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ORIGINAL PAPER

Hypolipidemic Effect of Avocado (*Persea americana* Mill) Seed in a Hypercholesterolemic Mouse Model

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Abstract Avocado seed contains elevated levels of phenolic compounds and exhibits antioxidant properties. We investigated the effect of Avocado Seed Flour (ASF) on the lipid levels in mice on a hyperlipidemic diet. The concentration of phenols was determined by high-performance liquid chromatography, antioxidant activity was evaluated using the Trolox equivalent antioxidant capacity method, and dietary fiber was measured using the Association of Official Analytical Chemists (AOAC) method. The LD_{50} of ASF was determined using Lorke's method and hypolipidemic activity was evaluated in a hypercholesterolemic model in mice. Protocatechuic acid was the main phenolic compound found in ASF, followed by kaempferide and vanillic acid. The total phenolic content in the methanolic extract of ASF was 292.00±9.81 mg gallic acid equivalents/g seed dry weight and the antioxidant activity resulted in 173.3 μmol Trolox equivalents/g DW. In addition, a high content of dietary fiber was found (34.8%). The oral LD_{50} for ASF was 1767 mg/kg body weight, and treatment with ASF significantly reduced the levels of total cholesterol, LDL-C, and prediction of the atherogenic index. Therefore, the antioxidant activity of phenolic compounds and dietary fiber in ASF may be responsible for the hypocholesterolemic activity of ASF in a hyperlipidemic model of mice.

Keywords Avocado · Dietary · Fiber · Hypolipidemic · Phenolic compounds · Seed

Abbreviations

ABTS	2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)
AI	Atherogenic Index
ASF	Avocado Seed Flour
BW	Body Weight

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GAE	Gallic Acid Equivalents
HDL-C	High-Density Lipoprotein Cholesterol
LDL-C	Low-Density Lipoprotein Cholesterol
LD_{50}	Median Lethal Dose
TC	Total Cholesterol
TG	Triglycerides
Trolox	6-hydroxy-2, 5, 7, 8 tetramethylchroman-2-carboxylic acid

Introduction

The treatment of hypercholesterolemia and related cardiovascular diseases with medicinal plants has increased in recent years [1]. Reasons for the increased popularity of these herbal medicines may include their relatively low cost compared to orthodox medicines, availability (since they are almost always derived from available plants in the local region), and efficacy. Although poisonous plants are ubiquitous throughout the world, herbal medicine is still used by up to 80% of the total population in developing countries. Despite widespread use, few scientific studies have explored the safety and efficacy of traditional herbal remedies [2] Substances such as chlortalidone and propranolol, which are used in the treatment of hypercholesterolemia, can have adverse effects that affect therapy compliance and total quality of life of the patient [3].

Cardiovascular disease is a growing health problem throughout the world and represents a leading cause of mortality and morbidity in humans [4]. Several factors, such as a high caloric diet, age, lack of exercise, smoking, alcohol consumption, and genetic predisposition have been linked with cardiovascular disease [1]. Elevated cholesterol levels predispose patients to a condition known as hypercholesterolemia [5], which increases the risk of fatal and nonfatal coronary heart disease in people over the age of 50 [6].

Several beneficial medicinal properties of compounds present in the avocado seed and peel have been reported, which are related to the elevated levels of phenolic compounds (64% in seed, 23% in peel, and 13% in pulp). In addition, the seeds and peels of avocado also contribute 57% and 38% of the antioxidant capacities of the entire fruit, respectively [7].

Several studies on the hypolipidemic effects of the avocado seed have been focused on methanolic extracts [1] and aqueous extracts [8]; however, the study of the hypolipidemic effects of the entire avocado seed provides an interesting alternative, since the seed represents 13-18% [9] of the avocado fruit and is discarded during avocado pulp processing. Therefore, the aim of this study was to investigate the effect of ASF on the lipid levels of mice on a hyperlipidemic diet.

Materials and Methods

Seed Flour Preparation

Fresh seeds of *Persea americana* Mill were obtained from an orchard in Uruapan, Michoacán, Mexico. The seeds were identified and authenticated with the number 15658 at the herbarium of the Centro Médico Nacional of the Instituto Mexicano del Seguro Social. The seeds were washed, cut into small pieces, and dried in an oven (Fisher Scientific, Isotemp model 718F, USA) at 40 °C for 48 h until achieving a moisture level of 4%. The small pieces were then crushed into powder with a hammer (Wiley Mill standard model No. 3, Arthur Thomas Co., USA) until the flour passed through US 20 mesh (0.844 mm).

Phenol Extraction

Phenol extraction was performed according to the method described by Asaolu et al. [1]. One hundred grams of seed flour were sloped into a beaker and then extracted with 75% methanol overnight in a soxhlet extractor (Electro thermal model ME466, England). The methanolic extract was concentrated and allowed to evaporate to dryness at 50 °C using a rotary evaporator (Büchi 461, Brinkmann Instruments, Switzerland). The extract was then dissolved in water at a concentration of 4 g/100 ml.

Total Phenolic Content

HPLC-PDA Analysis of Phenolic Compounds

Twenty microlitres of the methanolic extract were analyzed using an HPLC-PDA system (Varian 920-LC) and a C18 column (Onmisher 5; 150×4.6 mm i.d.). The solvent mixture system contained a mixture of 5% formic acid in water (A) and 100% methanol (B), with a flow rate of 0.9 ml/min, and the gradient flow was as follows: 0 min—5% B; 3 min—15% B; 13 min—25% B; 25 min—30% B; 35 min—35% B; 39 min—45% B; 42 min—45% B; 44 min—50% B; 45 min—70% B; 50 min—70% B; 56 min—75% B; and 61 min—80% B. Detection was achieved with a photo diode array detector. Spectrophotometric data from all peaks were monitored at 220–280 nm and chromatograms were recorded at 340 nm. The data were processed with Varian Galaxie™ Chromatography Data Software version 1.9.302.952 (Agilent Technologies USA). Phenolic compound quantification was determined using the retention times and absorbance recorded in the chromatograms relative to external standards [10].

Total phenol content was quantified using the Folin Ciocalteu [11]. Gallic acid was used as a standard for the calibration curve. The results were expressed as gallic acid equivalents (GAE) mg/g seed dry weight (DW).

Antioxidant Activity

Total antioxidant capacity was evaluated using the method reported by Re et al. [12] with Trolox as the standard. A stock solution was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate to generate the ABTS cation chromophore. This solution was diluted with absolute ethanol until reaching an absorbance of 0.7±0.02 at 734 nm. A sample of 10 μl of ASF extract was added to 990 μL of ABTS solution and the reaction was followed over a 7 min time course. Total antioxidant activity or capacity was calculated relative to the reactivity of Trolox as the standard under the same conditions. The results were expressed as μmol Trolox equivalent/g of seed DW.

Proximate Analysis

Moisture, protein, ether extract, ash, and crude fiber content in ASF were determined in triplicate following the standard methods from the Association of Official Analytical Chemists International (AOAC) [13], and total carbohydrate content was calculated as the difference to a total of 100%. Dietary fiber was also determined in triplicate using AOAC methods 997.08 and 999.03 [13].

Animals

For the hypolipidemic activity assay, 40 8-10-week-old adult male CD-1 mice with an average weight of 28±2 g were obtained from the Centro de Investigación y Estudios Avanzados (CINVESTAV) at the Instituto Politécnico Nacional (IPN-México). Each mouse was weighed and randomly assigned to groups by body weight (BW). Each group of mice was housed in cages with wooden chip bedding and maintained under a 12 h light/dark cycle. Mice were fed with standard laboratory chow (5001 Lab Rodent Diet, PMI Nutrition International, Inc., Bienwood, MO) and provided water *ad libitum*. The animal experiments and study design were approved by the Laboratory Animal Care Committee of IPN and were conducted in compliance with the Official Mexican standard NOM-062-ZOO-1999 regarding technical specifications for production, care, and use of laboratory animals [14].

Oral Acute Toxicity

Acute toxicity tests were conducted according to Lorke's methodology [15].

Hypolipidemic Activity of ASF

Five groups of CD-1 mice (8 mice per group) were formed, with group 1 serving as the control. A diet rich in cholesterol was supplied to the animals *ad libitum* for six days in order to induce hypercholesterolemia [16, 17]. The diet formula used is shown in Table 1. Group 2–5 received the hypercholesterolemic diet, which was administered with distilled water (group 2) or different doses of ASF (groups 3, 4 and 5 received 125, 250, and 500 mg ASF/kg BW, respectively, once a day) by gavage. The doses were chosen according to the acute toxicological study divided by a security factor of 10.

At the end of six days, the TC, HDL-C, LDL-C and TG concentrations were determined according to the methodology described by Argüelles et al. [17].

Statistical Analysis

All data for acute toxicity were statistically analyzed by the Student's *t*-test using Sigma-Stat version 3.5 (Jandel San Raphael, CA) and $P<0.001$ was considered statistically significant. Hypolipidemic activity data were statistically analyzed by one-way analysis of variance (ANOVA) and Tukey's test using Sigma-Stat version 3.5 (Jandel San Raphael, CA). The data were reported as mean ± standard deviation (SD). A $P<0.05$ was considered statistically significant for hypolipidemic activity data.

Results and Discussion

Identification of Phenolic Compounds

Analysis of methanolic extract from avocado seed by HPLC-PDA identified eleven major peaks. Seven phenolic compounds were identified using external standards, spectra characteristics, and retention time. Protocatechuic acid (128.18±0.01 μg/g DW) was the main phenolic compound identified, followed by kaempferide (107.42±0.04 μg/g DW) and vanillic acid (28.67±0.001 μg/g DW). In addition, clorogenic acid, syringic acid, rutin, and kaempferol were present in small amounts (Table 2). Recently, Rodríguez-Carpena et al. [18] analyzed and classified phenolic compounds from two avocado varieties as catechins, (sum of catechin and epicatechin), hydroxybenzoic acids

Table 1 Formulation for the hypercholesterolemic diet

Ingredients	% of total weight
Lab rodent diet 5001	53.5
Sucrose	30
Casein	10
Butter	5
Cholesterol	1
Sodium cholate	0.5

Table 2 Phenolic compounds in methanolic extract from *Persea americana* Mill

Peak number	Rt (min)	UV (nm)	μg/g
1 Protocatechuic acid	6.12	243, 322	128.18±0.01
2 Clorogenic acid	7.37	242,278,439	0.516±0.02
3 Syringic acid	8.98	242,314, 443	2.51±0.002
4 Vanillic acid	9.87	242,380,436	28.67±0.001
5 NI	11.00	242,307,446	–
6 Rutin	11.67	242,277,319,386	9.63±0.008
7 NI	13.28	242,315,363	–
8 NI	14.48	242,318,446	–
9 Kaempferol	16.30	242,311,386,429	2.19±0.002
10 Kaempferide	23.81	216,242	107.42±0.04
11 NI	51.71	241,334,380	–

Data expressed as mean ± standard deviation; n=3. NI=not identified

(p-coumaric, caffeic, ferulic, and sinapic), hydroxycinnamic acids (p-hydroxybenzoic, protocatechuic, vanillic, syringic, and gallic), flavonols, and procyanidins (sum of dimers, oligomers, and polymers). These authors reported that the seed and peel contained the highest amount of phenols in the entire fruit. Moreover, Terpinc et al. [19] reported that flavonoids, rutin, catechin, and quercetin are widespread in nature and may act as powerful antioxidants.

These findings and our results provide evidence for the importance of phenols present in avocado seed, since phenolic compounds have been shown to reduce plasma lipid levels in human body through the upregulation of LDL receptor expression, inhibition of hepatic lipid synthesis and lipoprotein secretion, and increase in cholesterol elimination through bile acids [20].

Total Phenolic Content and Antioxidant Activity of ASF

The total phenolic content and antioxidant activity of the methanolic extract of ASF was determined to be 292.00± 9.81 mg GAE/g seed DW and 173.3 μmol Trolox equivalents/g seed DW, respectively. Rodríguez-Carpena et al. [18] previously reported a total phenolic content of 351.1± 9.88 mg GAE/g seed for the Hass variety and 416.4± 10.48 mg GAE/g seed dry matter for the Fuerte variety. According to Wang et al. [7], seeds contain the strongest antioxidant properties and highest phenol and procyanidin content compared to the pulp. Soong & Barlow [21] reported a significantly higher total antioxidant capacity and phenolic content of fruit seeds than the edible portions. In most fruits, the contribution of the fruit seed fraction compared to the total antioxidant activity and phenolic content was more than 95%, and therefore these authors suggested that the fruit seeds should be further utilized rather than just discarded as waste [21]. Importantly, our results are in agreement with the findings by Wang et al. [17] and Soong & Barlow [21] showing that the antioxidant activity of fruit seeds components may be responsible for the hypocholesterolemic activity observed.

Proximate Analysis and Dietary Fiber

We found that the ASF preparation contained 4.0±0.8 moisture, 2.2±0.14 ash, 4.75±0.01 protein, 6.39±0.5 crude fiber, 4.38±0.8 ether extract, and 79.10±0.8 carbohydrates (data expressed as mean ± standard deviation g/100 g sample fresh weight; n=3). The low oil content of the ASF suggested that oil and its fatty acids could have a minimal effect on cholesterol and LDL-C reduction, since it only represented a small portion of the total daily oil intake of the treated mice. Nijjar et al. [22] found that nuts are a good source of mono and polyunsaturated fatty acids and also contain dietary fiber, phytosterols, and polyphenols. These components likely combine to a reduction in LDL-C levels beyond the effects predicted by equations based solely on fatty acid profiles. Nevertheless, the high crude fiber content (6.39 g/100 g DW sample) of ASF could have a beneficial effect on total cholesterol and LDL-C reduction in the plasma of the groups of mice treated [23].

The ASF preparation was found to contain 34.8±3.4 g dietary fiber/100 g DW sample, which is relevant in this study, since the natural gel-forming or viscous fibers (pectin, gums, mucilage, algal polysaccharides, some storage polysaccharides, and some hemicelluloses) are water-soluble and resistant to digestion by human gastrointestinal enzymes that are part of the dietary fiber. Moreover, this content has been shown to be associated with a cholesterol-lowering effect [24]. The dietary fiber content of the avocado seed is similar to another Mexican seed called chia [25]. Reyes-Caudillo et al. [25] reported that chia seeds from Jalisco and Sinaloa States contain a total dietary fiber content of 39.9% and 36.9%, respectively. Therefore, the dietary fiber content in chia seeds is of sufficient level to promote beneficial health effects, including a reduction of cholesterolaemia, modification of the glycemic and insulinaemic responses, changes in intestinal function, and antioxidant activity. The high content of dietary fiber in ASF found in the present study suggests that dietary fiber could play an important role in the hypocholesterolemic activity in mice.

Oral Acute Toxicity

In the first stage of the oral acute toxicity study, the animals did not exhibit any toxicological signs, including depression, writhing, diarrhea, hypermotility, or aggression

Table 3 Daily food and water consumption and relative weight of liver and kidney in CD-1 mice treated with different doses of avocado seed flour

Groups	Dose (mg/kg)	Daily food consumption (g)	Daily fluid consumption (ml)	LBW (%)	KBW (%)
Control 1st step[a]	–	38.3±10.2	40.8±12.8	6.80±0.15	1.58±0.09
ASF	10	28.4±10.4[a]	34.4±14.3	6.75±0.37	2.67±0.02 [a]
	100	31.9±8.3	47.1±11.2	5.48±0.11[a],[b]	1.83±0.05[a,b]
	1000	33.7±7.3	50.2±10.4[a]	5.04±0.22[a],[b]	1.75±0.09[a,b]
Control 2nd step[c]	–	35.5±6.2	53.4±9.7	5.50±0.28	1.54±0.16
ASF	1250	40.8±8.0	46.9±7.6	5.14±0.14	1.44±0.10
	2500	21.3±14.5[c]	28.1±15.8[c]	ND	ND

Data expressed as mean ± standard deviation; n=8. ASF, Avocado Seed Flour; LBW, liver-to-body weight ratio; KBW, kidney-to-body weight ratio. [a] $P<0.001$, significant difference with respect to control 1st step,. [b] $P<0.001$; significant difference with respect to 10 mg/kg of ASF; [c] $P<0.001$, significant difference with respect to control 2nd step. ND: Not determined.

compared to the control group. No signs of toxicity or death were observed in any of the animals, and all animals survived to the end of the 14 day study period. Weight gain in the control animals was minimal (< 4%), while the treated animals exhibited a slight increase in weight, although there was no significant difference in the percent weight change between the groups ($P<0.05$). In the second stage of the study, we observed 100% mortality by day six in the group fed with 2500 mg ASF/kg BW. Table 3 lists the effects of different doses of ASF on daily food and water intake and on the weight of the main organs, which was expressed as a ratio of relative weight (RW) to total body weight. Mice administered 100 and 1000 mg ASF/kg BW exhibited significant differences ($P\leq0.001$) compared with the control, whereby liver weight was lower and kidney weight was higher than control group. An increase in the RW of the kidney has also been reported by Ozolua et al. [8] in adult rats fed aqueous seed extract from avocado. In addition, Brai et al. [26] found that liver weights were significantly increased in albino rats fed avocado aqueous leaf extract after induction of a hyperlipidemic diet compared to normal control rats, which was accompanied with a significant increase in liver cholesterol level. These findings together with the results of this study suggest that the compounds present in avocado leaves and seeds are different and have an opposite influence on the liver.

In the second stage of the acute toxicity study, no significant differences ($P>0.001$) were found in daily food and water intake between mice treated with 1250 mg ASF/kg BW and control mice; however, significant differences ($P\leq0.001$) were found in daily food and water intake between mice treated with 2500 mg ASF/kg BW and control mice.

Based on these results, we determined the oral LD_{50} for ASF to be 1767 mg/kg BW by using the geometric mean of the dose that caused 100% mortality and the dose that caused no mortality, as suggested by Lorke [15]. The oral LD_{50} of ASF in mice indicated that it exhibited a low toxicity [17]. It has previously been shown that ether and aqueous extracts of *Persea americana* Mill seed administered by intraperitoneal injection in rats also had low toxicity, with LD_{50} values of 751.6 mg/kg BW and 10 g/kg BW, respectively [8, 27].

Hypolipemic Activity of ASF

To determine the hypolipemic activity of ASF, mice were dosed according to the LD_{50} of ASF found in this study. The

Table 4 Effect of avocado seed flour on lipid profile of mice

Treatment	ASF Dosis (mg/kg)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	TG (mmol/L)	AI
Normocholesterolemic	–	31.9±7.16	12.50±3.02	18.92±4.25	1.05±0.13	1.6±0.05
Hypercholesterolemic	–	106.7±9.70	92.1±10.26	15.00±2.1	0.852±0.09	7.9±1.86
ASF	125	70.9±4.49[a]	55.8±5.35[a]	15.6±1.79	0.872±0.09	3.9±0.59[a]
	250	69.0±5.15[a]	56.1±5.28[a]	13.5±0.78	0.914±0.07	4.3±0.58[a]
	500	67.6±4.92[a]	54.4±5.37[a]	13.8±1.21	0.930±0.12	4.3±0.72[a]

Data expressed as mean ± standard deviation; n=8, analyzed by ANOVA and Tukey-Kramer test.[a] $P\leq0.0001$; significant difference with respect to the hypercholesterolemic control. ASF, Avocado Seed Flour. TC, Total Cholesterol; LDL-C, Low-density Lipoprotein Cholesterol; HDL-C, High-density Lipoprotein Cholesterol; TG, Triglycerides; AI, Atherogenic Index.

hypolipidemic effect of ASF was evaluated at doses at 125, 250, and 500 mg/kg BW. Acute supplementation of cholesterol produced a significant ($P \leq 0.05$) elevation in plasma cholesterol levels in the hypercholesterolemic control compared to the normocholesterolemic control. In addition, the TC increased from 31.9±7.16 to 106.7± 9.70 mmol/L, LDL-C increased from 12.5±3.02 to 92.1± 10.26 mmol/L, and the calculated AI increase from 1.6± 0.05 to 7.9±1.86 between the two groups, respectively (Table 4). No significant ($P > 0.05$) changes were found in the plasma HDL-C (18.92±4.25 vs 15.0±2.1 mmol/L, respectively) and TG (1.05±0.13 vs. 0.85±0.09 mmol/L, respectively) between the two groups. These observations could be associated with insulin activity [28].

Similar results were reported by Asaolu et al. [1] in normocholesterolemic and hypercholesterolemic groups for TC (3.12±0.83 mmol/L vs 7.52±1.11 mmol/L, respectively) and LDL-C (0.36 mmol/L vs 5.79±2.10 mmol/L, respectively) using a methanol extract of avocado seeds. In addition, the AI was significantly increased in the hypercholesterolemic group compared to the normocholesterolemic group of that study (4.3 vs 1.3, respectively).

Treatment of mice with 125 mg ASF/kg BW significantly ($P \leq 0.05$) reduced the elevated levels of TC by 33% (106.7± 9.70 to 70.9±4.49 mmol/L) and LDL-C by 39.4% (92.1± 10.26 to 55.8±5.35 mmol/L). In addition, treatment with 250 mg ASF/kg BW reduced TC and LDL-C by 34 and 39%, respectively, while treatment with 500 mg ASF/kg BW reduced the TC and LDL-C levels by 36 and 41%, respectively. A similar effect was reported by Asaolu et al. [1] in Albino rats administered 200 mg of avocado seed extract (75% methanol)/kg BW, where they observed a significant reduction in TC, LDL-C, and TG levels by 47, 69, and 44%, respectively, compared to hypercholesterolemic control mice. In addition, it was reported that the cholesterol levels of hypertensive rats treated with 500 mg/kg BW of avocado aqueous seed extract were reduced by 19.2, 42.5, 47.9, and 13.6% in the plasma, kidney, heart, and liver, respectively, compared to hypertensive control mice [29]. In addition, significant reductions in LDL-C and triglycerides were also observed. These studies together with our results indicate that aqueous or methanol seed extract or seed flour of avocado can be used as an effective supplement in mice and rats for treating hyperlipidemia.

Conclusion

In this study, we found that ASF has low toxicity and can significantly reduce the cholesterol and LDL-C levels in hypercholesterolemic mice. This effect could be attributed to the phenolic content, antioxidant activity, and/or dietary and crude fiber content of the seed. Further research is required in order to identify the components of ASF that are responsible for the observed hypocholesterolemic effects.

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References

1. Asaolu MF, Asaolu SS, Oyeyemi AO, Aluko BT (2010) Hypolipemic effects of methanolic extract of *Persea americana* seeds in hypercholesterolemic rats. J Med Sci 1(14):126–128
2. Veerappan A, Miyazaki S, Kadarkaraisamy M, Ranganathan D (2007) Acute and subacute toxicity studies of Aegle marmelos Corr., an Indian medicinal plant. Phytomedicine 14(2–3):209–215
3. Fogari R, Zoppi A (2004) Effect of antihypertensive agents on quality of life in the elderly. Drugs Aging 21(6):377–393
4. Crews DE (2007) Senescence, aging, and disease. J Physiol Anthropol 26(3):365–372
5. Durrington P (2003) Dyslipidaemia. Lancet 362(9385):717–731
6. Marks D, Thorogood M, Neil HA, Humphries SE (2003) A review on the diagnosis, natural history, and treatment of familial hypercholesterolaemia. Atherosclerosis 168(1):1–14
7. Wang W, Bostic TR, Gu LW (2010) Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars. Food Chem 122(4):1193–1198
8. Ozolua RI, Anaka ON, Okpo SO, Idogun SE (2009) Acute and sub-acute toxicological assessment of the aqueous seed extract of *Persea americana* Mill (Lauraceae) in rats. Afr J Tradit Complement Altern Med 6(4):573–578
9. Ortiz MA, Dorantes AL, Galindez MJ, Cárdenas SE (2004) Effect of a novel oil extraction method on avocado (*Persea americana* Mill) pulp microstructure. Plant Foods Hum Nutr 59(1):11–14
10. Ferreres F, Gomes D, Valentao P, Goncalves R, Pio R, Chagas EA, Seabra RM, Andrade PB (2009) Improved loquat (*Eriobotrya japonica* Lindl.) cultivars: Variation of phenolics and antioxidative potential. Food Chem 114(3):1019–1027
11. Sun T, Xu Z, Wu CT, Janes M, Prinyawiwatkul W, No HK (2007) Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). J Food Sci 72(2):S98–102
12. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26(9–10):1231–1237
13. AOAC (2000) Official methods of analysis of AOAC international, vol 14. William Horwitz. AOAC International, Washington, D.C.
14. Norma Oficial Mexicana 062-ZOO-1999. Especificaciones Técnicas para la producción, cuidado y uso de los animales de laboratorio. Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, Estados Unidos Mexicanos
15. Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275–287
16. Pineiro V, Ortiz-Moreno A, Mora-Escobedo R, Hernandez-Navarro MD, Ceballos-Reyes G, Chamorro-Cevallos G (2010) Effect of L-arginine oral supplementation on response to myocardial infarction in hypercholesterolemic and hypertensive rats. Plant Foods Hum Nutr 65(1):31–37

17. Argüelles N, Sánchez-Sandoval E, Mendieta A, Villa-Tanaca L, Garduño-Siciliano L, Jiménez F, MdC C, Medina-Franco JL, Chamorro-Cevallos G, Tamariz J (2010) Design, synthesis, and docking of highly hypolipidemic agents: *Schizosaccharomyces pombe* as a new model for evaluating α-asarone-based HMG-CoA reductase inhibitors. Bioorg Med Chem 18 (12):4238–4248
18. Rodríguez-Carpena JG, Morcuende D, Andrade MJ, Kylli P, Estevez M (2011) Avocado (*Persea americana* Mill.) phenolics, *in vitro* antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. J Agric Food Chem 59(10):5625–5635
19. Terpinc P, Polak T, Makuc D, Ulrih NP, Abramovic H (2012) The occurrence and characterisation of phenolic compounds in *Camelina sativa* seed, cake and oil. Food Chem 131(2):580–589
20. Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP, Phivthong-Ngam L, Ratanachamnong P, Srisawat S, Pongrapeeporn KU (2008) The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves. J Ethnopharmacol 116 (3):439–446
21. Soong YY, Barlow PJ (2004) Antioxidant activity and phenolic content of selected fruit seeds. Food Chem 88(3):411–417
22. Nijjar PS, Burke FM, Bloesch A, Rader DJ (2010) Role of dietary supplements in lowering low-density lipoprotein cholesterol: A review. J Clin Lipidol 4(4):248–258
23. Solà R, Bruckert E, Valls RM, Narejos S, Luque X, Castro-Cabezas M, Doménech G, Torres F, Heras M, Farrés X, Vaquer JV, Martínez JM, Almaraz MC, Anguera A (2010) Soluble fibre (*Plantago ovata* husk) reduces plasma low-density lipoprotein (LDL) cholesterol, triglycerides, insulin, oxidised LDL and systolic blood pressure in hypercholesterolaemic patients: A randomised trial. Atherosclerosis 211(2):630–637
24. Theuwissen E, Mensink RP (2008) Water-soluble dietary fibers and cardiovascular disease. Physiol Behav 94(2):285–292
25. Reyes-Caudillo E, Tecante A, Valdivia-López MA (2008) Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. Food Chem 107(2):656–663
26. Brai BIC, Odetola AA, Agomo PU (2007) Effects of *Persea americana* leaf extracts on body weight and liver lipids in rats fed hyperlipidaemic diet. Afr J Biotechnol 6(8):1007–1011
27. Taha NA, Alkhawajah AAM, Raveesha KK (2008) Acute and subacute toxicity studies of *Persea americana* Mill (Avocado) seed in rats. Int Jour Med Toxicol Leg Med 11(2):31–36
28. Foufelle F, Ferre P (2002) New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: A role for the transcription factor sterol regulatory element binding protein-1c. Biochem J 366(Pt 2):377–391
29. Imafidon KE, Amaechina FC (2010) Effects of aqueous seed extract of *Persea americana* Mill. (Avocado) on blood pressure and lipid profile in hypertensive rats. Adv Biol Res 4(2):116–121