Effect of avocado seed extract on lipid profile and atherogenic index in cyclosporine-treated rats

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Abstract

Hypertension is a global health issue because of its high prevalence and its association with increased risk of cardiovascular disease. This work evaluated the anti-dyslipidemic potential of aqueous extract of avocado seed (APE) (50 – 100 mg/kg) in adult male wistar rats. Twenty-five rats were used for this work and divided into five groups of 5 rats each (n= 5). Group 1 (normal control), group 2 (cyclosporine alone), group 3 (cyclosporine + 5 mg of lisinopril), group 4 and 5 (cyclosporine + 50 mg/kg and 100 mg/kg b.wt of extract respectively). Treatments lasted for 7 days and the rats were sacrificed by cervical dislocation. Plasma samples from the rats were used for lipid profile analysis such as; total cholesterol, triglyceride, and high density lipoprotein. The results showed significant ($p < 0.05$) increase in triglyceride (TG), low density lipoprotein (LDL), and total cholesterol (TCH), as well as significant ($p < 0.05$) decrease in HDL and atherogenic index in cyclosporine only treated rats compared with the normal control group. However, treatment with aqueous extract of avocado seed (APE) caused a significant decrease in TG, LDL, and total cholesterol, with concomitant increase in HDL concentration and atherogenic index in a dose-dependent manner. The findings of this study suggest that avocado seed extract has anti-dyslipidemic potential which could be useful in the management of hypertension and other diseases arising from dyslipidemia.

Keywords: Avocado pear, atherogenic index, lipid profile, cyclosporine.

Introduction

Dyslipidemia is a major risk factor for the onset of cardiovascular disease, accounting for the highest morbidity and mortality (Asadi *et al*., 2019). Dyslipidemia poses a serious health problem throughout the world because of its high prevalence and its association with increased risk of cardiovascular disease (CVD). It is usually taken as that level of arterial blood pressure associated with doubling of long-term cardiovascular risk (Reiner *et al*., 2017). It is widely accepted that CVD is associated with dyslipidemia such as high blood level of low-density lipoprotein (LDL), total cholesterol (TC), and triglycerides (TG). In contrast, a low level of high density lipoprotein (HDL) is a risk factor for mortality from CVD (Darroudi *et al*., 2018).

Traditional and alternative medicines involving application of medicinal plants have attracted numerous attentions globally.

Various parts of plants such as seeds, leaves, stems, roots and barks have been reported useful for the treatment of diseases affecting humans (Tremocoldi *et al*., 2018). This is as a result of the continuous need for less expensive means of disease prevention and control. Furthermore, most conventional drugs commonly used today are expensive and usually have associated side effects. Plants represent resources with medicinal properties that are cheap and readily available, with minimal side effects. Indeed, about 25% of the prescription drugs dispensed contain at least one active ingredient derived from plant material. Avocado (*Persea americana*) is one of the many medicinal plants used in the treatment of several human diseases. Avocados are a rich source of nutrients and phytochemicals. Some scientific records on the pharmacological activities of the avocado pear include its vasorelaxant activity (Unegbu *et al*., 2017), antihypertensive activity, analgesic and anti-inflammatory activity (Araujo *et al.*, 2018), antiviral activity, anticonvulsant effect among others (Calderon-Oliver *et al*., 2016). There are lots of research on the effects of avocado seeds, oil and pulp on hypertension and other CVD related diseases (Ramos *et al.*, 2017; Dabas *et al*., 2013). Furthermore, medical application of cyclosporine-A has been reported to cause some unwanted side effects which include; hypertension, nephrotoxicity and hepatotoxicity (Alimazroo *et al*., 2017). Despite these reported activities of Avocado seed and adverse effects of cyclosporine-A, the effects of aqueous extract of Avocado pear on lipid profile and atherogenic index in cyclosporine-treated rats has not been examined. Thus, this study sought to assess the effects of Avocado seed extract on lipid profile and atherogenic index in cyclosporine-treated rats.

Materials and methods

Sample collection and preparation

Fresh Avocado fruits were purchased from Owena main market in Oriade Local Government Area of Osun state, Nigeria, in December, 2020. The seeds were removed from the fruits, cut into smaller pieces and air-dried to constant weight. The dried seeds were pulverized to fine powder using domestic blender. One hundred gram (100 g) of powdered sample was soaked in 500 mL of distilled water in a beaker for 8 h under continuous stirring on a shaker. The homogenate was filtered through a piece of clean white cloth. The filtrate was freeze-dried and the residues kept in a refrigerator (-4^{o}C). The yield was 13.4%. From the stock, doses (50 and 100 mg/kg) were calculated based on the weight of the animals whenever needed.

Experimental design

Twenty-five rats weighing 180-200 g were obtained from the Animal house, Biochemistry Department of Federal University of Technology, Akure, Nigeria. The animals were housed in stainless steel cages and kept in a room where 12 hours light/dark was maintained throughout the period of the experiment. The animals were given free access to commercial diets and water *ad libitum*. Animals were handled in accordance with international guidelines and approval of institutional ethical committee (FUTA/ETH/21/10). The rats were acclimatized for two weeks. The rats were subsequently divided into five groups (n=5) as follows:

Group 1: (Normal control)

Group 2: (Cyclosporine (CSA) induced rats; 25 mg/kg)

Group 3: (CSA + 5 mg Lisinopril, standard drug)

Group 4: (CSA + 50 mg/kg of APE)

Group 5: (CSA + 100 mg/kg of APE)

The experiment lasted for seven days. The choice of dose of cyclosporine (25 mg/kg) was in accordance with previous work of El-Kenawy (2010); while 50 mg/kg and 100 mg/kg of APE were given according to the report of Dwi *et al.* (2020).

Sample Collection

After the treatment period of 7 days, the animals were sacrificed 24 hours after the last dose under light ether anesthesia. After anaesthetizing, laparotomy was carried out to expose the internal organs. Blood samples were collected using a 5 mL syringe into EDTA bottles and centrifuged at 5000 rpm for 10 min to separate the plasma. The supernatants was collected into sample bottles and refrigerated for further analyses.

Biochemical Analysis

Determination of total cholesterol concentration

Principle: The cholesterol was determined according to the principle described by Allain *et al.,* (1974). The cholesterol was determined after enzymatic hydrolysis and oxidation. The quinoneimine was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Procedure: 1 mL of the reacting mixture containing 4-aminoantipyrine, phenol, peroxidase, cholesterol esterase, cholesterol oxidase and 80 mM pipes buffer pH 6.8 was mixed with 10 µL of sample and incubated for 5 min at 37 ^{o}C. The absorbance at 500 nm was then taken against the reagent blank within 60 min. The concentration of cholesterol in the sample was subsequently calculated against a standard.

Determination of high-density lipoprotein (HDL) – cholesterol concentration

Principle: Low-density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high-density lipoprotein) fraction, which remains in the supernatant, is determined.

Procedure: 200 µL of sample was mixed with 500 µL of the precipitant (0.55 mmol of phosphotungstic acid and 25 mmol magnesium chloride) these were mixed and allowed to sit for 10 mins at 25 ^{o}C. Thereafter, it was then centrifuged for 10 mins at 4,000 rpm. The supernatant was separated within two hours and the cholesterol content was determined by the method described by Allain (1974).

Determination of triglyceride concentration

Principles:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerolphosphate oxidase}} \text{Dihydroxyacetone} + \text{Phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + HCl + 4H_2O$$

Procedure: The triglyceride concentration was determined using the colorimetric method as described by Tietz (1982). Briefly, 10 µL of the sample was mixed with 1 mL of Pipes reagent (40 mM phosphate buffer, 5.5 mM 4-chlorophenol and 17.5 mM Mg^{2+}) and 1mL of enzyme reagent (4-aminophenazone, adenosinetriphosphate, lipase, glycerolkinase, glycerol-3-phosphate oxidase and peroxidase). Thereafter the mixture was incubated for 5 min at 37 ^{o}C and the absorbance at 546 nm was taken against reagent blank within 60 min. The triglyceride concentration was subsequently calculated against the standard.

Determination of Low Density Lipoprotein

LDL was calculated using the formula below as described by Akinyemi *et al*. (2016).

LDL = Total CHOL – HDL – TG/5

Determination of Atherogenic index of plasma (AIP)

Atherogenic index was calculated as previously described (Akinyemi *et al*., 2016).

AIP =log (TG/HDL-c)

Statistical Analysis

Data analysis and graph construction were performed using Graphpad prism version 5.00 for windows (GraphPad Prism Software Inc., USA). The results were analyzed by one-way ANOVA followed by the Dunnette's multiple comparison tests to determine the difference among treatments, considering a significance level of $p < 0.05$. All data were expressed as mean values ± standard deviation.

Results

The results in Figure 1 showed that there was no significant difference ($p > 0.05$) in the total cholesterol level of animals in the normal control group compared with rats induced with cyclosporine alone. Similarly, the total cholesterol of the groups treated with standard drug, lisinopril and 50 mg/kg avocado seed extract were reduced by 14.23 and 17.09% respectively but were not significantly different ($p > 0.05$) when compared with that of the group treated with cyclosporine alone. On the other hand, the animals treated with 100 mg/kg of avocado seed extract

showed a significant difference ($p < 0.05$) with 22.45% reduction in total cholesterol level when compared with the group induced with cyclosporine alone.

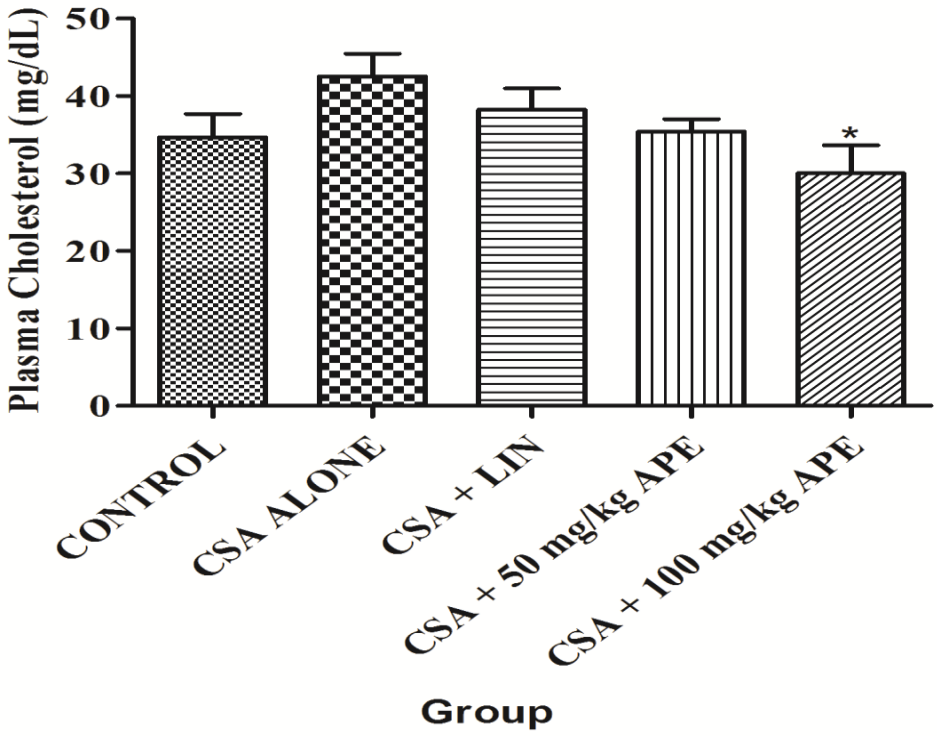


Figure 1**:** Effect of aqueous extract of avocado pear seed on total cholesterol concentration in cyclosporine (CSA) treated rats. Bars represent mean ± standard deviation (n=5).*p< 0.05 compared with cyclosporine alone.

Results presented in figure 2 showed the effects of various treatments on high density lipoprotein of rats. There was a significant ($p < 0.05$) decrease in HDL level of the group treated with cyclosporine alone when compared with other groups. The normal control group showed a significant increase ($p < 0.05$) in HDL level when compared with the untreated cyclosporine group. In addition, the group treated with standard anti-hypertensive drug, lisinopril showed a significant ($p < 0.05$) increase in HDL level. Also, the groups treated with 50 mg/kg and 100 mg/kg of avocado seed extract (APE), showed significant increased ($p < 0.05$) in HDL (Figure 2).

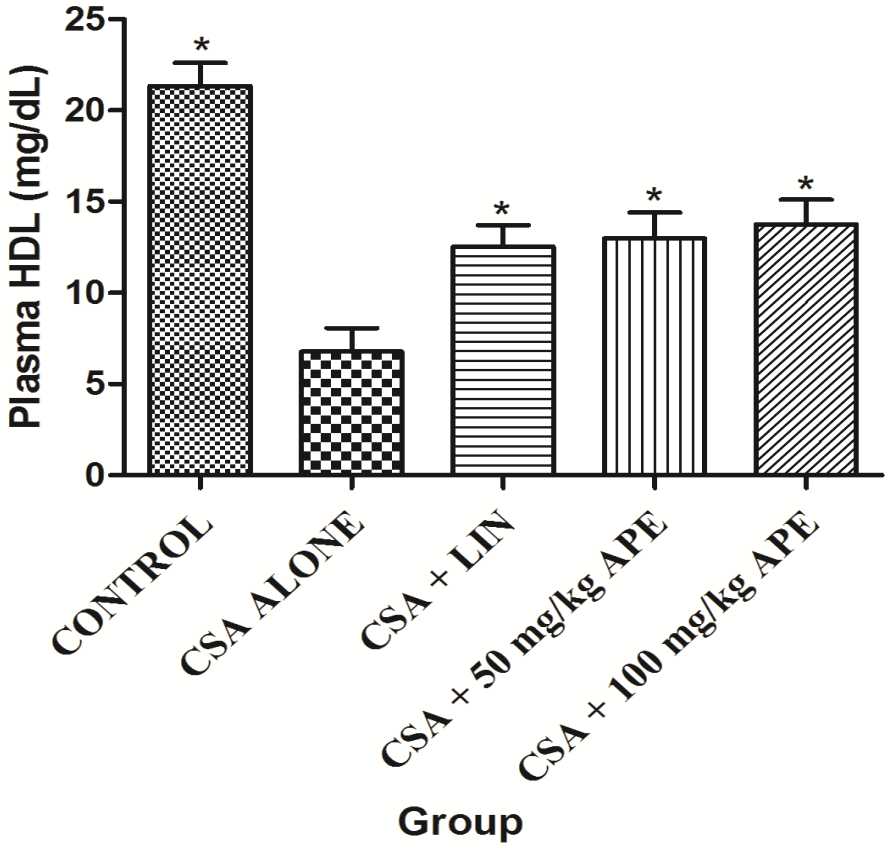


Figure 2: Effect of aqueous extract of avocado seed on the plasma high density lipoprotein (HDL) concentration in cyclosporine treated rats. Bars represent mean ± standard deviation (n=5).*p< 0.05 compared with cyclosporine alone.

The plasma triglyceride level of the group treated with cyclosporine alone increased significantly ($p < 0.05$) compared with the control group. However, treatment with lisinopril and avocado seed extract (100 mg/kg) significantly ($p < 0.05$) lowered the plasma triglyceride level in comparison with cyclosporine treated group. The group treated with 50 mg/kg of avocado seed extract also showed a decrease in triglyceride level but not significantly ($p > 0.05$) different from cyclosporine treated group (Figure 3).

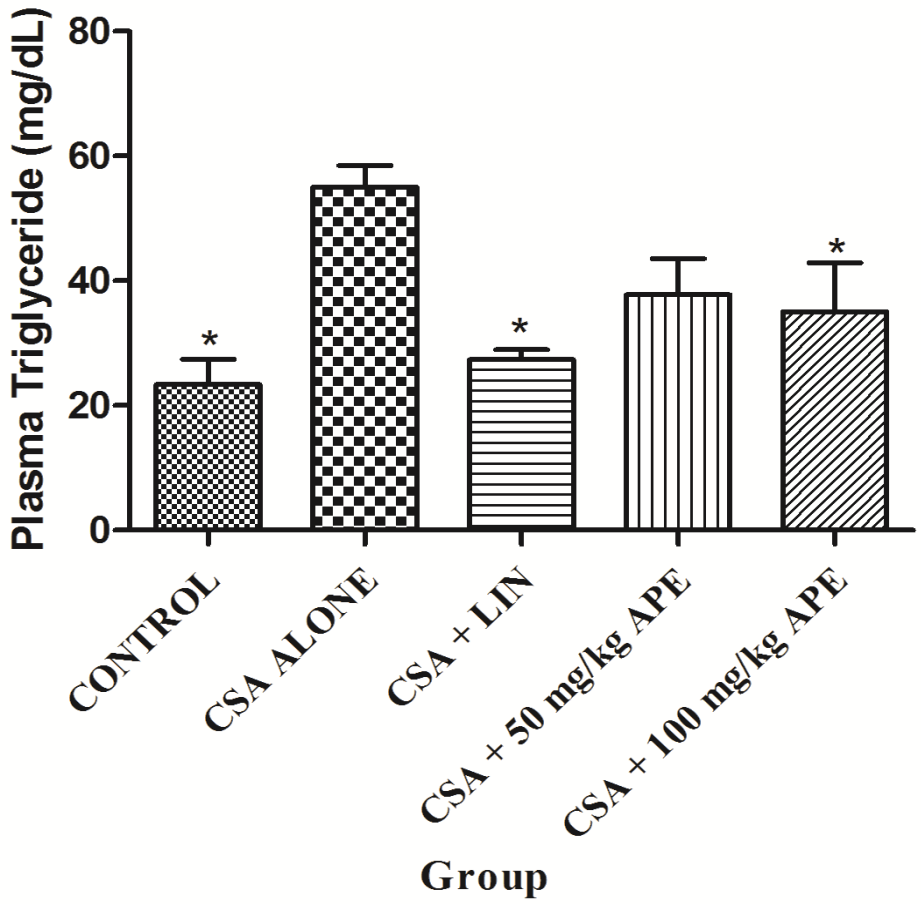


Figure 3: Effect of aqueous extract of avocado seed on the plasma triglyceride concentration in cyclosporine treated rats. Bars represent mean ± standard deviation (n=5).*$p < 0.05$ compared with cyclosporine alone.

In figure 4, the plasma LDL level of cyclosporine treated group increased significantly ($p < 0.05$) when compared with the control group. However, treatment with avocado seed extract and lisinopril significantly reduced plasma LDL level in comparison with the cyclosporine treated group. Avocado seed extract lowered plasma LDL level in a dose-dependent manner.

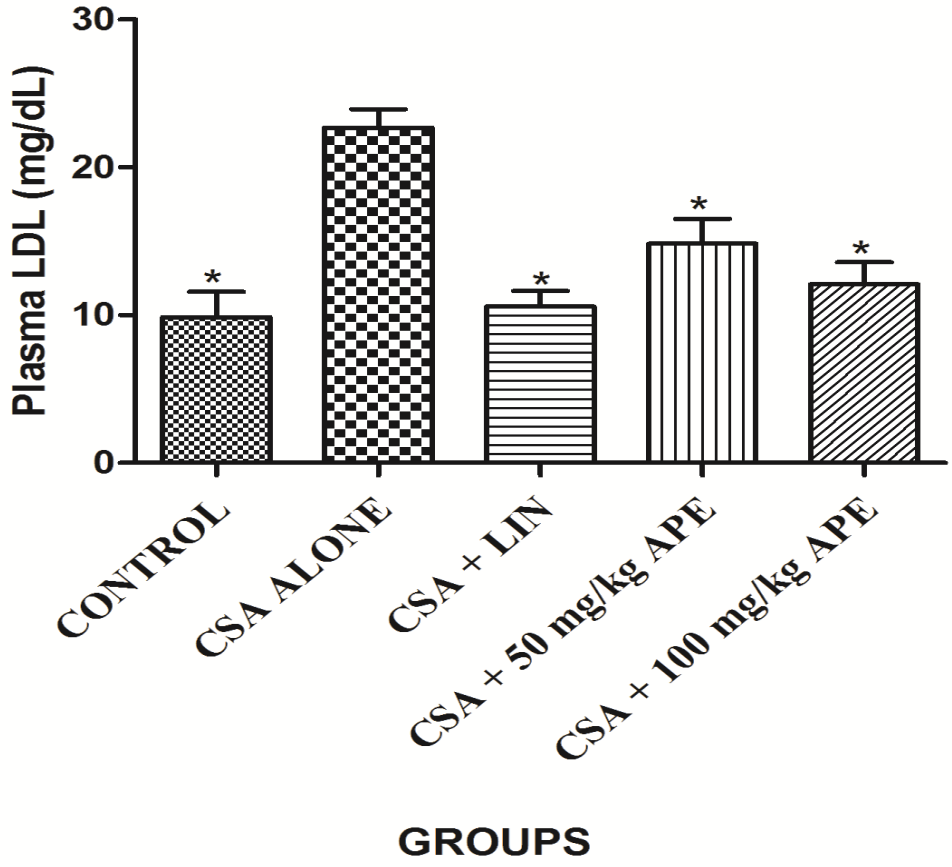


Figure 4: Effect of aqueous extract of avocado seed on the plasma low density lipoprotein (LDL) concentration in cyclosporine treated rats. Bars represent mean ± standard deviation (n=5).*p< 0.05 compared with cyclosporine alone.

Figure 5 showed a statistically significant ($p < 0.05$) increase in atherogenic index of group treated with cyclosporine alone when compared with normal, lisinopril, and extract (50 – 100 mg/kg) treated group. However, there was a significant ($p < 0.0$) reduction in atherogenic index level of the normal control group compared with the group induced with cyclosporine alone. In a similar manner, animals treated with standard drug, Lisinopril also showed a significant difference ($p < 0.05$) in AIP level when compared with CSA alone group. Though, there was a significant ($p < 0.05$) increase in the AIP level of the cyclosporine treated group, however, treatment with 50 mg/kg and 100 mg/kg of avocado seed extract (APE) reversed the increased level of AIP observed in the CSA alone group.

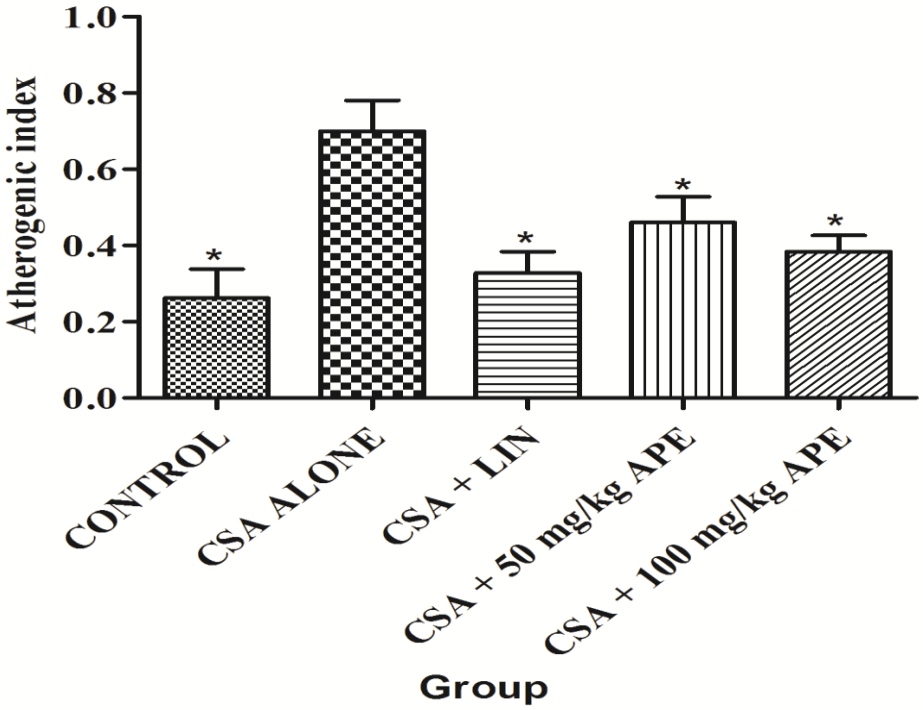


Figure 5: Effect of aqueous extract of avocado seed on atherogenic index in cyclosporine treated rats. Bars represent mean ± standard deviation (n=5).*p< 0.05 compared with cyclosporine alone.

Discussion

Previous studies have reported the antioxidant and antihypertensive properties of Avocado pear *in vitro* (Oboh *et al*., 2016; Odubanjo *et al*., 2016). Besides, the phenolic and flavonoid compositions of Avocado pear seed have been documented (Figueroa *et al*., 2017; Rosero *et al*., 2019). Some of phenolic compounds present in avocado pear are caffeic acid, Vanilin, p-Coumaric acid, sinapic acid, ferulic acid, quercetin, rutin, kaempferol, among others. The main causative factors for hypertension and other cardiovascular diseases are the disturbances occurring in lipid metabolism. Despite the presence of different hypertensive and hypolipidaemic drugs in the market, their therapeutic application is usually associated with severe side effects (Araujo *et al.*, 2018). Hence effort is being made to find safer and more efficient anti-hypertensive and anti-hyperlipidaemic drugs. In that respect, medicinal plants have been considered as promising resources for the discovering of new drugs.

In this study, the effect of aqueous extract of avocado seed (APE) on the lipid profile (TG, TC and HDL-C) was evaluated in the cyclosporine treated rats. Cholesterol is an essential structural element of the biological membranes. In addition, it is the precursor of many compounds such as the starting materials for the synthesis of bile acids, steroid hormones, and vitamins among others. Despite this knowledge, high concentration of serum cholesterol increases the risk of developing CVD (Ramos-Aguilar *et al.*, 2019). This study showed that rats induced with cyclosporine alone showed a higher concentration of serum total cholesterol compared with normal control group. However, avocado seed extract (APE)

reduced the high level of total cholesterol in rats treated with 50 mg/kg and 100 mg/kg. It has been reported that low plasma triglyceride (TG) levels are associated with lower risks of cardiovascular diseases (Hippe *et al*., 2018). This study showed that, the administration of APE to cyclosporine induced rats significantly lowered their serum triglyceride levels.

It is widely accepted that elevation of plasma LDL levels are major risk factors for CVD (Yusuf *et al*., 2020). Direct correlation between LDL and CVD and also the reversibility of the related pathological events by lowering serum level of LDL have already been reported by many research groups (Rekha and Prasad 2016). This study indicated that, the high concentration of LDL-C in cyclosporine induced rats was significantly reduced by administration of APE. Therefore, APE might constitute a good candidate for the treatment and management of CVD by lowering serum LDL-C level. Another risk factor for developing hypertension and other related CVD is the reduced serum level of HDL-C. This effect of HDL is largely attributed to its central function in the reverse cholesterol transport, a process whereby excess cell cholesterol is taken up and processed by HDL particles for further delivery to the liver for metabolism (Berman *et al*., 2019). Therefore, it is logical that an increase in HDL level can contribute to lower risk of CVD (Berman *et al*., 2019). The results of this study showed clearly that APE is capable of increasing the serum level of good cholesterol (i.e. HDL-C) in cyclosporine induced rats treated with 50 mg/kg and 100 mg/kg of the extract.

Atherogenic index of plasma (AIP) is defined as the logarithm (log) of the ratio of plasma concentration of TG to HDL-C. It is a critical index that can be used as a stand-alone index for cardiac risk estimation (Gao *et al*., 2017). In this study, the reduction in AIP showed that aqueous extract of avocado seed at dose 100 mg/kg has the potential of decreasing plasma atherogenic index compared with the rats induced with cyclosporine alone with high AIP level.

Conclusion

The findings presented in this study suggest that avocado seed extract could be considered to have anti-dyslipidemic effect and thus could prevent abnormal lipid metabolism that gives rise to hypertension. Hence, avocado seed could serve as dietary regimen in the management of dyslipidemia that may eventually cause hypertension. However, further studies and clinical trials are recommended.

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