a powder with a mortar and pestle. The powdered samples were acid digested to decompose the organic and inorganic components of the samples and release the elements

from the sample matrix [26]. The solutions were then cooled and diluted with distilled water to make a sample concentration of 33.33 mg/mL. Then, the concentrations of boron (B), sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), molybdenum (Mo), selenium (Se), arsenic (As) and chromium (Cr) were determined with an inductively coupled plasma-optical emission spectrometer (ICP-OES). Where readings were above the linear dynamic range, the samples were diluted further. Standards of the elements were obtained from SMM instruments; Johannesburg, South Africa (SA), Industrial analytical; Johannesburg, South Africa (SA) and Teknolab Sorbent AB; Kungsbacka, Sweden, and were used at the concentration of 50 µg/mL.

2.2. In Vivo Assays

Ethical approval to conduct this study was obtained from the Animal Ethics Committee of the University of Pretoria (V038-15) and the Animal Research Ethics Committee of Tshwane University of Technology (AREC2015/10/005(2)). This study was conducted following the South African Medical Research Council's guidelines on ethics for medical research (use of animals in research and training [27]), and the South African National Standard (the care and use of animals for scientific purposes [28]). Other than the researcher, the animals were under the care of a certified animal technician with indirect supervision of a veterinarian. The animals were observed for any signs of distress such as behavioral changes, tremors, salivation, diarrhea, lethargy, sleep, convulsions or coma throughout this study. At the end of this study, the animals were euthanized under anesthesia.

2.2.1. Animal Model

Eight-to-twelve-week-old male Sprague Dawley rats sourced from South African Vaccine Producers (Johannesburg; South Africa) were used as animal models in this study. The rats were kept at the University of Pretoria Biomedical Research Centre in conventional cages (one rat per cage with sawdust bedding, facial tissues, egg containers, shredded paper as nesting material, wooden sticks as gnawing material and 15 cm PVC pipe for hiding). The room was maintained at a temperature of ±22 °C, controlled relative humidity (50–60%) and a light/dark cycle of 12 h [29]. The animals received a high-fat diet (HFD) to induce obesity for 10 weeks, after which they were randomly assigned to 2 groups of 8 animals per group. Rats in one group (n = 8) continued with a HFD (control group) for 6 more weeks, while the treatment group (n = 8) received a high-fat diet with avocado seed (HFD-A; treatment group) for 6 weeks. The animals were weighed at the beginning of the study (week 0), then weekly and the weight for each animal was recorded. The weight gain for each animal was calculated as the difference between its weekly weight and its weight at week 0 of the treatment (Equation (1)), while percentage weight gain was calculated using Equation (2). Food intake was monitored by weighing the amount of food that was given and the amount that remained after every 48 h to determine food consumption using Equation (3).

$$\text{Weekly weigh gain} = WweekX - Wweek0 \quad (1)$$

where W = weight, X = week number and 0 = initial

$$\%\text{weight gain} = \frac{\text{Weekly weight gain}}{\text{Initial weight}} \times 100 \quad (2)$$

$$\text{Daily food intake} = \frac{Mass\ food\ in - Mass\ of\ food\ left}{2\ days} \quad (3)$$

The animals were euthanized at the end of week 16 (thus, 10 weeks of obesity induction and 6 weeks of treatment). The animals were sacrificed by cardiac puncture under isoflurane anesthesia [30]. After the animal was confirmed dead, the kidneys, liver, heart, adipose tissue and brain were isolated and weighed. Blood samples were collected and sent to the pathology lab for liver function tests (ALT, AST and ALP) and cholesterol analysis (total cholesterol, high-density lipoproteins, low-density lipoproteins and triglycerides).

2.2.2. Histopathology

Histopathology was conducted on 3 randomly selected rat samples from each group by IDEXX laboratory (Pretoria North, South Africa). Organ specimens were collected and fixed in 10% buffered formalin. The organs were cut, and the tissues were processed in an automated histological tissue processor overnight. Histological sections of 5 µm were cut on a microtome and the resultant slides were stained with hematoxylin and eosin dyes in an automated histological stainer. The slides were examined, and morphological findings recorded.

2.3. Phytochemical Analysis

To determine the phytochemicals responsible for the obesity prevention effects of avocado seed powder observed in this study, avocado seed powder methanol extract was subjected to analysis by liquid chromatography coupled with mass spectroscopy (LC-MS), as described before [31].

2.4. Data Analysis

Results were reported as mean ± standard deviation (n = 8). Data collected were analyzed using Stata software version 14 (StataCorp LLC: College Station, TX, USA). A generalized least squares (GLS) regression model was used to determine significant statistical differences between the treatment groups and the control group. This method was utilized because it improved statistical efficiency and minimized the chances of reporting erroneous inferences. The results were considered significantly different at $p < 0.05$.

3. Results

3.1. Nutritional and Elemental Analysis

Results of the nutritional composition of the formulated pellets are shown in Table 1. Although HFD showed a higher protein than HFD-A, HFD-A showed a higher fat content than HFD. For elemental composition, a solution obtained from the acid digestion of HFD-A showed a higher content of Mg, K, Ca, Mn and Fe than a solution from HFD (Table 2).

Table 1. Nutritive value of pellets in %.

Analysis	HFD	HFD-A
Moisture (%)	9.30	8.10
Protein (%)	16.09	13.70
Ash (%)	5.46	4.82
Fat (%)	19.50	24.50
Fiber (%)	4.60	4.70

Table 2. Elemental content of the solution obtained from acid digestion of the pellets in mg/L.

Trace Elements (mg/L)	B	Cu	Se	Mo	As	Cr	Na	Mg	K	Ca	Mn	Fe
HFD	0.22	0.60	0.23	<−0.17	0.01	<−0.08	34.01	17.08	69.03	42.75	0.52	1.89
HFD-A	0.25	0.34	0.16	<−0.18	0.01	<−0.07	30.26	22.52	>105.0	57.08	0.76	2.52

3.2. In Vivo Study

3.2.1. Animal Food Intake and Effects of Avocado Seed Powder on Animal Weights

The food intake by animals in both groups was comparable, as animals in HFD consumed an average 22.18 ± 0.66 g/day while animals in HFD-A consumed an average of 28.04 ± 3.11 g/day. The difference in food intake by the treatment group was not significant ($p > 0.05$) when compared with food intake by the control group. The weekly weights of the

animals for the treatment period are presented in Table 3, with weight gain/loss presented in Figure 1.

Table 3. Weekly weights of the animals across the treatment period (mean ± SD; n = 8).

Week	HFD (Control) Group Weight (g)	HFD-A (Treatment) Group Weight (g)
0	345.88 ± 9.16	306.75 ± 4.06
1	340.00 ± 11.48	288.38 ± 6.65
2	350.00 ± 11.77	288 ± 6.59
3	355.88 ± 10.37	286.88 ± 7.28
4	362.38 ± 12.93	289.88 ± 9.51
5	363.63 ± 15.84	293.25 ± 9.22
6	379.75 ± 12.67	314.5 ± 9.72

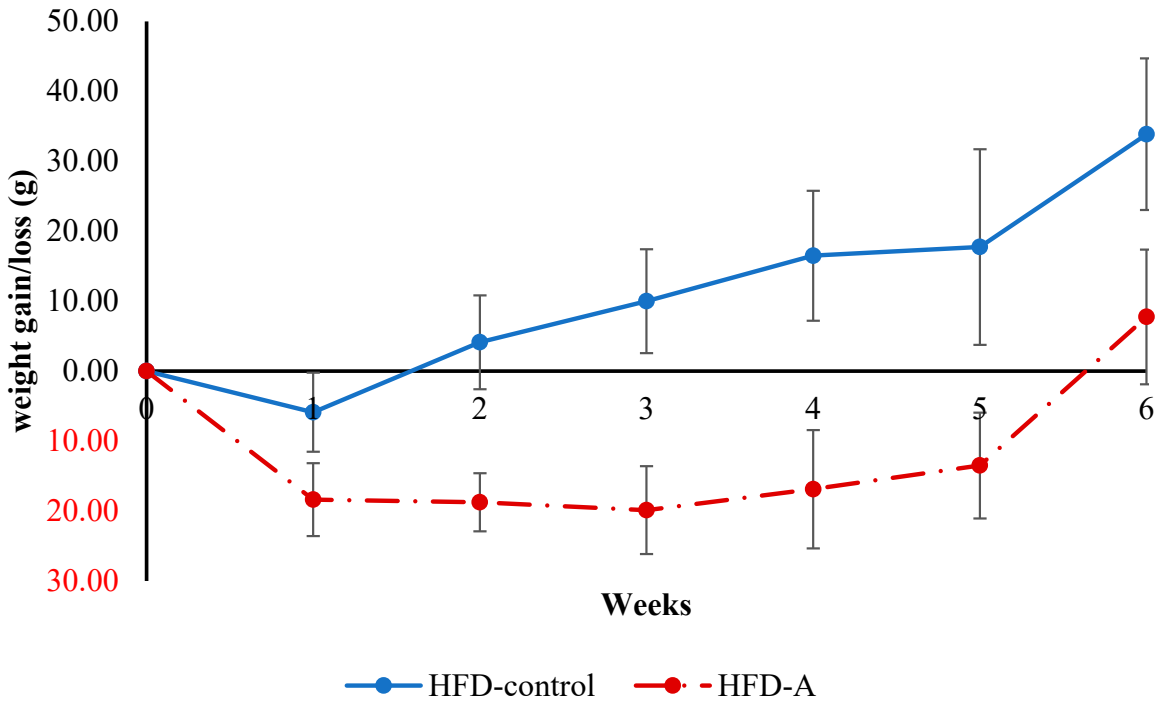

Figure 1. Weight gain per group during treatment period of this study (error bars represent the standard deviation of mean (n = 8)).

There was a significant weight loss in the first week of treatment for the animals in the HFD-A group (Figure 1). The animals in this group did not gain weight until week 3, after which they showed minimal weight gain compared with the control group ($p < 0.05$) until the end of this study. HFD-A gained 7.75 ± 9.63 g, while HFD gained 33.88 ± 10.84 g by the end of this study. Percentage weight gain for animals was 9.81% and 2.53% for HFD and HFD-A, respectively, by the end of this study. A significant difference in average animal weights between the two groups was observed throughout this study.

3.2.2. Effects of Avocado Seed Powder on Animal Organ Weight and Biochemical Parameters

There was no significant difference in the relative heart weight between the two groups (Figure 2). HFD-A presented with a significantly higher brain weight than the control. Adipose tissue was significantly lower in HFD-A compared with the control.

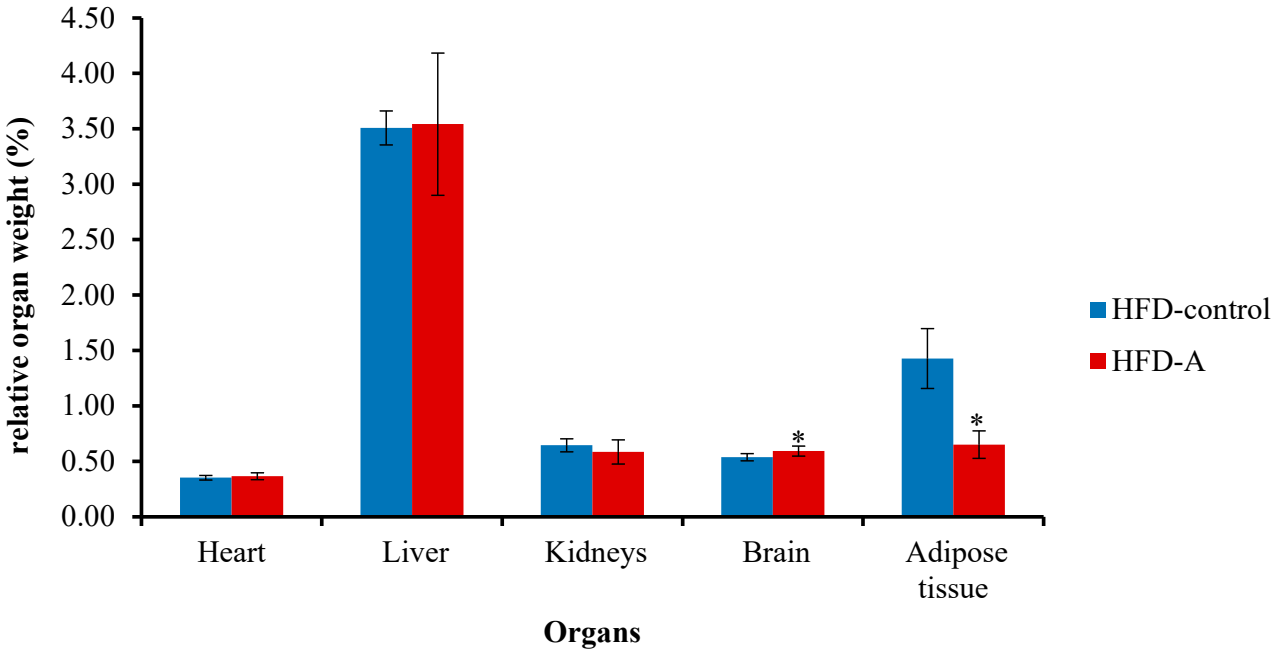

Figure 2. Percentage organ weight relative to the body weight of the animals. * Statistical significance ($p < 0.05$) when treatment group is compared with HFD-control.

Regarding biochemical parameters (Table 4), the treatment group showed triglyceride levels significantly lower ($p < 0.05$) than that of the control group, with comparable HDL and LDL. Both groups presented comparable total cholesterol levels. The ALP in HFD-A group was significantly higher than in the control group.

Table 4. Biochemical parameters of the animals at the end of this study (mean ± SD; n = 8).

Analysis	HFD	HFD-A
Total cholesterol (mmol/L)	2.38 ± 0.13	2.23 ± 0.32
Triglycerides (mmol/L)	0.49 ± 0.10	0.34 ± 0.05 *
HDL (mmol/L)	0.98 ± 0.05	1.00 ± 0.08
LDL (mmol/L)	0.59 ± 0.08	0.69 ± 0.11
ALT (U/L)	65.75 ± 14.67	70.75 ± 12.93
ALP (U/L)	130.00 ± 22.81	175.88 ± 28.40 *
AST (U/L)	83.63 ± 18.95	74.29 ± 10.13

* Statistical significance ($p < 0.05$) when HFD-A is compared with HFD.

3.2.3. Post-Mortem Results

All the animals were in good health with no abnormalities detected in both groups. The livers of some animals in both groups were histologically within normal limits, while some revealed mild vascular changes with varying distribution patterns in the hepatocyte cytoplasm. Macroscopic pathology was not detected with all other tissues.

3.3. Phytochemical Analysis

Figure 3 presents LC-MS chromatogram for the avocado seed powder extracts. Further analysis of peaks 309 and 351 fragments showed the presence of cinnamic acid. Fragments of peak 371 showed the presence of ferulic acid, neochlorogenic acid and flavone. Peak 702 fragments showed the presence of isoferulic acid-3-O-glucoronide and rothindin. From the LC-MS chromatograms, it was established that the main constituents of avocado seed powder extracts were ferulic acid, neochlorogenic acid and flavone.

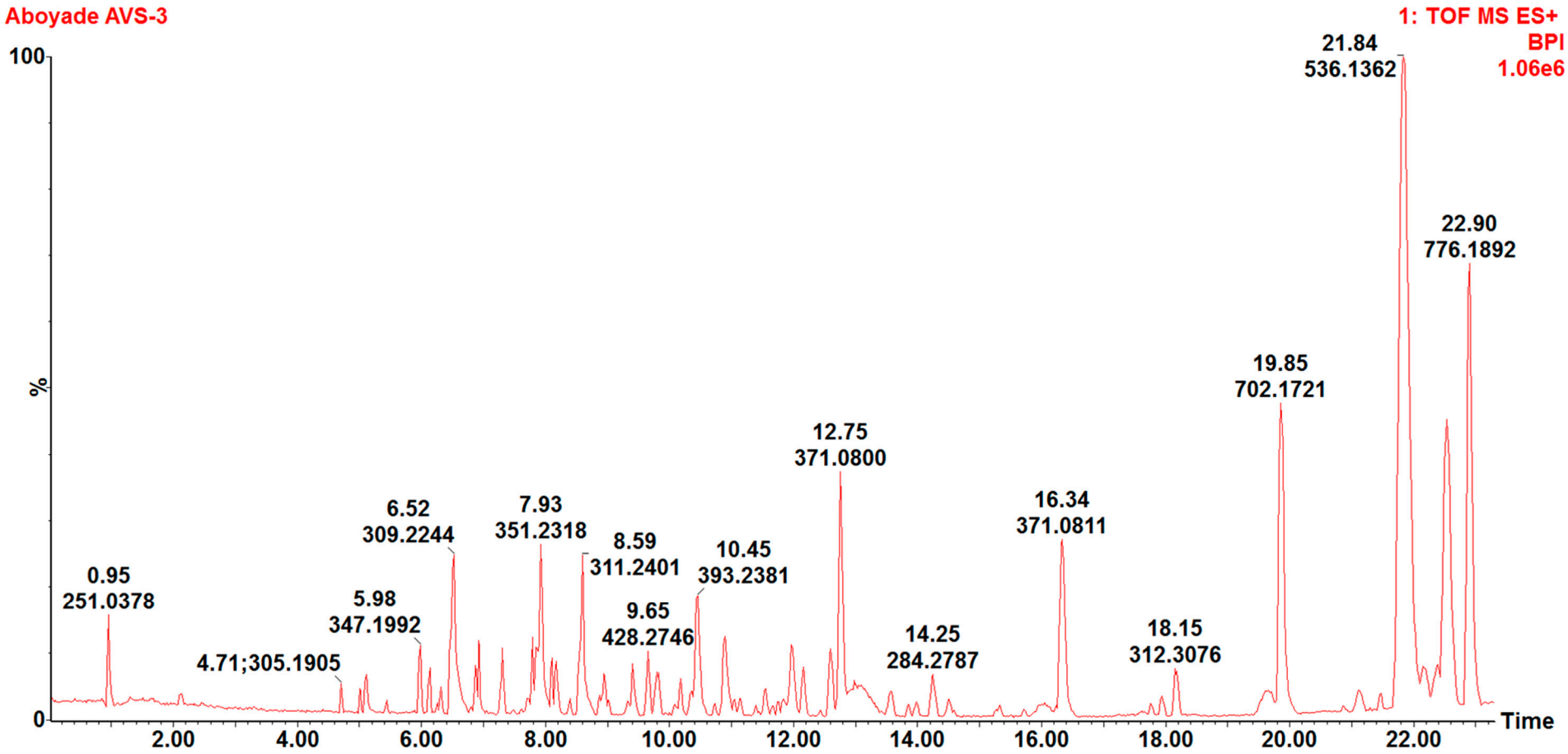

Figure 3. LC-MS chromatogram for avocado seed.

4. Discussion

Rats are considered the best models for studying human metabolic syndrome as they have similar metabolic patterns as humans [32]. The animals maintained their good health throughout this study and none of them became sick or died from the treatment.

Food intake determines calorie intake, which is one of the risk factors of obesity development. High food intake might lead to a higher calorie intake than energy expenditure, leading to the accumulation of body fat and obesity development. Sprague Dawley rats have shown the ability to become obese when placed on a HFD [33]. HFD has also been used to induce obesity in Zucker rats and mice [34–36].

The treatment group gained significantly less weight than the control group in this study, showing the potential of avocado seed to reduce weight gain. A previous study showed that avocado fruits (not avocado seed) resulted in drastic weight loss and reduction in BMI in individuals who received avocados daily for 6 months in an obesity reduction study [9]. Avocado seed oil may be responsible for the reduction of weight observed in animals fed with HFD-A (shown by the high crude fat content of HFD-A compared with HFD). Avocado seed oil is full of monounsaturated fatty acids, beneficial for the management of obesity and other inflammatory disorders. These fatty acids increase the secretion of adiponectin and reduce the plasma levels of pro-inflammatory cytokines [37].

Animals in the treatment group showed significantly less adipose tissue when compared with the control in this study. Similar results were previously obtained with *Cosmos caudatus* Kunth leaf [32]. Animals on a high-fat diet might present with high-fat deposits regardless of their weight [38]. Since the accumulation of adipose tissue in obesity is due to an increase in the number of adipocytes (hyperplasia), which follows increased adipocyte differentiation and size (hypertrophy), a decrease in adipose tissue mass might be associated with a reduction in adipocyte differentiation [35]. This is shown by a decrease in the mRNA level of differentiation biomarkers such as PPARγ, C/EBPα and adiponectin in weight reduction [35]. Therefore, avocado seed powder might possibly inhibit one or more of the processes involved in adipocyte differentiation and proliferation [39].

Trace elements such as zinc, iron, calcium, copper, manganese and selenium, play a major role in the regulation of metabolic pathways, including lipid regulation [40], and act as co-factors for enzymes. Disturbances in their homeostasis have been noted in obesity [41,42] and may lead to oxidative stress, reduction in immunity and increased expression of inflammatory mediators. Their supplementation to the required concentration may, therefore, play a role in ameliorating obesity. Avocado seed powder showed the presence of Fe, K, Ca, Mn and Mg in this study. Talabi et al. (2016) reported avocado seed to contain calcium, phosphorus, potassium and sodium [20]. While the other study reported the presence of sodium, potassium, calcium, magnesium, iron, manganese and zinc in avocado seed [43]. According to Bouglé et al. (2009), trace elements decrease oxidative and inflammatory stress, thereby preventing obesity and its co-morbidities. Calcium suppresses 1-α-25-dihydroxycholecalciferol, leading to increased adipocyte differentiation and lipogenesis from fatty acids and inhibits lipolysis [42]. The role of magnesium and calcium in obesity modulation has been demonstrated with deep sea water (DSW), for instance. DSW resulted in weight reduction in mice, decreased levels of adiponectin and PPARγ and decreased levels of circulating adipogenic proteins in the DSW group compared with the tap water group and this was attributed to the presence of Mg and Ca [44]. In addition to regulating insulin production and action, magnesium is a co-factor for different enzymatic reactions involved in glucose modulation [45].

In this study, HFD-A showed a potential to decrease triglycerides, with LDL, HDL and total cholesterol comparable to animals fed with HFD. Obesity is associated with various biochemical abnormalities such as high triglyceride levels and low HDL [46]. High-fat-containing meals increase the level of cholesterol and also alter lipid profiles [47]. Higher levels of cholesterol and triglycerides were previously obtained from rodents on a HFD (21% fat content of the food) than with the control (5% fat content) [48]. A good treatment would therefore be able to decrease total cholesterol, triglyceride and LDL while

increasing the level of HDL. Avocado oil has been found to alleviate non-alcoholic fatty liver disease, hyperglycemia and dyslipidemia in rats fed with a high-fat/high-fructose diet by improving mitochondrial function and reducing the levels of reactive oxygen species, pro-inflammatory factors and lipid peroxidation [10].

Though the mechanism of obesity prevention was not established from this study, phytochemicals such as apigenin (flavone), hesperetin (flavanone), cyanidin (anthocyanins) and curcumin (phenolic acid) among others have been found to combat obesity in different ways [49]. These phytochemicals have been reported to reduce the digestion of fat (probably through lipase inhibition), hence reduce lipid absorption from the gastro-intestinal tract, and also effect anti-inflammatory actions [49]. Polyphenols also cause a decrease in lipogenesis and increase lipolysis [17,39,50,51]. The flavonoids and saponins in avocado are associated with inhibition in the inflammatory pathways involved in obesity development and the pathophysiology of its comorbidities [51,52]. These phytochemicals may be responsible for the observed anti-obesity effects of avocado seed powder, as the LC-MS analysis indicated the presence of cinnamic acid, ferulic acid, neochlorogenic acid, isoferulic acid-3-O-glucoronide and rothindin in this study. Ferulic acid and chlorogenic acid have been reported to elicit their anti-obesity effects through the inhibition of pancreatic lipase [53]. As obesity is associated with inflammation, agents with anti-inflammatory effects would be beneficial. The reduction of inflammatory cytokines has been reported as one of the mechanisms the phytochemicals follow in obesity management. This mechanism was reported for cinnamic acid [54], ferulic acid [55] and neochlorogenic acid [56], in preventing or reversing obesity. It was reported that the anti-obesity activity of ferulic acid was due to its potential to inhibit inflammatory markers such as tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCH-1) [55]. This was also observed in a study by Tian et al., 2022, where ferulic acid reduced the production of the inflammation cytokines TLR-4, NF-κB, TNF-α, IL-1β and IL-6 and reduced weight gain in mice fed a high-fat diet [57]. In addition, ferulic acid reduced leptin levels and increased ghrelin levels in mice fed with HFD [55].

Avocado seed has been reported to contain catechins, hydroxybenzoic acids, hydroxycinnamic acids, flavonols and procyanidins [13]. Another study showed the presence of 5-O-caffeoylquinic acid, quercetin 3-O-glucoside, quercetin 3-O-galactoside, quercetin 3-O-rutinoside, quercetin-3,4′-diglucoside and quercetin 3-O-arabinoside [58]. These phytochemicals have been reported to play a significant role in the treatment of obesity and its co-morbidities [59]. Hence, the potential anti-obesity properties shown by avocado seed powder in this study may be attributed to these phytochemicals. The phytochemical composition of plants is affected by genetic, environmental and physical factors [60]. Parameters for extraction methods such as temperature, duration of extraction, solvent-to-solid ratio and storage conditions affect the phytochemical content [61]. These may explain the differences in the phytochemical composition of the avocado seed from this study and the other studies.

Although animals in the treatment group showed increased brain weight, both the post-mortem and histology examination did not confirm injury to any other organ and did not reveal any lesions due to toxic effects in any of the organs in both groups. As a major organ of metabolism, the liver is more prone to drug toxicity [62]. Liver injury can increase the permeability of hepatocytes, as they lose their integrity, accompanied by the leakage of the enzymes into the bloodstream [63]. The increase in liver enzymes in the blood might therefore be directly proportional to the extent of the injury. The results of the liver enzymes showed that the animals on HFD-A had higher ALP than those on HFD. Apart from its use in hepatic function analysis, ALP is also elevated in bone disease [64]. An increase in ALP levels of the treatment group may therefore require further investigations. An increase in ALT and AST levels on animals fed avocado seeds has been reported previously [65]. However, another study found no significant changes in liver enzymes associated with avocado seed [66]. The changes in liver enzymes might indicate possible liver damage in this group. However, the histopathology results did not show any detectable liver

injury or injury to any other organ. The vascularization observed in this study might be due to microvesicular lipidosis, which has been reported before in mice on HFD [67]. Vascularization might also show hepatocellular damage under oxidative stress [68]. Since other findings (clinical and anatomical) did not point towards any clinically significant hepatic changes, the importance of the observed changes was uncertain due to the degree of the hepatic lesions (varying between normal, minimal/slight and mild).

In this study, avocado seed showed the potential to reduce weight gain in rats fed with a high-fat diet. Avocado seed should be investigated further for its mechanisms of action and safety.

5. Conclusions

Avocado seed powder contains different phytochemicals and trace elements with potential for use in management of obesity. In this study, avocado seed powder has shown potential to prevent obesity and reduce triglycerides. However, the long-term effects of the avocado seed powder ingestion still needs to be investigated. Further studies should be conducted over a long period to determine the mechanisms of action of avocado seed in managing obesity and its safety in humans. Until that is done, we would not recommend it's chronic use in supplements.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets for this study are not available online; however, they can be found upon request by interested researchers to the corresponding author.

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References

1. Klein, S.; Gastaldelli, A.; Yki-Järvinen, H.; Scherer, P.E. Why does obesity cause diabetes? *Cell Metab.* **2022**, *34*, 11–20. [CrossRef] [PubMed]
2. Pi-Sunyer, X. The Medical Risks of Obesity. *Postgrad. Med.* **2009**, *121*, 21–33. [CrossRef]
3. Tzenios, N. Obesity as a Risk Factor for Different Types of Cancer. *EPRA Int. J. Res. Dev.* **2023**, *8*, 97–100. [CrossRef]
4. Popkin, B.M.; Ng, S.W. The nutrition transition to a stage of high obesity and noncommunicable disease prevalence dominated by ultra-processed foods is not inevitable. *Obes. Rev.* **2021**, *23*, e13366. [CrossRef] [PubMed]
5. WHO. *World Obesity Day 2022—Accelerating Action to Stop Obesity*; WHO: Geneva, Switzerland, 2022.

6. Iwu, M.M. Introduction: Therapeutic Agents from Ethnomedicine. In *Advances in Phytomedicine*; Elsevier: Amsterdam, The Netherlands, 2002; pp. 1–22.
7. Ruchi, S. Role of nutraceuticals in health care: A review. *Int. J. Green Pharm.* **2017**, *11*. [CrossRef]
8. Nijhawan, P.; Behl, T. Nutraceuticals in the management of obesity. *Obes. Med.* **2019**, *17*, 100168. [CrossRef]
9. Devalaraja, S.; Jain, S.; Yadav, H. Exotic fruits as therapeutic complements for diabetes, obesity and metabolic syndrome. *Food Res. Int.* **2011**, *44*, 1856–1865. [CrossRef]
10. García-Berumen, C.I.; Vargas-Vargas, M.A.; Ortiz-Avila, O.; Piña–Zentella, R.M.; Ramos-Gómez, M.; Figueroa–García, M.d.C.; Mejía-Zepeda, R.; Rodríguez–Orozco, A.R.; Saavedra–Molina, A.; Cortés-Rojo, C. Avocado oil alleviates non-alcoholic fatty liver disease by improving mitochondrial function, oxidative stress and inflammation in rats fed a high fat–High fructose diet. *Front. Pharmacol.* **2022**, *13*, 1089130. [CrossRef]
11. Ochoa-Zarzosa, A.; Báez-Magaña, M.; Guzmán-Rodríguez, J.J.; Flores-Alvarez, L.J.; Lara-Márquez, M.; Zavala-Guerrero, B.; Salgado-Garciglia, R.; López-Gómez, R.; López-Meza, J.E. Bioactive molecules from native Mexican avocado fruit (*Persea americana* var. drymifolia): A review. *Plant Foods Hum. Nutr.* **2021**, *76*, 133–142. [CrossRef]
12. Alhassan, A.J.; Sule, M.S.; Atiku, M.K.; Wudil, A.M.; Abubakar, H.; Mohammed, S.A. Effects of aqueous avocado pear (*Persea americana*) seed extract on alloxan induced diabetes rats. *Greener J. Med. Sci.* **2012**, *2*, 005–011. [CrossRef]
13. Pahua-Ramos, M.E.; Ortiz-Moreno, A.; Chamorro-Cevallos, G.; Hernández-Navarro, M.D.; Garduño-Siciliano, L.; Necoechea-Mondragón, H.; Hernández-Ortega, M. Hypolipidemic effect of avocado (*Persea americana* Mill) seed in a hypercholesterolemic mouse model. *Plant Foods Hum. Nutr.* **2012**, *67*, 10–16. [CrossRef] [PubMed]
14. Wang, W.; Bostic, T.R.; Gu, L. Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars. *Food Chem.* **2010**, *122*, 1193–1198. [CrossRef]
15. Ezejiofor, A.N.; Okorie, A.; Orisakwe, O.E. Hypoglycaemic and tissue-protective effects of the aqueous extract of *Persea americana* seeds on alloxan-induced albino rats. *Malays. J. Med. Sci.* **2013**, *20*, 31–39. [PubMed]
16. Henry, L.N.; Mtaita, U.Y.; Kimaro, C.C. Nutritional efficacy of avocado seeds. *Glob. J. Food Sci. Technol.* **2015**, *3*, 192–196.
17. Leite, J.J.G.; Brito, H.S.; Cordeiro, R.A.; Brilhante, R.S.N.; Sidrim, J.J.C.; Bertini, L.M.; de Morais, S.M.; Rocha, M.F.G. Chemical composition, toxicity and larvicidal and antifungal activities of *Persea americana* (avocado) seed extracts. *Rev. Soc. Bras. Med. Trop.* **2009**, *42*, 110–113. [CrossRef]
18. Sebastián, D.F.-S.; Alaniz-Monreal, S.; Rabadán-Chávez, G.; Vázquez-Manjarrez, N.; Hernández-Ortega, M.; Gutiérrez-Salmeán, G. Anthocyanins: Potential Therapeutic Approaches towards Obesity and Diabetes Mellitus Type 2. *Molecules* **2023**, *28*, 1237. [CrossRef]
19. Aloo, S.-O.; Ofosu, F.K.; Kim, N.-H.; Kilonzi, S.M.; Oh, D.-H. Insights on Dietary Polyphenols as Agents against Metabolic Disorders: Obesity as a Target Disease. *Antioxidants* **2023**, *12*, 416. [CrossRef]
20. Talabi, J.Y.; Osukoya, O.A.; Ajayi, O.O.; Adegoke, G.O. Nutritional and antinutritional compositions of processed Avocado (*Persea americana* Mill) seeds. *Asian J. Plant Sci. Res.* **2016**, *6*, 6–12.
21. Dreher, M.L.; Davenport, A.J. Hass avocado composition and potential health effects. *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 738–750. [CrossRef]
22. González-Domínguez, Á.; Domínguez-Riscart, J.; Millán-Martínez, M.; Lechuga-Sancho, A.M.; González-Domínguez, R. Exploring the association between circulating trace elements, metabolic risk factors, and the adherence to a Mediterranean diet among children and adolescents with obesity. *Front. Public Health* **2023**, *10*, 1016819. [CrossRef]
23. Imafidon, K.; Amaechina, F. Effects of aqueous seed extract of *Persea americana* Mill. (avocado) on blood pressure and lipid profile in hypertensive rats. *Adv. Biol. Res.* **2010**, *4*, 116–121.
24. Agoreyo, B.; Obansa, E.; Obanor, E. Comparative nutritional and phytochemical analyses of two varieties of Solanum melongena. *Sci. World J.* **2012**, *7*, 5–8.
25. Dolan, S.P.; Capar, S.G. Multi-element analysis of food by microwave digestion and inductively coupled plasma-atomic emission spectrometry. *J. Food Compos. Anal.* **2002**, *15*, 593–615. [CrossRef]
26. Mester, Z.; Sturgeon, R.E. *Sample Preparation for Trace Element Analysis*; Elsevier: Amsterdam, The Netherlands, 2003; Volume 41.
27. SAMRC. *Guidelines on Ethics for Medical Research: Use of Animals in Research and Training 2004*; South African Medical Research Council: Cape Town, South Africa, 2004; pp. 11–16.
28. South African National Standard. *Care and Management of Laboratory Animals—Rodents (Mice, Rats and Hamsters) in the Care and Use of Animals for Scientific Purposes*; SABS: Pretoria, South Africa, 2008; pp. 180–187.
29. Olaokun, O.O.; McGaw, L.J.; van Rensburg, I.J.; Eloff, J.N.; Naidoo, V. Antidiabetic activity of the ethyl acetate fraction of *Ficus lutea* (Moraceae) leaf extract: Comparison of an in vitro assay with an in vivo obese mouse model. *BMC Complement. Altern. Med.* **2016**, *16*, 110. [CrossRef]
30. Parasuraman, S.; Raveendran, R.; Kesavan, R. Blood sample collection in small laboratory animals. *J. Pharmacol. Pharmacother.* **2010**, *1*, 87–93. [CrossRef]
31. Mokhele, M.; Tswaledi, D.; Aboyade, O.; Shai, J.; Katerere, D. Investigation of Aloe ferox leaf powder on anti-diabesity activity. *S. Afr. J. Bot.* **2020**, *128*, 174–181. [CrossRef]
32. Rahman, H.A.; Sahib, N.G.; Saari, N.; Abas, F.; Ismail, A.; Mumtaz, M.W.; Hamid, A.A. Anti-obesity effect of ethanolic extract from *Cosmos caudatus* Kunth leaf in lean rats fed a high fat diet. *BMC Complement. Altern. Med.* **2017**, *17*, 122. [CrossRef]

33. Nilsson, C.; Raun, K.; Yan, F.-F.; O Larsen, M.; Tang-Christensen, M. Laboratory animals as surrogate models of human obesity. *Acta Pharmacol. Sin.* **2012**, *33*, 173–181. [CrossRef]
34. Kalupahana, N.S.; Claycombe, K.; Newman, S.J.; Stewart, T.; Siriwardhana, N.; Matthan, N.; Lichtenstein, A.H.; Moustaid-Moussa, N. Eicosapentaenoic acid prevents and reverses insulin resistance in high-fat diet-induced obese mice via modulation of adipose tissue inflammation. *J. Nutr.* **2010**, *140*, 1915–1922. [CrossRef]
35. Moon, M.-H.; Jeong, J.-K.; Lee, J.-H.; Park, Y.-G.; Lee, Y.-J.; Seol, J.-W.; Park, S.-Y. Antiobesity activity of a sphingosine 1-phosphate analogue FTY720 observed in adipocytes and obese mouse model. *Exp. Mol. Med.* **2012**, *44*, 603–614. [CrossRef] [PubMed]
36. Tsuda, T.; Horio, F.; Uchida, K.; Aoki, H.; Osawa, T. Dietary cyanidin 3-O-β-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *J. Nutr.* **2003**, *133*, 2125–2130. [CrossRef]
37. Hamouda, A.F. Study on the effect of avocado on apoptosis, oxidative stress and injuries induced by diethyl nitrosamine in rat liver. *J. Pharm. Pharmacol.* **2015**, *3*, 243–252. [CrossRef]
38. Cha, M.C.; Chou, C.J.; Boozer, C.N. High-fat diet feeding reduces the diurnal variation of plasma leptin concentration in rats. *Metabolism* **2000**, *49*, 503–507. [CrossRef]
39. Hasani-Ranjbar, S.; Jouyandeh, Z.; Abdollahi, M. A systematic review of anti-obesity medicinal plants—An update. *J. Diabetes Metab. Disord.* **2013**, *12*, 28. [CrossRef]
40. Li, C.-P.; Song, Y.-X.; Lin, Z.-J.; Ma, M.-L.; He, L.-P. Essential trace elements in patients with dyslipidemia: A meta-analysis. *Curr. Med. Chem.* **2024**, *31*, 3604–3623. [CrossRef]
41. Nimrouzi, M.; Abolghasemi, J.; Sharifi, M.H.; Nasiri, K.; Akbari, A. Thyme oxymel by improving of inflammation, oxidative stress, dyslipidemia and homeostasis of some trace elements ameliorates obesity induced by high-fructose/fat diet in male rat. *Biomed. Pharmacother.* **2020**, *126*, 110079. [CrossRef]
42. Bouglé, D.; Bouhallab, S.; Bureau, F.; Zunquin, G. Effects of trace elements and calcium on diabetes and obesity, and their complications: Protective effect of dairy products–A mini-review. *Dairy Sci. Technol.* **2009**, *89*, 213–218. [CrossRef]
43. Alagbaoso, C.A.; Tokunbo, I.I.; Osakwe, O.S. Comparative study of antioxidant activity and mineral composition of methanol extract of seeds of ripe and unripe avocado pear (*Persea americana*, Mill.). *NISEB J.* **2015**, *15*, 123–127.
44. Hwang, H.S.; Kim, H.A.; Lee, S.H.; Yun, J.W. Anti-obesity and antidiabetic effects of deep sea water on ob/ob mice. *Mar. Biotechnol.* **2009**, *11*, 531–539. [CrossRef] [PubMed]
45. Barbagallo, M.; Dominguez, L.J. Magnesium and type 2 diabetes. *World J. Diabetes* **2015**, *6*, 1152. [CrossRef] [PubMed]
46. El-Awdan, S.A.; Jaleel, G.A.A.; Saleh, D.O. Grape seed extract attenuates hyperglycaemia-induced in rats by streptozotocin. *Bull. Fac. Pharm. Cairo Univ.* **2013**, *51*, 203–209. [CrossRef]
47. Angéloco, N.; Deminice, R.; Leme ID, A.; Lataro, R.C.; Jordão, A.A. Bioelectrical impedance analysis and anthropometry for the determination of body composition in rats: Effects of high-fat and high-sucrose diets. *Rev. Nutr.* **2012**, *25*, 331–339. [CrossRef]
48. Chadli, F.K.; Nazih, H.; Krempf, M.; Nguyen, P.; Ouguerram, K. Omega 3 fatty acids promote macrophage reverse cholesterol transport in hamster fed high fat diet. *PLoS ONE* **2013**, *8*, e61109. [CrossRef]
49. Carrera-Quintanar, L.; Lopez Roa, R.I.; Quintero-Fabián, S.; Sánchez-Sánchez, M.A.; Vizmanos, B.; Ortuño-Sahagún, D. Phytochemicals that influence gut microbiota as prophylactics and for the treatment of obesity and in-flammatory diseases. *Mediat. Inflamm.* **2018**, *2018*, 9734845. [CrossRef]
50. Chandrasekaran, C.V.; Vijayalakshmi, M.A.; Prakash, K.; Bansal, V.S.; Meenakshi, J.; Amit, A. Review article: Herbal approach for obesity management. *Am. J. Plant Sci.* **2012**, *3*, 1003–1014. [CrossRef]
51. Mohamed, G.A.; Ibrahim, S.R.; Elkhayat, E.S.; El Dine, R.S. Natural anti-obesity agents. *Bull. Fac. Pharm. Cairo Univ.* **2014**, *52*, 269–284. [CrossRef]
52. Wintola, O.A.; Afolayan, A.J. Phytochemical constituents and antioxidant activities of the whole leaf extract of Aloe ferox Mill. *Pharmacogn. Mag.* **2011**, *7*, 325–333. [CrossRef]
53. Kasabri, V.; Al-Hallaq, E.K.; Bustanji, Y.K.; Abdul-Razzak, K.K.; Abaza, I.F.; Afifi, F.U. Antiobesity and antihyperglycaemic effects of *Adiantum capillus-veneris* extracts: In vitro and in vivo evaluations. *Pharm. Biol.* **2016**, *55*, 164–172. [CrossRef] [PubMed]
54. Lee, A.G.; Kang, S.; Im, S.; Pak, Y.K. Cinnamic Acid Attenuates Peripheral and Hypothalamic Inflammation in High-Fat Diet-Induced Obese Mice. *Pharmaceutics* **2022**, *14*, 1675. [CrossRef] [PubMed]
55. de Melo, T.; Lima, P.; Carvalho, K.; Fontenele, T.; Solon, F.; Tomé, A.; de Lemos, T.; Fonseca, S.d.C.; Santos, F.; Rao, V.; et al. Ferulic acid lowers body weight and visceral fat accumulation via modulation of enzymatic, hormonal and inflammatory changes in a mouse model of high-fat diet-induced obesity. *Braz. J. Med. Biol. Res.* **2017**, *50*, e5630. [CrossRef] [PubMed]
56. Singdam, P.; Naowaboot, J.; Senggunprai, L.; Boonloh, K.; Hipkaeo, W.; Pannangpetch, P. The mechanisms of neochlorogenic acid (3-caffeoylquinic acid) in improving glucose and lipid metabolism in rats with insulin resistance induced by a high fat-high fructose diet. *Trends Sci.* **2023**, *20*, 6455. [CrossRef]
57. Tian, B.; Geng, Y.; Wang, P.; Cai, M.; Neng, J.; Hu, J.; Xia, D.; Cao, W.; Yang, K.; Sun, P. Ferulic acid improves intestinal barrier function through altering gut microbiota composition in high-fat diet-induced mice. *Eur. J. Nutr.* **2022**, *61*, 3767–3783. [CrossRef] [PubMed]
58. Kosińska, A.; Karamac, M.; Estrella, I.; Hernández, T.; Bartolomé, B.; Dykes, G.A. Phenolic compound profiles and antioxidant capacity of *Persea americana* Mill. peels and seeds of two varieties. *J. Agric. Food Chem.* **2012**, *60*, 4613–4619. [CrossRef] [PubMed]
59. Zhang, Y.; Huang, C. Targeting adipocyte apoptosis: A novel strategy for obesity therapy. *Biochem. Biophys. Res. Commun.* **2012**, *417*, 1–4. [CrossRef]

60. Li, H.; Tsao, R.; Deng, Z. Factors affecting the antioxidant potential and health benefits of plant foods. *Can. J. Plant Sci.* **2012**, *92*, 1101–1111. [CrossRef]
61. Michiels, J.A.; Kevers, C.; Pincemail, J.; Defraigne, J.O.; Dommes, J. Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices. *Food Chem.* **2012**, *130*, 986–993. [CrossRef]
62. Hardisty, J.F.; Brix, A.E. Comparative hepatic toxicity: Prechronic/chronic liver toxicity in rodents. *Toxicol. Pathol.* **2005**, *33*, 35–40. [CrossRef]
63. Wintola, O.; Sunmonu, T.; Afolayan, A. Toxicological evaluation of aqueous extract of Aloe ferox Mill. in loperamide-induced constipated rats. *Hum. Exp. Toxicol.* **2010**, *30*, 425–431. [CrossRef]
64. Kunutsor, S.K.; Bakker, S.J.L.; Kootstra-Ros, J.E.; Gansevoort, R.T.; Gregson, J.; Dullaart, R.P.F. Serum alkaline phosphatase and eisk of incident cardiovascular disease: Interrelationship with high sensitivity C-reactive protein. *PLoS ONE* **2015**, *10*, e0132822. [CrossRef]
65. Zakariya, U.A.; Umar, U.A.; Dambazau, S.M.; Sulaiman, A. Comparative Hepatotoxic Effects of Aqueous and Phenolic Extracts of Avocado (*Persea americana*) Seed in Wistar Albino Rats. *Int. J. Biochem. Res. Rev.* **2016**, *10*, 1–6. [CrossRef]
66. Ozolua, R.; Anaka, O.; Okpo, S.; Idogun, S. Acute and sub-acute toxicological assessment of the aqueous seed extract of *Persea americana* Mill (Lauraceae) in rats. *Afr. J. Tradit. Complement. Altern. Med.* **2009**, *6*, 573–578. [CrossRef] [PubMed]
67. Kanfi, Y.; Peshti, V.; Gil, R.; Naiman, S.; Nahum, L.; Levin, E.; Kronfeld-Schor, N.; Cohen, H.Y. SIRT6 protects against pathological damage caused by diet-induced obesity. *Aging Cell* **2010**, *9*, 162–173. [CrossRef] [PubMed]
68. Bhushan, B.; Saxena, P.N.; Saxena, N. Biochemical and histological changes in rat liver caused by cypermethrin and be-ta-cyfluthrin. *Arch. Ind. Hyg. Toxicol.* **2013**, *64*, 57–67.