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Full Length Research Paper

Hypolipidemic and antioxidant activities of avocado fruit pulp on high cholesterol fed diet in rats

Mohammed S. Al-Dosari

Department of Pharmacognosy, College of Pharmacy, P. O. Box 2457, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia. E-mail: msdosari@yahoo.com.

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Avocado, *Persea americana* Mill. (Lauraceae) is a widely consumed fruit in many countries for its nutritional and medicinal benefits. In the present study, avocado fruit pulp (AFP) was investigated for its anti-hyperlipidemic and antioxidant activities in Wistar albino rats. Hyperlipidemia in the animal was induced by feeding high cholesterol diet (HCD) for 70 days in standard chow diet. Rats on HCD showed significant increase in serum liver marker enzymes (GOT, GPT, GGT, ALP) and bilirubin levels; cholesterol, Increased low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerides (TG) levels in serum were also elevated. Besides, an increase in lipid peroxidative indicator malondialdehyde (MDA) level, there was a decrease in serum HDL-C; non-protein sulfhydryl (NP-SH) and total protein (TP) in both liver and heart tissues. Treatment with AFP (1 and 2 ml/rat/day, orally) showed significant decrease in serum cholesterol, LDL-C, VLDL-C, TG, glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and bilirubin levels, while liver and heart MDA was also significantly decreased. After ingestion of AFP, significant increase in non-protein sulfhydryl (NP-SH), total protein (TP) contents in both tissues were observed. Assessment of liver and heart pathology showed that AFP administration caused an improvement in fatty acid changes of the tissues caused by heavy-chain disease (HCD). These results suggest that AFP possesses hypocholesterolemic and antioxidant properties due to its phytoconstituents contents and substantiates its use in folkloric practices to control dyslipidemia.

Key words: Avocado, *Persea Americana*, hypolipidemia, anti-oxidant.

INTRODUCTION

A high level of serum cholesterol has been identified clearly as a risk factor for atherosclerosis and coronary heart disease (Chen et al., 2004). High cholesterol diet is however regarded as an important factor in the development of cardiac diseases since it leads to development of hyperlipidemia, atherosclerosis, and ischemic heart disease. Moreover, hypercholesterolemia is shown to be one of the major risk factors of atherosclerosis by increasing plasma low-density lipoprotein (LDL) levels (Levine et al., 1995).

Consumption of fruits and vegetables is associated with a lowered risk of cardiovascular diseases and even cancer (Block et al., 1992; Riboli and Norat et al., 2003). Natural medicines have been used empirically to lower the cholesterol levels. Avocado (*Persea Americana* Mill.; Family: Lauraceae) fruit is widely consumed throughout the world as food, as well as medicinal purposes. Avocado ranks as the 14th most commonly consumed raw fruit in the United States (Duester, 2001). The health benefits of avocado may be due to its contents of over 20 essential nutrients and various disease-curing potential phytochemicals. Avocado fruit and leaves have been used in Latin American folk medicine, including Mexico to treat a variety of diseases. Hot water infusion from its leaves has been used as a diuretic, to induce menstruation and to treat hypertension (Adeboye et al., 1999; Ross, 1999). A previous study among Mexican population has shown that avocado consumption decreases serum total cholesterol, LDL-cholesterol and triglycerides, and increases HDL-cholesterol levels compared to control diet subjects (Alvizouri-Muñoz et al., 1992; LópezLedesma et al., 1996). In an earlier study,

avocado oil-rich diet has been shown to modify the fatty acid content in cardiac and renal cell membranes. Some studies on lipophilic extarcts of avocado inhibited prostate cancer cell apoptosis (Butt et al., 2006) and suppressed liver injury (Kawagishi et al., 2001). However, the cholesterol-lowering effects of avocado fruit pulp in hypercholesterolemic and hyperlipidemic animal models have not been studied. In this present study, the effect of fresh fruit pulp of avocado (AFP) has been carried out on serum liver enzymes as well as lipid profiles in high cholesterol-fed rats.

MATERIALS AND METHODS

Plant material

The fresh fruits of avocado were purchased from the local fruit and vegetable market in Riyadh, Saudi Arabia. Botanical authentication was confirmed by an expert taxonomist at the Department of Pharmacognosy, College of Pharmacy, King Saud University (KSU), Riyadh, Saudi Arabia. The voucher specimen (MSD #1210) has been deposited for the future reference in the herbarium of the college.

Preparation of the dosage form

The fresh avocado was peeled off and the creamy pulp was homogenized using electric blender. The obtained viscous slurry-like pulp was orally administered to the rats.

Acute toxicity studies

Acute toxicity study on avocado pulp was conducted as per the OECD guidelines (OECD, 1993), using Wistar albino rats. Each animal was administered the pulp by oral route. The animals were observed continuously for the first 2 h for any toxic effects and upto 24 h for mortality.

Animals

Male albino rats of Wistar strain weighing between 120 and 150 g were used in this study. The animals were housed under standard environmental conditions (temperature 22±2°C; humidity 55%) and 12 h light/dark cycle at the Experimental Animal Care Center of the College. The animals had free access to water *ad libitum*. All animal experiments were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, KSU. After a 7 day adaptation period, the animals were divided into five groups (n=6 each) and the following treatments were given simultaneously to the concerned groups for 10 weeks.

Group-I: Served as control (normal) rats and given standard purina chow diet.
Group-II: Rats fed with 1% cholesterol mixed pellets diet for 10 weeks to induce hypercholesterolemia.
Group-III: Animals fed with 1% cholesterol mixed pellets diet plus avocado pulp, 1 ml/day/rat for 70 consecutive days.
Group-IV: Animals fed with 1% cholesterol mixed pellets diet plus avocado pulp, 2 ml/day/rat for 70 consecutive days.
Group-V: In this group, rats were fed with 1% cholesterol mixed pellets diet plus Simvastatin at a dose of 10 mg/kg body weight once daily for 10 weeks.

Cholesterol supplemented diet

Purina chow diet pellets (Grain Silos and Flour Mills Organization, Saudi Arabia) were pulverized, cholesterol (1% w/w) (AVONCHEM, UK)powder was thoroughly mixed and the pellets were reconstituted with water, and dried properly to avoid any fungal contaminations.

Estimation of liver marker enzymes

After the termination of the experiments (feeding), the animals were subjected to overnight fasting and subsequently sacrificed under ether euthanasia. Serum glutamate oxaloacetate transaminase (SGOT) (Reitman and Frankel, 1957), Serum glutamate pyruvate transaminase (SGPT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (King and Armstrong, 1988), γ-glutamyltransferase (GGT) (Fiala et al., 1972) and bilirubin (Stiehl, 1982) were determined using Reflotron® Plus Analyzer and Roche kits.

Estimation of the lipid profile

Blood samples were collected from overnight fasted rats. Serum total cholesterol, triglycerides and high-density lipoprotein-cholesterol (HDL-C) levels were determined by commercially available spectrophotometric assay kits (Crescent Diagnostics, Jeddah, Saudi Arabia).

Determination of malondialdehyde (MDA)

The method reported by Utley et al. (1967) was followed. The liver and heart tissues were removed and each tissue was homogenized in 0.15 M KCl (at 4°C; Potter-Elvehjem type C homogenizer) to give a 10% w/v homogenate. Aliquots of homogenate (1 ml) were incubated at 37°C for 3 h in a metabolic shaker. Then 1 ml of 10% aqueous trichloroacetic acid (TCA) was added and mixed. The mixture was then centrifuged at 800 g for 10 min. 1 ml of the supernatant was removed and mixed with 1 ml of 0.67% thiobarbituric acid in water and placed in a boiling water bath for 10 min. The mixture was cooled and diluted with 1 ml distilled water. The absorbance of the solution was then read at 535 nm. The content of malondialdehyde (nmol/g wet tissue) was then calculated, by reference to a standard curve of malondialdehyde solution.

Estimation of non-protein sulfhydryls (NP-SH)

Hepatic and cardiac non-protein sulfhydryl was measured according to the method of Sedlak and Lindsay (Sedlak and Lindsay, 1968). The liver and heart was homogenized in ice-cold 0.02 mmol/L ethylenediaminetetraacetic acid (EDTA). Aliquots of 5 ml of the homogenates were mixed in 15 ml test tubes with 4 ml of distilled water and 1 ml of 50% trichloroacetic acid (TCA). The tubes were shaken intermittently for 10 min and centrifuged at 3000 rpm. Two milliliters of supernatant was mixed with 4 ml of 0.4 mol/L Tris buffer (pH 8.9). 0.1 ml of 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) was added and the sample was shaken. The absorbance was measured within 5 min of addition of DTNB at 412 nm against a reagent blank.

Determination of total protein (TP)

Total protein was estimated by the kit method, supplied by Crescent Diagnostics, Jeddah, Saudi Arabia.

Table 1. Effect of AFP on HCD-induced hepatic enzymes changes in rats.

Treatment group (n=6)	SGOT(U/L)	SGPT(U/L)	GGT(U/L)	ALP(U/L)	Bilirubin(mg/dl)
Normal control)	79.35±3.83	32.33±2.42	3.68±0.26	257.66±13.42	0.49±0.04
HCD only	255.33±7.25***[a]	198.66±22.76***[a]	8.31±0.61***[a]	407.5±9.69***[a]	1.14±0.04***[a]
AFP(1 ml)+HCD	223.5±5.74**[b]	152.16±4.25[b]	6.41±0.46*[b]	367.83±11.13*[b]	0.89±0.04**[b]
AFP(2 ml)+HCD	174.83±8.73***[b]	102.95±10.26**[b]	5.41±0.40**[b]	324.5±9.13***[b]	0.72±0.02***[b]
Simvastatin (30 mg/kg)+HCD	162.00±9.17***[b]	119.71±7.71**[b]	6.63±0.31*[b]	313.5±6.53***[b]	0.81±0.05***[b]

All values represent mean ± SEM. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett,s multiple comparision test. [a] As compared with normal group.[b] As compared with CCl_4 only group.

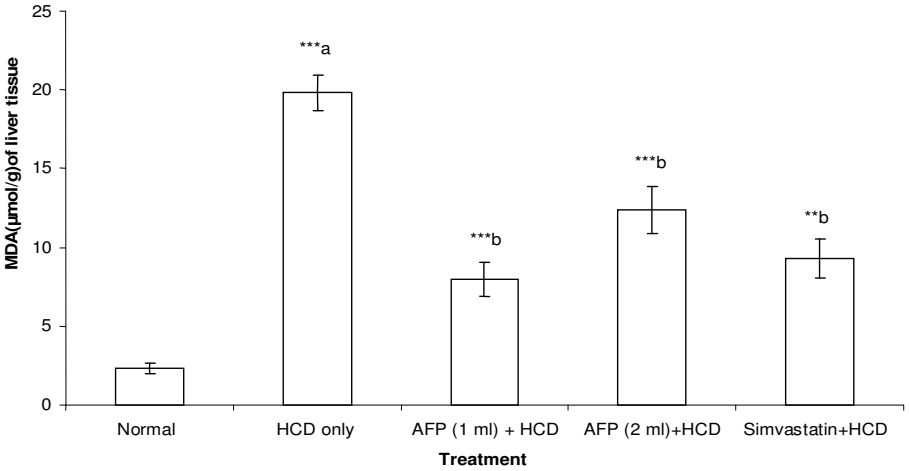

Figure 1A. Effect of AFP on HCD-induced hepaticMDA concentration in rats. All values represent mean ± SEM. **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparision test. [a] As compared with normal group. [b] As compared with cholesterol diet only group.

Histopathological evaluation

Liver and heart tissue samples were fixed in 10% neutral buffered formalin for 24 h. Sections of the tissues were histologically examined. The processed tissues were then embedded in paraffin blocks and sections of about 5 µm thickness were cut by employing an American optical rotary microtome. These sections were stained with haematoxylin and eosin using routine procedures (Culling, 1974).

Statistical analysis

Values are given as arithmetic means ± standard error of the mean (S.E.M.). Data was statistically analyzed by using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

RESULTS

Acute toxicity test

No toxicity symptoms were recorded, only mild diarrhea was observed. The LD_{50} value by oral route could not be determined as no lethality was observed up to 3 g/kg of the AFP in mice.

Effect of AFP on marker enzymes and bilirubin in serum

The effect of AFP pretreatment on the HCD-induced elevation of the activities of serum GOT, GPT, ALP, GGT and bilirubin are shown in Table 1. Oral administration of HCD significantly elevated the GOT, GPT, ALP, GGT and bilirubin levels in serum. Pretreatment of rats with AFP significantly prevented the elevation of GOT, GPT, ALP, GGT and bilirubin at both doses used. Simvastatin on the other hand, significantly diminished the enhanced levels of all marker enzymes and bilirubin in this group as compared to the only HCD-fed group II animals.

Effect of AFP on hepaticand cardiac lipid peroxidation

As depicted in Figure 1A and B, the MDA in liver and heart tissues was estimated; rats fed with HCD was shown to have significant increase in MDA concentration

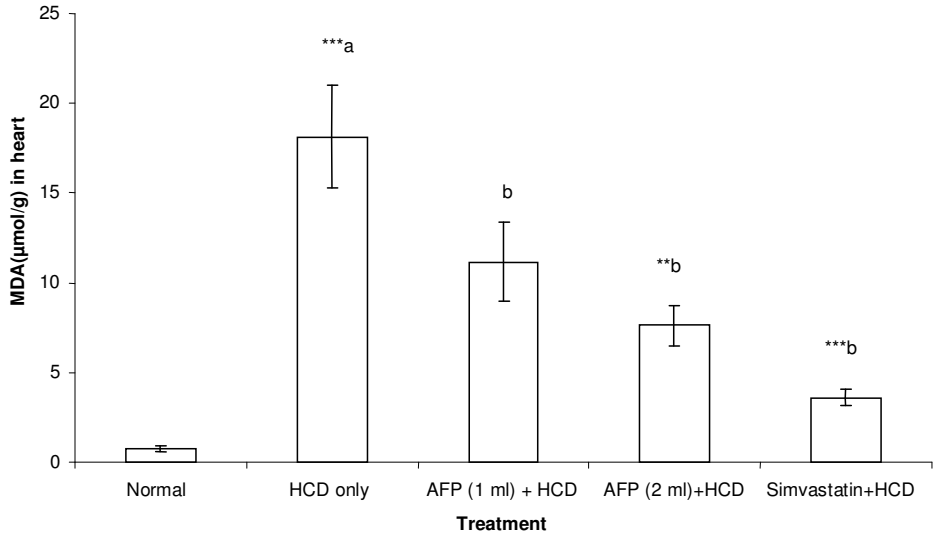

Figure 1B. Effect of AFP on HCD-induced cardiacMDA concentration in rats. All values represent mean ± SEM. **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparision test. [a] As compared with normal group. [b] As compared with cholesterol diet only group.

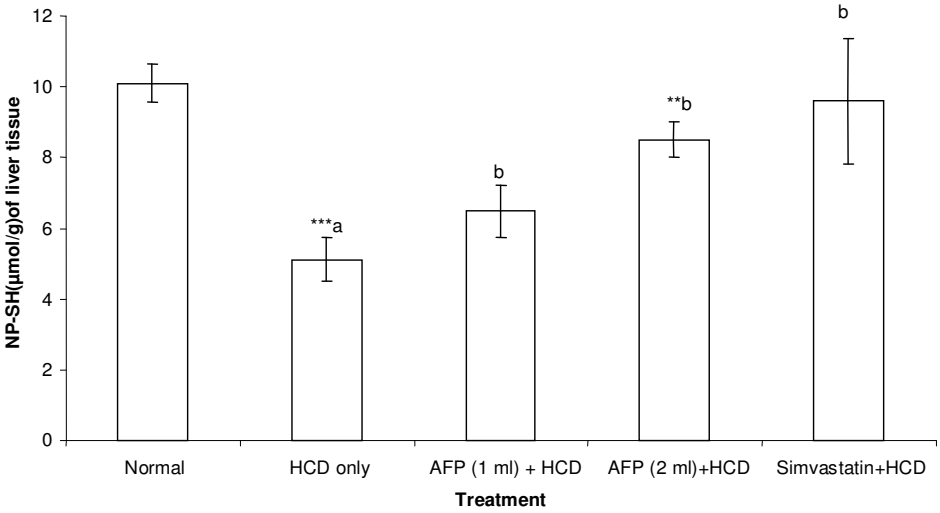

Figure 2A. Effect of AFP on HCD-induced hepatic NP-SH level in rats. All values represent mean ± SEM. **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparision test. [a] As compared with normal group. [b] As compared with cholesterol diet only group.

when compared with the normal control rats (Group I). Pretreatment of rats with AFP resulted in a significant and dose-dependent decrease in the concentration of MDA. Simvastatin treatment also caused a significant reduction in MDA level.

Effect of AFP on hepatic and cardiac NP-SH

As shown in Figure 2A and B, the reduced level of NP-SH caused by HCD was significantly and dose-dependently elevated in the liver and cardiac tissue by AFP at both doses used. Simvastatin-treatment also showed a significant enhanced level of NP-SH in this group.

Effect of AFP on hepatic and cardiac TP

Figure 3A and B demonstrated that the total protein level

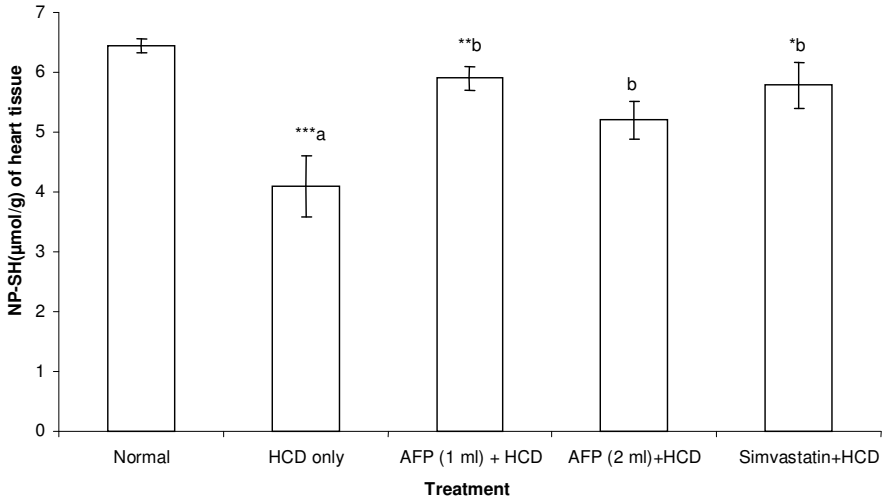

Figure 2B. Effect of AFP on HCD-induced cardiacNP-SH level in rats. All values represent mean ± SEM. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparision test. [a] As compared with normal group.[b] As compared with cholesterol diet only group.

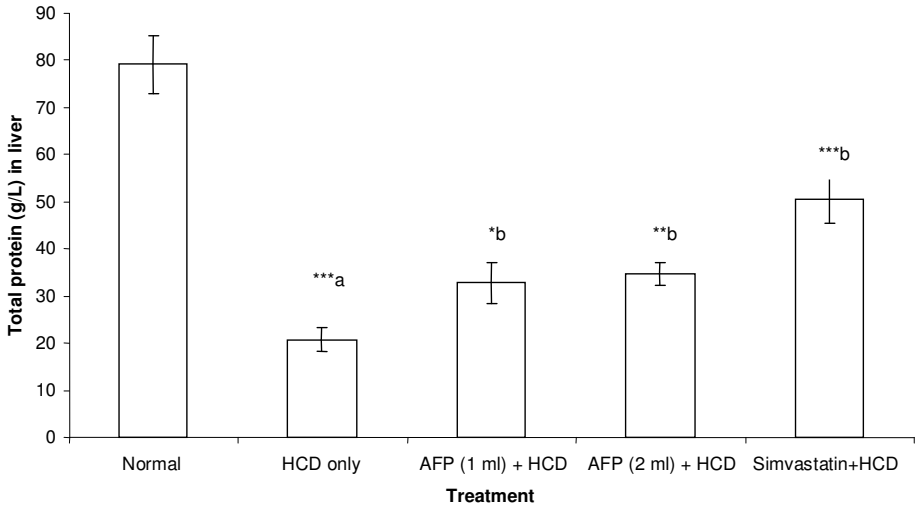

Figure 3A. Effect of AFP on HCD-induced hepatic TP changes in rats. All values represent mean ± SEM. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparision test. [a] As compared with normal group.[b] As compared with cholesterol diet only group

was significantly decreased in only HCD-fed group. AFP at both doses used, afforded to significantly and dose-dependently elevate the protein concentration in liver and cardiac tissues. Simvastatin-treated rats also showed a significant in the increase level of total protein.

Effect of AFP on histopathological evaluation

The light microscopy examination showed no significant abnormalities in the liver (Figure 4 (a-e)) and heart muscles (Figure 4(f-j)) of rats treated with AFP. HCDonly fed rats, resulted in the marked fatty acid degeneration and vascular cytoplasm. Simvastatin also showed degenerative changes in liver tissues, while heart muscles showed normal architecture.

DISCUSSION

The obtained results showed that the cholesterol-enriched diet-fed for 10 weeks, markedly enhanced the

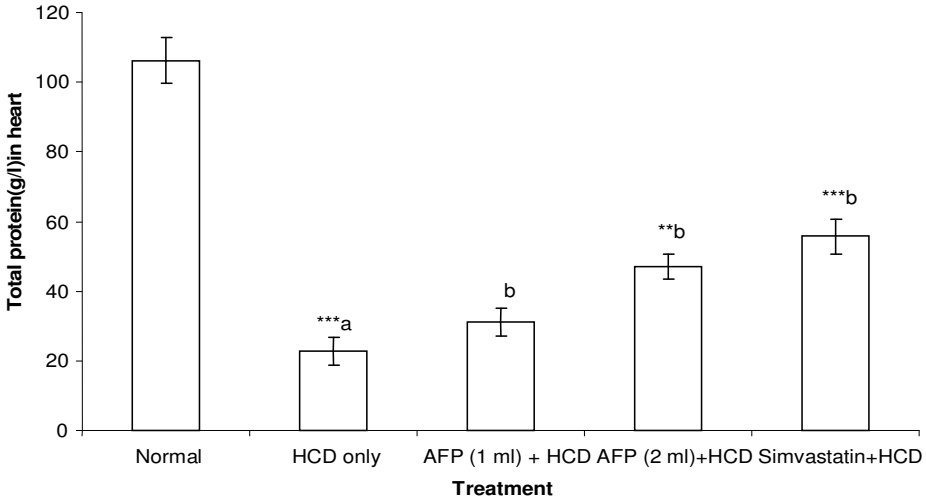

Figure 3B. Effect of AFP on HCD-induced cardiacTP changes in rats. All values represent mean ± SEM. **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparision test.[a] As compared with normal group.[b] As compared with cholesterol diet only group.

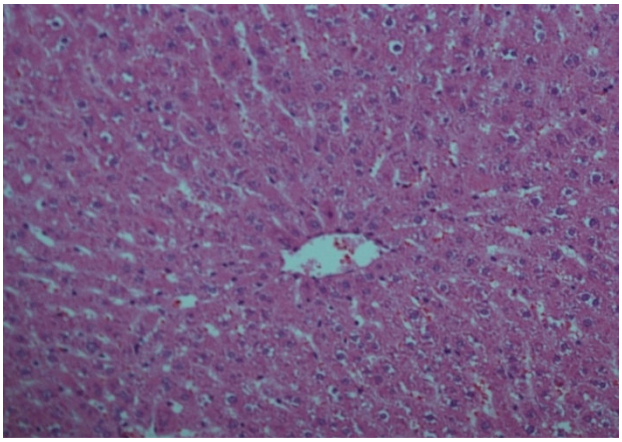

Figure 4a. Control rat liver showing normal liver cells and central vein with central lobule. H&E. 200x.

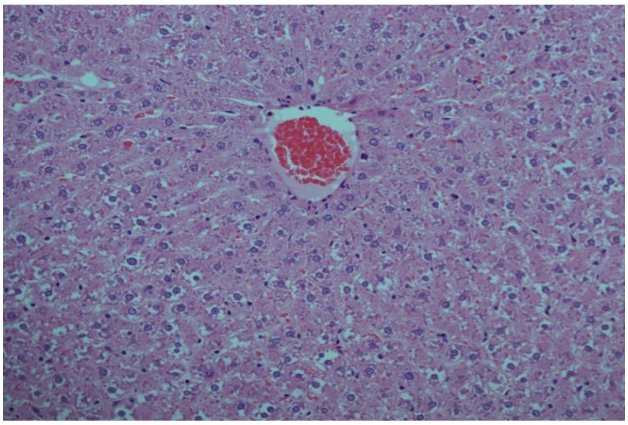

Figure 4c. HCD plus AFP 1 ml/rat/day-treated rat liver showing decreased hepatocyte degeneration. H.E. 200x.

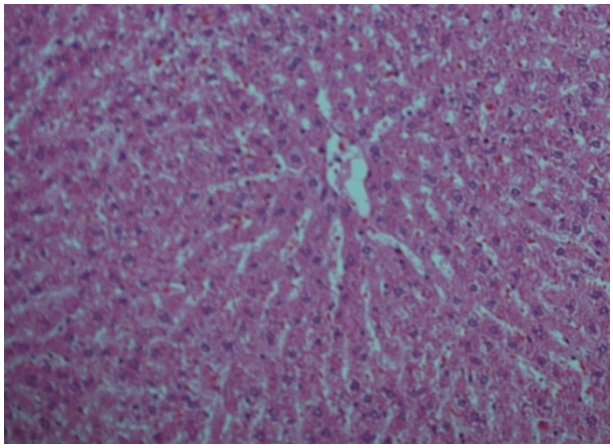

Figure 4b. HCD only fed rat liver showing early feathery degeneration and vascular cytoplasm. H&E. 200x.

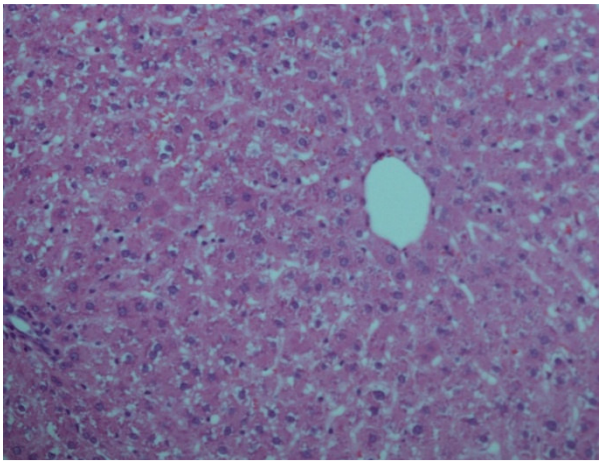

Figure 4d. HCD plus AFP 2 ml/rat/day-treated rat liver showing marked improvement in hepatocytes. H&E. 200x.

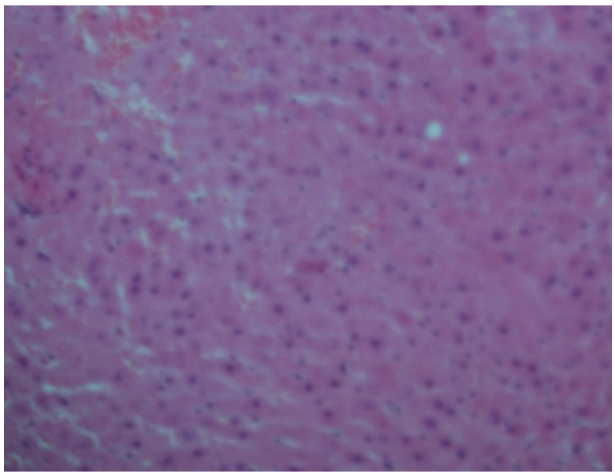

Figure 4e. HCD plus Simvastatin-treated rat liver showing individual cellular necrosis and degenerative changes. H&E. 200x.

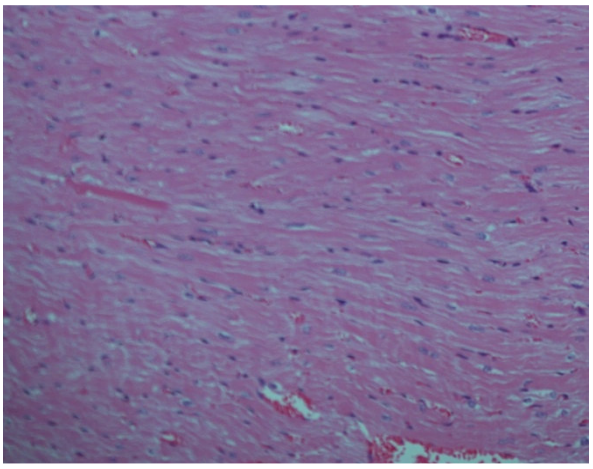

Figure 4f. Normal heart muscle cells. H&E. 200x.

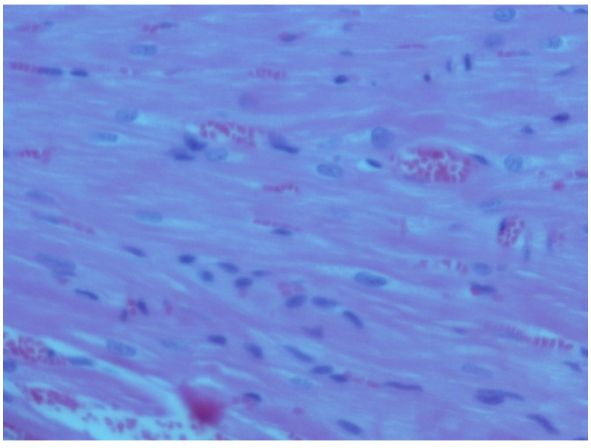

Figure 4g. HCD only-fed rats showing degenerative changes in heart muscle with the presence of large nuclei. H&E. 200x.

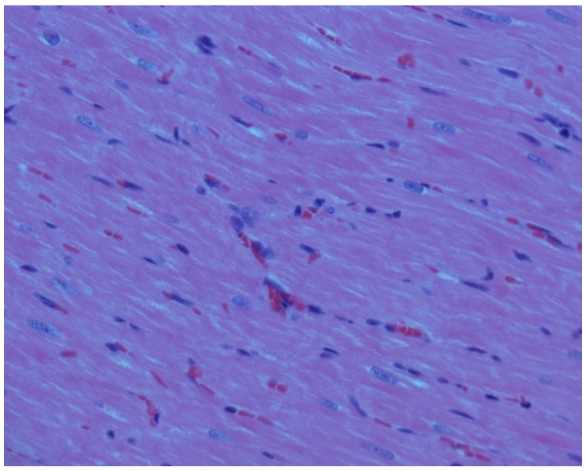

Figure 4h. HCD plus AFP 1 ml/rat/day-treated rat showing normal heart muscle. H&E. 200x.

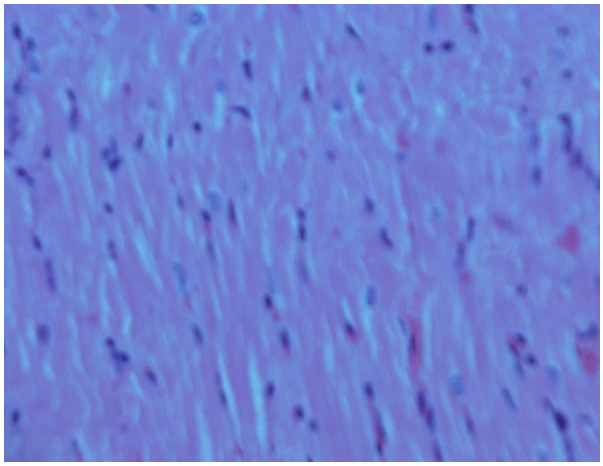

Figure 4i. HCD plus AFP 2 ml/rat/day-treated rat showing normal heart muscle. H&E. 200x.

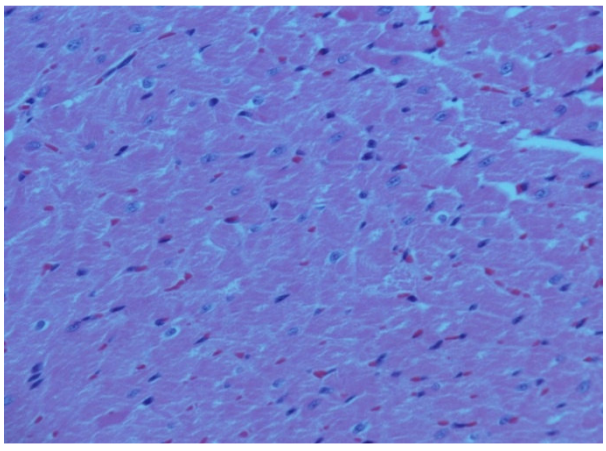

Figure 4j. HCD plus simvastatin-treated rat showing no effect on heart muscle. H&E. 200x.

liver marker enzymes and bilirubin levels, including GOT, GPT, GGT and ALP. Liver enzymes are considered as the biochemical indicators for assessing liver function. HCD-fed hepatic dysfunction is evidenced by an elevation in the serum marker enzymes (Beneyenet al., 1986). Avocado fruit pulp (AFP) treatment reduced the elevated levels of these marker enzymes showing that avocado pulp has the ability to preserve normal liver function.

It is now established that the high-cholesterol diet (HCD) and resultant hyperlipidemia causes an increase in TC, LDL-cholesterol levels to enhance the risk for the development of atherosclerosis (Genest et al., 1992; Kamal, 1975). Additionally, serum TG is also considered a risk factor for atherosclerosis (Jones and Chambliss, 2000). High levels of LDL-cholesterol (bad cholesterol) are also a high risk factor of coronary heart diseases (Smith et al., 2004). The results of the present study revealed that the AFP when administered orally at doses 1 and 2 ml/day/rat for ten weeks causes a significant decrease in the serum lipids, including TC, TG and liver enzymes levels. Besides, the serum lipoproteins including LDL-cholesterol and VLDL-cholesterol levels also significantly declined and a highly significant rise in HDL-cholesterol levels in the AFP-fed rats can be considered beneficial in the prevention of cardiovascular diseases. Simvastatin was included in this study as a standard drug in order to compare with the AFP activity.

There is a mounting evidence that high-cholesterol-diet has an increasing effect on lipid peroxidation in plasma and tissues (Mahfouz and Kummerow, 2000; Uysal et al., 1988; Balkan et al., 2002). Tests frequently used to evaluate products of lipid peroxidation in tissues include the determinations of malondealdehyde (MDA) (Sattler et al., 1998). In the current study, cholesterol-enriched diet caused an increase in MDA levels in both liver and heart tissues. It was also observed that such diet decreased the level of nonprotein-sulfhydryl (NP-SH) in both heart and liver tissues. The 10 weeks treatment of rats with AFP significantly reduced the MDA concentration, besides replenishing NP-SH contents in both tissues. These results convincingly prove the anti-oxidant property of AFP in experimental rats.

It has been reported that avocados are rich in monounsaturated fatty acids, fiber, Vitamins B and E and phytosterols (Wang et al., 2010) which have been shown to exert potent anti-oxidant activities. Avocado consumption also provides more β-sitosterol compared to other edible fruits (Weihrauch and Gardner, 1978). β-sitisterol is also known as anticholesterolemic agent (Moghadasian and Frohlich, 1999). The underlying mechanism of the serum cholesterol-lowering effect of phytosterols involves inhibition of intestinal cholesterol absorption and decrease in hepatic cholesterol synthesis (Ikeda and Sugano, 1983). Avocado is also reported to contain several carotenoids and is one of the richest sources of leutin among commonly eaten fruits. Avocado also contain γ-tocopherols and other bioactive phytochemical substances which are known to possess potent antioxidant properties and beneficial in chronic diseases including prostate cancer and heart diseases (Lu et al., 2005). The observation of the present investigation was further supported by the histological findings, as the prolong consumption of AFP produced remarkable protection of liver and heart tissues against oxidative stress provoked by high-cholesterol diet in rats.

The results conclude that the avocado fruit pulp has a definite anti-hyperlipidemic and hepato-cardiac protective potential and substantiate its use in folk medicine and recommendations of the dietitians in various diseases and in hypercholesterloemic conditions.

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