RESEARCH

23(100), November - December, 2019

ISSN 2321–7359 EISSN 2321–7367

Medical Science

Hypocholesterolemic and antioxidant effects of *Persea americana* leaf extract on hypercholesterolemic rats

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Article History

Received: 21 July 2019

Reviewed: 26/July/2019 *to* 03/September/2019

Accepted: 07 September 2019

Prepared: 12 September 2019

Published: November - December 2019

Citation

Maha A Althaiban. Hypocholesterolemic and antioxidant effects of *Persea americana* leaf extract on hypercholesterolemic rats. *Medical Science*, 2019, 23(100), 920-928

ABSTRACT

Plants are a major source of substances with therapeutic abilities. However, only a little number of plants around the world had been phytochemically examined. Hypocholesterolemic and antioxidant activity of *Persea americana* leaves methanolic extract (PALE) was assessed *i*n this research. Hypercholesterolemia was induced by feeding the animals diets enrich with cholesterol (2%) for four weeks. Fifty male albino rats had been distributed into five equivalent groups. Group 1 was held as a non-treated group (negative control) Group 2, which was held as a hypercholestrolemic group (positive control) (cont. (+), groups (3), (4) and (5) received orally Atorvastatine (AT) (40 mg/ kg), PALE in doses of twenty and forty mg/kg/day respectively, for 4 weeks. At the last day, blood was collected for biochemical analysis from all groups. The heart was also examined histopathologically. The results illustrated that the PALE significantly decreased serum levels of lipid profile, total cholesterol (TC), triglycerides (TG), liver enzymes and lipid peroxidation (MDA) but there were an increased in the antioxidant enzymes of hypercholesterolemic rats compared to control rats.

There was also an improvement in histopathological changes observed in the heart of hypercholesterolemic rats. Therefore, the administration of PALE has antioxidant and anti-hypercholesterolemic effects on hypercholesterolemic rats.

Keywords: Hypercholesterolemia - *Persea americana*- Rats – Statins- antioxidant enzymes – Histopathology-lipid profile.

1. INTRODUCTION

A well-known risk factor for cardiovascular disease (CVD) was hypercholesterolemia (Wang *et al*., 2011). The ministry of health in Saudi Arabia (2013) revealed that hypercholesterolemia in the majority of the population was 65 years and older. Hypercholesterolemia is classified among death- causing variables as number eight in KSA (Basulaiman, 2013). Death rates from CVD are projected to rise by the year 2030 and stay the world's leading cause of mortality (WHO, 2008). Several illnesses have been impacted by increased lipid profile levels such as coronary heart disease (CHD), diabetes and hypertension (Navar-Boggan, 2015). Statins (Atorvastatin) is one of the drugs used to treat hypercholesterolemia ,which act as anti-hypercholesterolemic agent which had severe side effects such as muscle or joint ache, soreness, weakness of liver and kidney problems (Ramkumar, 2016), rash, breathing difficulties, increased body temperature, unusual fatigue and loss of appetite (Mancini *et al*., 2016).

Therefore, recent researches focus on natural plants. The use of medicinal plants is currently growing depending on their availability, promising effectiveness similar to the often-high price and inaccessibility, as well as adverse effects associated with conventional synthetic drugs (Opara and Al-Ani, 2010).

The use of fresh fruits and vegetables to enhance human health was primarily attributed to their elevated content of useful phytochemicals and other micronutrients. These phytochemicals, mainly phenolic compounds (such as phenolic acids, saponins, flavonoids, and tannins), received considerable attention due to their high antioxidant activity by scavenging free radicals (Boyer and Liu, 2004).

This generates the need for natural antioxidants in the target food to be supplemented with antioxidant characteristics (Erukainure *et al*., 2012). Among such crops, *Persea americana* (PA) is commonly known as avocado. It is commonly grown all over the globe for its edible fruits and financial and therapeutic uses (Gomez-Flores *et al*., 2008). The plant that bears avocado pear as fruit contributes to improving health and may contain some significant chemicals such as ascorbic acid and soluble phenolic compared to most popular vegetables and fruits (Garcia-Alvarado *et al*., 2001). It was revealed that PA was efficient against hepatotoxicity, inflammation, cancer, hypertension, etc. (Brai *et al*., 2014). Because of the significance of this plant in traditional medicine, the plant can be used for these health advantages. To evaluate the influence of PALE on hypercholesterolemia the current study was undertaken.

2. MATERIAL AND METHODS

Material

Plant material

Persea americana Leaf was purchased from Abazeer organic store, Jeddah, KSA.

Kits and chemicals

Cholesterol, triglycerides, lipid profiles and liver enzymes, as well as antioxidant kits were purchased from Sigma Aldrich, Germany. All solvents with high analytical grade were obtained from Scientific Fisher, Germany. Statins (Atorvastatine) was purchased from local pharmacy Jeddah, Saudi Arabia.

Rats and diet

Fifty male abino rats (160 – 180 g) were provided from King Fahd Research Center, KAU. Basal diet ingredients were obtained from Baghafar Company for Pharmaceutical and Chemical, Jeddah, KSA.

Ethical approval

The experimental study was adhering under rules of Canadian ethic upon approval for biomedical committee, KFMRC, KAU, KSA.

Methods

Preparation of PALE

Persea americana Leaves were air dried then blended to a fine powder and the methanolic extract prepared by the method of Soxhlet extraction. Extract was clarified using proper filter. The yield was 9.7% w/w, then the extract was concentrated at 40 °C using the rotary evaporator and the residue was freeze-dried (Brai *et al*., 2007).

Phytochemical screening of PALE

The phytochemical analysis of PALE was carried out to detect the presence of the major chemical components, including alkaloids, flavonoids, glycosides, steroids, saponins and tannins, using conventional analytical procedures (Goupy *et al*., 1999).

Basal diet and cholesterol induction

Diet has been established as outlined in Reeves *et al.*, (1993). Then, by using the method described by Shinnick *et al.,* (1990), the hypercholesterolemic diet containing cholesterol (2%) and bile salts (0.5%) was prepared. The feeding period was 4 weeks.

Experimental protocol

After acclimatization period (one week), rats were separated unsystematically into 5 groups, 10 rats/ group. First group was kept as a control negative group. groups from (2-5) were consumed hypercholesterolmic diet for 4 weeks then blood samples were collected to ensure that rats have hypercholesteremia (TC ≥5.2 mmol / L) (Iqbal *et al*., 2011).

Group (1)	Control negative orally given purified water.
Group (2)	Cont. (+) orally given purified water by gavage + high fat diet.
Group (3)	Given orally Statins (AT) at (40 mg/ kg) + high fat diet.
Group (4 & 5)	Given PALE by gavage at doses of (20 and 40 mg/kg) + high fat diet (Kolawole *et al*., 2012).

In last day of experimental period (4 weeks), all rats were fasting for 12 hours, blood was collected for biochemical analysis and then all rats were sacrificed to collect heart for histopathological examination.

Liver enzymes assay

Serum liver function activities of alanine and aspartate aminotransferase (ALT and AST), as well as alkaline phosphatase (ALP) were assessed as described in protocol provided in ELSA kits obtained from MyBioSource, USA.

Serum lipid levels assay

Serum lipid levels were assessed as described in protocol provided in ELSA kits obtained from My BioSource, USA.

Determination of antioxidant enzymes activity

Antioxidant enzymes as well as malondialdehyde level (MDA) were determined by the protocol provided in ELSA kits obtained from My BioSource, USA.

Histopathological examination

Heart was dipped in neutral formalin (10%) for histopathological examination.

Statistical analysis

Results analysis by ANOVA one way analysis of variance, values were presented as mean ± SMD for 10 rats/ group.

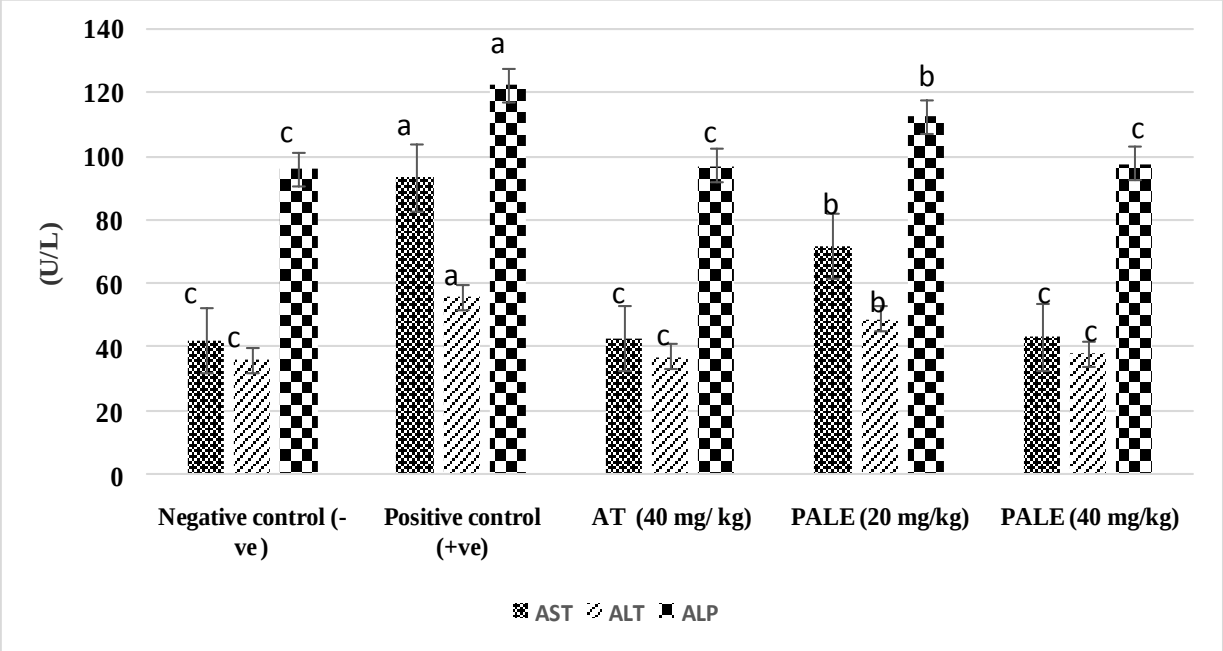
3. RESULTS

The phytochemical screening of alcoholic PALE revealed that it contains large amounts of phenols, oxalate and steroids; moderate amounts of alkaloids, phytate and tannins and a few saponins and glycosides as depicted in Table (1).

Table 1 Phytochemical screening of PALE

Phytochemical	Test results
Alkaloids	++
Phytate	++
Phenols	+++
Oxalate	+++
Saponins	+
Tannins	++
Glycosides	+
Steroids	+++

The following symbol indicated the intensity of active compounds: a small amount (+), a moderate amount (++), and large amount (+++).



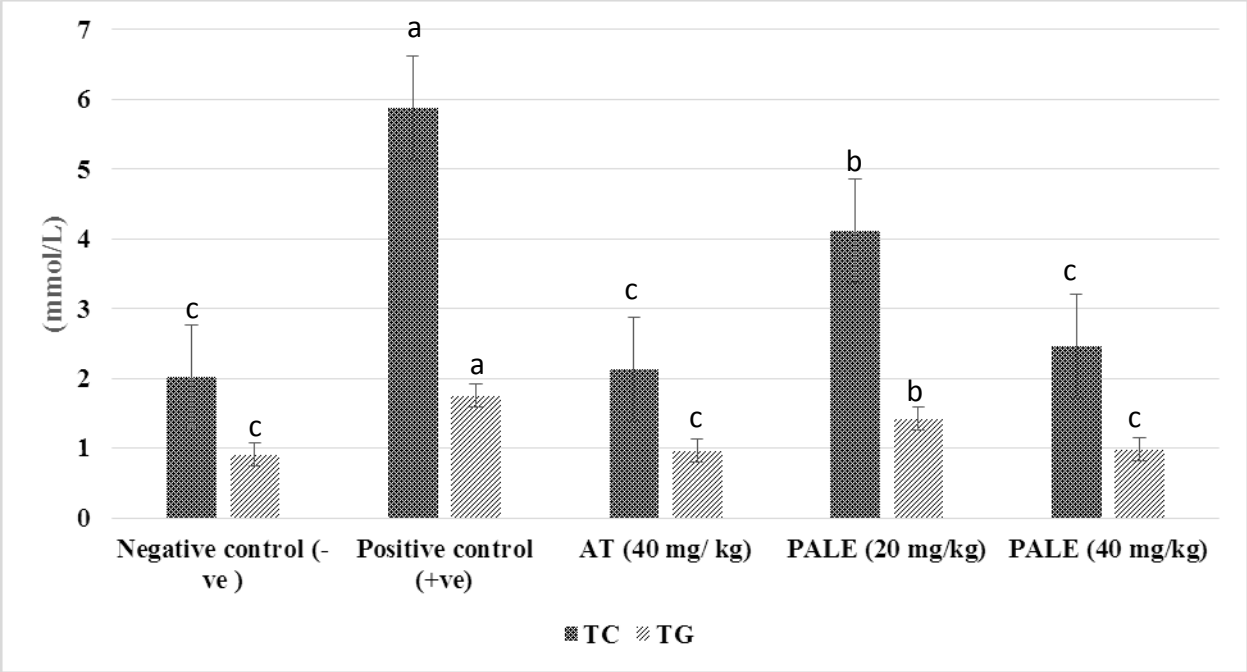
- Values are presented as mean±SMD.
- Values with different superscript letters within a column are significantly different at P<0.05.

Figure 1 Hypocholesterolemic and antioxidant effects of PALEon liver enzymes of hypercholesterolemic rats

Figure 1 shows that cont (+) group had significant increases (P<0.05) in the serum concentrations of liver enzymes (AST, ALT and ALP) (93.63±5.23, 56.11 ± 3.39 and 122.66 ± 7.38) respectively compared to healthy rats (42.32 ± 3.28, 36.21 ± 2.12 and 96.11 ± 4.59) respectively. Oral administration of AT and PALE in both dosage concentrations to cont (+) rats considerably (P<0.05) decreased serum liver enzymes and become near the control level, particularly in drug and high PALE dose compared with cont (+) group.

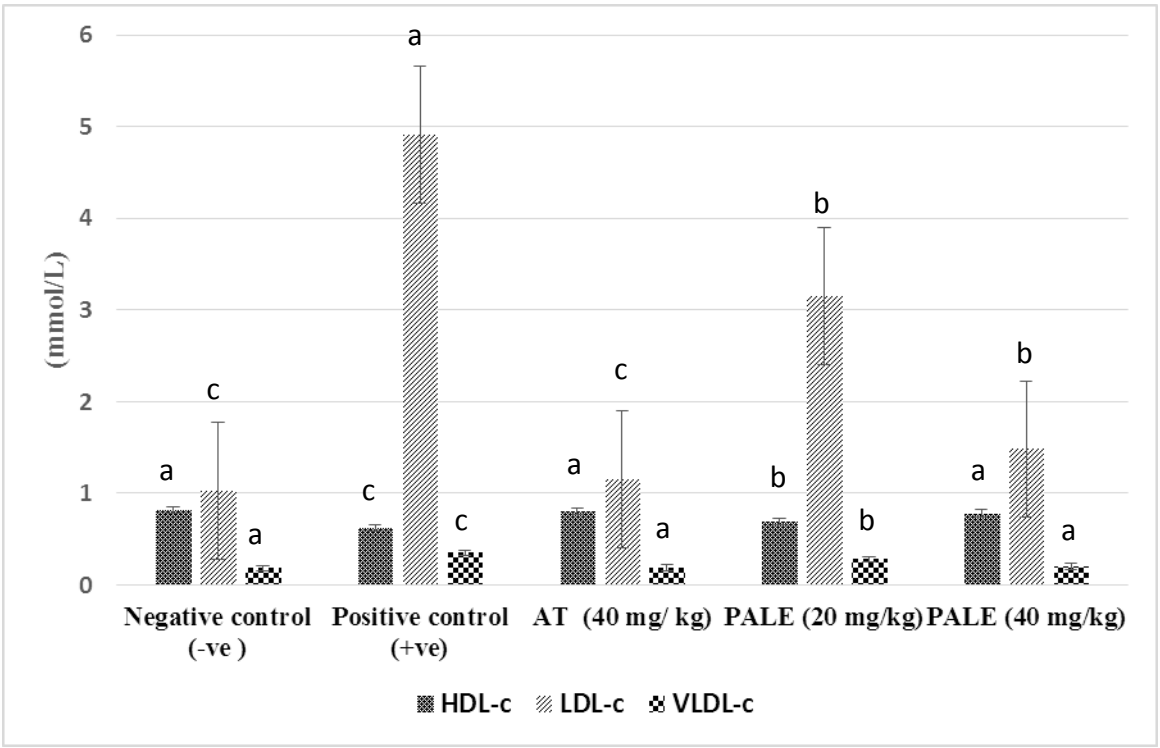
Figure 2 demonstrated the effect of PALE on TC and TG levels against hypercholesrolemia in male rats. High cholesterol diet (cont. (+)) caused a significant increase in both TC (5.88±0.24) (P<0.05) and TG levels (1.75±0.05) (P<0.05) respectively as compared with the control negative group (2.03 ± 0.02 and 0.91± 0.01).Oral administration of the drug and PALE at a high dose produced a

highly significant changes in TC and TG levels (2.14±.0.03, 0.96±0.05, 2.46±0.13 and 0.98±0.04) respectively ($p<0.05$) as compared with the cont. (+) group (211.76 ±3.95 and 134.82±2.95) ($p<0.05$).



- Values are presented as mean±SED.
- Values with different superscript letters within a column are significantly different at $P<0.05$.

Figure 2 Hypocholesterolemic and antioxidant effects of PALEon cholesterol (TC) and triglyceride (TG) levels of hypercholesterolemic rats.



- Values are presented as mean±SMD.
- Values with different superscript letters within a column are significantly different at $P<0.05$.

Figure 3 Hypocholesterolemic and antioxidant effects of PALEon lipid profile of hypercholesterolemic rats.

Figure 3 confirmed the effect of PALE on lipid profile against hypercholesrolemia in male rats, caused a significant decrease in HDL (0.62±0.04) (P<0.05) associated with significant increases in both LDL and VLDL (4.91±0.04 and 0.35±0.02) (P<0.05) as compared with the control negative group (0.82 ± 0.04, 1.03±.0.01and 0.18± 0.01) for HDL, LDL, and VLDL levels, respectively. Oral administration of the AT and PALE at a high dose produced a significant changes in HDL, LDL and VLDL levels (0.80±.0.03, 1.15±0.01, 0.19± 0.03 and 0.78±0.02, 1.48±0.03 and 0.02±0.01, respectively (p<0.05) as compared with the cont. (+) group (0.62±0.04, 4.91±0.04 and 0.35±0.02, respectively) (p<0.05).

The results revealed that cont. (+) group recorded significant increase (p<0.05) in MDA and significant decreases in CAT, SOD and GPx (p<0.05) comparing with the control negative group after 4 weeks of treatment. Treatment with drug and PALE at the two doses used induced significant improvement (p<0.05) in all parameters value compared with cont. (+) group. The high dose had the better effect compared with the low dose in reducing the MDA and increasing the antioxidant enzymes level (Table 2).

Table 2 Hypocholesterolemic and antioxidant effects of PALE on serum MDA, CAT, SOD and GPx levels of hypercholesterolemic rats.

Groups	MDA (nmol/g protein)	CAT (U/mL)	SOD (U/mL)	GPx(U/mL)
Negative control (-ve)	12.46±1.22c	0.16 ±.04a	59.22± 1.21a	0.82 ± 0.02a
Positive control (+ve)	24.65±1. 51a	0.12 ±0.08c	28.76 ± 1.23c	0.41 ±0.02 c
AT (40 mg/ kg)	13.88±1. 27c	0.16 ± 0.02a	58.66± 1.16 a	0.79 ± 0.04a
PALE (20 mg/kg)	20.44±1.21b	0.14± 0.06b	44.95± 2.13 b	0.56 ± 0.05 b
PALE (40 mg/kg)	14.43±1.12c	0.16 ± 0.05a	57.99± 2.41a	0.74 ±0.07a

- Values are presented as mean ± SMD.
- Values with different superscript letters within a column are significantly different at P<0.05.

Histopathological results

Heart sections of normal control rats showed normal cardiac myocytes (Fig. 4A). Heart of the positive control group showing cardiac myocyte necrosis and infiltration of inflammatory cells (Fig. 4B & C). While the heart of hypercholesterolemic rat treated with the AT showing no histopathological changes (Fig.4D). The PALE at low dose showing slight myocardial blood vessel congestion (Fig.4 E). Heart Sections of rats received orally forty mg/kg b.wt. PALE showing normal tissue structure (Fig.4F).

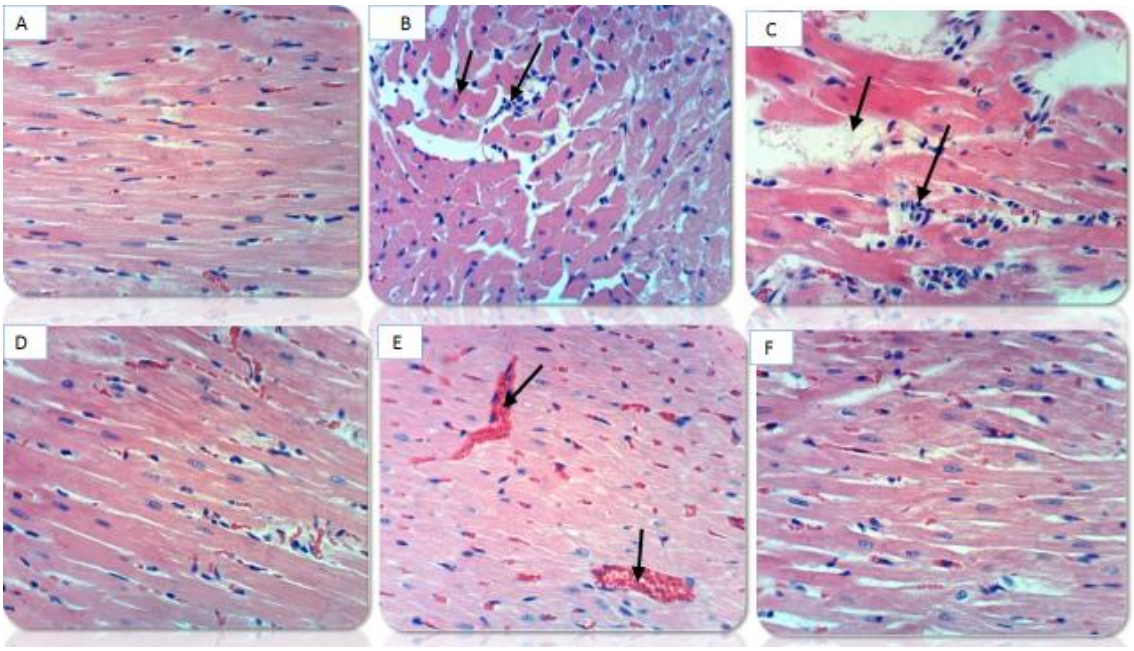


Figure 4 Photomicrography illustrating H&E-stained sections of heart in different groups. Heart of control negative rats showing normal cardiac myocytes(A). In hypercholesterolemic rats, heart sections showing cardiac myocyte necrosis and infiltration of inflammatory cells (B and C).In hypercholesterolemic rat heart treated with the AT showingno histopathological changes (D). Heart of rats received orally 20 mg/kg b.wt. PALE showing slight myocardial blood vessel congestion (E), while the heart of rats resaved PALE in a dose of 40 mg/Kg showing normal tissue structure (F). (H and E x 400).

4. DISCUSSION

The elevation of total cholesterol in the blood is known as hypercholesterolemia. It is the type of "hyperlipidemia" (high blood lipid concentration) and hyperlipoproteinemia (high blood lipoprotein concentration) (Insull, 2009). Hypercholesterolemia may result in the accumulation, oxidation, and alteration of lipids in the vascular endothelium resulting in endothelial dysfunction, chronic inflammation, and cardiovascular diseases (CVD) (Ito *et al*., 2011).

Antioxidants from plant are of increasing interest to consumers because of their roles in the maintenance of human health. Several phenolic compounds such as saponins, flavonoids and phenolic acids possess diverse biological activities and are thought to be beneficial for reducing tissue damage induced by oxidative stress (Mohana *et al*., 2012). This study aimed to demonstrate the potential treatment ability of PALE on hypercholesterolemic rats.

The present findings showed that AT or PALE administration to hypercholesterolemic rats improved all tested parameters versus cont. (+). The PALE possess hypocholesterolemic effect, due to its high amounts of phenols, oxalate and steroids in addition, there is moderate amounts of alkaloids, phytate and tannins as depicted in Table (1). The results were in the same line with Dabas et al., (2013) who reported the abundance of phenol in avocado that plays a major role in an individual's body system. By decreasing oxidative and inflammatory stress (Chong et al., 2010), the existence of phenol in avocado fruit helps to decrease the risk of cardiovascular disease (CVD).

This study verified that cont (+) group had marked elevations in liver enzymes compared to control rats, while the treatment of rats with PALE induced significant declines in the level of liver enzymes. The significant reduction in the enzymes of liver after the treatment with PALE suggested that the extract is hepatoprotective (Sakr *et al*., 2011). Therefore, it was suggested that PALE's hepatoprotection mechanism might be partially due to its antioxidant effect and components including, tannins, triterpenoids, alkaloids and Flavonoids (Gupta *et al*., 2004). Flavonoids are known to be antioxidants, free radical scavengers and anti-lipoperoxidants, which cause hepato-protection (Mankani *et al*., 2005).

Hyperlipidemia is a significant risk factor in atherosclerosis, pathogenesis it is a physiological disorder affecting the circulation of the coronary, brain and peripheral arteries (Gordon *et al*., 2007). Hyperlipidemia may be manifested by elevation of TC, TG and LDL concentrations and a decrease in HDL concentration. It was also shown that lowering LDL level significantly lowered the risk of CHD [28]. In addition, elevated plasma HDL concentration is associated with reduced CHD danger and it is commonly thought that HDL protects against atherosclerosis by enabling inverse cholesterol transport (Adaramoye *et al*., 2008).

The current results showed that there were significant changes in the plasma levels of TC, TG, LDL, VLDL and HDL of cont. (+), when compared with normal control. These changes were reversed by AT and PALE in a dose-dependent manner as confirmed by histopathological examination of the heart. These results may be due to phytochemicals such as luteolinrutin, orhamnetin, apigenin and quercetin isolated from avocado leaves, which may be assist to avoid the progression of multiple oxidative stress- related disease (Adaramoye *et al*., 2008).

Suppression of TG level suggested that the bile acid synthesis was reversed. By this TG reduction by the extract. Therefore, since TG level was significantly reduced by this extract, it is pertinent that VLDLC synthesis will be disturbed (Van et al., 2009). The VLDLC is indirectly involved in LDLC generation, so VLDLC increases plasma LDLC (Adaramoye *et al*., 2008).

There is an inverse association between plasma TG concentration and HDLC levels (Singh *et al*., 2007) and therefore, there is reduction in the TG concentration by the PALE administration, it should expect an increase in the plasma concentration of HDLC. These results showed that PALE could be used as an effective supplement in hyperlipidemic patients.

The PALE lowers serum lipid by other possible mechanisms. It could reduce the biosynthesis of cholesterol by inhibiting the activity of 3-hydroxy-3-methylglutaryl coenzyme-A reductase (HMG-CoA reductase), the key enzyme in cholesterol synthesis. The PALE could also behave by raising the activity of lecithin-cholesterol acyl transferase. This enzyme plays a significant role in the incorporation of free cholesterol in HDL, promoting reverse cholesterol transport and competitively inhibiting LDL absorption by endothelia cells (Geetha *et al*., 2011).

Oxidative stress plays a major role in the pathogenesis of atherosclerosis (Adaramoye *et al*., 2005). In this study, there was a significant increase in MDA and significant decreases in antioxidant enzymes of hyperlipidemic animals. Elevated MDA would lead to the generation of free radicals that weaken membrane functions, and eventually led to ultimately results in macrovascular and microvascular complications (Virella –Lopes *et al*., 2003). The increase in MDA and the decrease in antioxidant enzymes level observed in hyperlipidemic rats were reversed to near normal level by PALE and AT administration. This could be due to PALE antioxidant effects. This is most likely the case because isolation of bioactive phyto constituents from the leaves of PALE has produced compounds with antioxidant properties such as luteolin, rutin, quercetin and apigenin (Owolabi *et al*., 2010).

5. CONCLUSION

It can be hypothesized that PALE decreases liver enzymes, lipid parameters, and lipid per oxidation while it increase antioxidant enzymes in hypercholesterolemic rats. However, it might be necessary to do more researches on PALE to insure that it will be beneficial in the treatment of hypercholesterolemia.

Conflict of interest

The author declares that no conflict of interest

Financial resources of the study

None

REFERENCE

1. Adaramoye OA. Akintayo O. AchemJ. Fafunso MA. Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. Vasc Health and Risk Manag 2008; 4(1), 235-241.
2. Adaramoye OA. Nwaneri VO. Anyanwu KC. Farombi EO. Emerole GO. Possible anti-atherogenic effect of kolaviron (a Garcinia kola seed extract) in hypercholesterolaemic rats. Clin Exp Pharmacol Physiol 2005; 32, 40-46.
3. Basulaiman M. Hypercholesterolemia and its associated risk factors—Kingdom of Saudi Arabia. Annals of epidemiology 2013; 24 (11): 801-808.
4. Boyer J. Liu R H. Apple phytochemicals and their health benefits. Nutrition Journal 2004; 12(3):5-9.
5. Brai BIC. Adisa RA. Odetola AA. Hepatoprotective properties of aqueous leaf extract of *Persea americana*, (Lauraceae) 'Avocado' against CCl4- induced damage in rats. African Journal of Traditional, Complementary and Alternative Medicine 2014; 11(2): 237–244.
6. Brai BIC. Odetola AA. Agomo PU. Effects of *Persea americana* leaf extracts on body weight and liver lipids in rats fed hyperlipidaemic. African Journal of Biotechnology 2007; 6 (8):1007-1011.
7. Chong MF. Macdonald R. Lovegrove JA. Fruit polyphenols and CVD risk a review of human intervention studies. British Journal of Nutrition 2010; 104:S28-S39.
8. Dabas D. Shegog RM. Ziegler GR. Lambert JD. Avocado (*Persea americana*) Seed as a Source of Bioactive Chemicals. Current Pharmaceutical Design 2013; 19: 6133-6140.
9. Erukainure OL. Oke OV. Owolabi FO. Kayode FO. Umanhonlen EE. Aliyu M. Chemical properties of *Monodora myristica* and its protective potentials against free radicals in vitro. Oxidants and Antioxidants in Medical Science 2012; 1(2): 127- 3.
10. Garcia-Alvarado JS. Verde-Star MJ. Heredia NL. Traditional uses and scientific knowledge of medicinal plants from Mexico and Central America. J Herbs Spices Med Plants 2001; 8:37-90.
11. Geetha G. Kalavalarasariel GP. Sankar V. Antidiabetic effect of *Achyranthes rubrofusca* leaf extracts on all oxan induced diabetic rats. Pak J Pharm Sci 2011; 24(2): 193-199.
12. Gomez-Flores R. Verastegui-Rodriguez L. Quintanilla-Licea R. Tamez-Guerra P. Tamez-Guerra R. Rodriguez-Padilla C. In vitro rat lymphocyte proliferation induced by *Ocinum basilicum, Persea americana, Plantago virginica* and Rosa spp extracts. J Med. Plant Res 2008; 2:5-10.
13. Gordon T. Castelli WP. Hjortland MC. Kannel WB. Dawber TR. High density lipoprotein as a protective factor against coronary heart disease: The Framingham study. Am J Med 2007; 62(5), 705-714.
14. Goupy P. Hugues M. Biovin P. Amiot MJ. Antioxidant, composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. J Sci Food Agric 1999; 79(12): 1625-1634.
15. Gupta M. MazumderUK.Kumar TS. Gomathi P. Kumar RS. Antioxidant and hepatoprotective effects of *Bauhinia racemosa* against paracetamol and carbon tetrachloride induced liver damage in rats. Iranian J Pharmacol Therapeutic 2004; 3:12–20.
16. InsullWJr.The pathology of atherosclerosis: Plaque development and plaque responses to medical treatment. The American Journal of Medicine 2009; 122(1):3-14.
17. Iqbal M. Kalsoom A. Jafri SA. Effect of *Punica granatum* Flowers Extract on Hypercholesterolemic and Alloxan Induced Diabetic Rats Global Journal of Biotechnology and Biochemistry 2011; 6(2):83-6.
18. Ito MK. Mc Gowan MP. Moriarty PM. Management of family's hypercholesterolemia in adult patients: recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. Journal of Clinical Lipidology 2011; 5 (3): 38–45.
19. Kolawole OT. Kolawole SO. Ayankunle AA. Olaniran IO. Methanol Leaf Extract of *Persea americana* Protects Rats against Cholesterol-Induced Hyperlipidemia. British Journal of Medicine and Medical Research 2012; 2(2): 235-242.

20. Mancini GB. Baker S. Bergeron J. Diagnosis, prevention and management of statin adverse effects and intolerance: Canadian Consensus Working Group update. Can J Cardiol 2016; 32:S35-65.
21. MankaniKL.Krishna V. Manjunatha BK. Vidya SM. Jagadeesh Singh SD. Manohara Y N. Raheman A. Avinash KR. Evaluation of hepatoprotective activity of stem bark of *Pterocarpus marsupium* Roxb. Indian J Pharmacol 2005; 37:165–168.
22. Mohana LS. Reddy UK. Rani KS. A review on medicinal plant for nephroprotective activity. Asion J of pharm and clinic Resh 2012; 5(4)124-132.
23. Navar-Boggan AM. Hyperlipidemia in Early Adulthood Increases Long-Term Risk of Coronary Heart Disease clinical perspective. Circulation 2015; 131(5): 451-458.
24. Opara UL. Al-Ani MR. Antioxidant contents of pre-packed fresh-cut versus whole fruit and vegetables. British Food Journal 2010; 112 (8):797 – 810.
25. Owolabi MA. Coker HAB.Jaja SI. Bioactivity of the phytoconstituents of the leaves of *Persea americana*. J Med Plant Res 2010; 4(12): 1130-1135.
26. Ram kumar S. Statin Therapy Review of Safety and Potential Side Effects. PMC 2016; 32 (6): 631–639.
27. Reeves PG. Nielsen FH. Fahey Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. Journal of Nutrition 1993; 123 (11):1939–1951.
28. Sakr SA. Mahran HA. Lamfon HA. Protective effect of ginger (*Zingiber officinale*) on adriamycin-induced hepatotoxicity in albino rats. J Med Plant Res 2011; 5:133–140.
29. Shinnick FL. Ink SL.Marie JA.Dose response to a dietary oat bran fraction in cholesterol-fed rats, The Journal of Nutrition 1990;120(6): 561-8.
30. Singh IM. Shishehbor MH. Ansell BJ. High-density lipoprotein as a therapeutic target: a systematic review. JAMA.2007; 298:786-798.
31. Van EC. Lievens J. Jacobs F. Feng Y. Snoeys J. Geest BA. Apolipoprotein A-I and lecithin cholesterol acyltransferase transfer induce cholesterol unloading. Gene therapy 2009; 16, 757-765.
32. Virella –Lopes MFL. Virella G. The role of immune and inflammatory processes in the development of macrovascular disease in diabetes. Frontiers in Biosc 2003; 8: 750-768.
33. Wang X, Hasegawa J, Kitamura Y, Wang Z, Matsuda A, Shinoda W, Miura N, Kimura K. Effects of hesperidin on the progression of hypercholesterolemia and fatty liver induced by high-cholesterol diet in rats. Journal of Pharmacological Sciences 2011; 117(3):129-38.
34. WHO, World Health Organization (2008). Cardiovascular diseases (CVDs), Access date, May 17, 2019, from: http://www.who.int/mediacentre.