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HYPOLIPIDEMIC AND ANTIOXIDANT EFFECTS OF PETROLEUM ETHER AND METHANOLIC FRACTIONS OF *PERSEA AMERICANA* MILL SEEDS IN WISTAR RATS FED A HIGH FAT-HIGH CHOLESTEROL DIET

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ABSTRACT

Background of study: The efficacy of methanolic and petroleum ether fractions of Avocado pear (*Persea americana* Mill*)* seeds in diet-induced hyperlipidemic rats was studied.

Aim: To determine hypolipidemic and antioxidant effect of petroleum ether and methanolic fraction of seeds of *Persea americana* on diet-induced hyperlipidemic rats.

Methodology: Eleven groups of five rats each were employed which included normal control, the high fat diet (HFD) control group, HFD + 0.2mg/kgbw atorvastatin, and groups treated with different doses of methanolic or petroleum ether fractions for four weeks.

Result: Quantitative phytochemical analysis revealed significantly ($p<0.05$) higher quantities of saponins, flavonoids, and phenols in the methanolic fraction. HFD caused significant increases in the total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), triacylglycerols, total lipids, total phospholipids, and the atherogenic index (LDL-c/HDL-c and TC/HDL-c); while it caused significant decrease in high density lipoprotein cholesterol (HDL-c). These effects were prevented in the fractions treated groups. There was a significant ($p<0.05$) increase in thiobarbituric acid reactive substances and significant ($p<0.05$) decrease in organ catalase and superoxide dismutase activities observed in the liver and kidney homogenate in the HFD control group. Serum alanine aminotransferase showed no significant ($p>0.05$) differences between the normal and HFD control groups, while there was significant ($p<0.05$) decrease in alkaline phosphatase level.

Conclusion: The serum urea level decreased ($p<0.05$) significantly in the treatment groups. The petroleum ether and methanolic fractions of *Persea americana* seeds have been shown to have hypolipidemic and hypocholesterolemic potentials as well as *in vivo* antioxidant activity in diet-induced hyperlipidemic rats.

Keywords: Antioxidant, High-fat-high-cholesetrol-diet**,** Hypercholesterolemic, Hyperlipidemic, *Persea americana*

INTRODUCTION

Hyperlipidemia is a risk factor for cardiovascular diseases which is one of the leading causes of mortality and morbidity in humans (Krieger, 1998). Hypercholesterolemia is the presence of high levels of cholesterol in the blood (Ghanta and Asthana, 1995), and it is closely related to the terms-“hyperlipidemia”and"hyperlipoproteinemia" (Durrington, 2003). *Persea americana*, otherwise known as the Avocado pear, Mexican avocado and so on, is a medium-sized, singlestemmed, terrestrial, erect, perennial, deciduous tree 15–20m

in height. Although a native of Central America (Mexico), it is now found in most tropical and subtropical countries of the world. In many parts of Africa, the fruits of the avocado are much sought after by humans and some animals as valuable foodstuff. The aqueous seed extract of *Persea americana* Mill (Lauraceae) is used by herbalists in Nigeria for the management of hypertension. Products of the plant have been effectively used for the management, control and/or treatment of amenorrhoea, anaemia, insomnia, hyperlipidaemia, diabetes mellitus, diarrhoea, dysentery, gastritis, peptic ulcers, bronchitis, cough, hepatitis, and so forth (Ross, 1999). In this study, the hypolipidemic effect and *in vivo* antioxidative capacities of petroleum ether and methanolic fractions of *Persea americana* seeds were investigated.

AIMS AND OBJECTIVES

Primary Aim: The aim is to investigate the hypolipidemic and antioxidant effects of petroleum ether and methanolic fractions of *Persea americana seed* in experimental animal paradigms fed a high-fat-high-cholesterol diet with a view to providing a pharmacological justification (or otherwise) for the ethnomedical uses of the plant seeds in the management, control and/or treatment of and certain cardiovascular disorders in some rural African communities.

Secondary objectives: To carry out the quantitative phytochemical analysis of petroleum ether and methanolic fractions of the seeds of *Persea americana* and to assess the effects of the extracts of *Persea americana* on hepatic and renal function markers in the serum of the diet-induced hyperlipidemic rats.

RESEARCH METHODOLOGY

Chemicals: All assays kits were purchased from Randox laboratories Ltd. Ardmore and every other chemical used was of analytical grade.

Plant material: Seeds of *Persea americana* were collected from Kafanchan, Kaduna State, Nigeria in June, 2011. The plant was identified at the herbarium unit of Biological Sciences Department Ahmadu Bello University, Zaria where a voucher specimen with number 992 was deposited. The powdered sample was extracted with petroleum ether ($60\text{-}80^0$c), and then extracted with 100% methanol and the extracts were used.

Quantitative determination of the phytochemical constituents

The quantitative phytochemical screening of the petroleum ether and methanol fractions of *Persea americana* was carried out. Total phenols was determined using spectrophotometric method, alkaloids determined using method described by Harborne, (1973). Tannins by Van-Burden and Robinson (1981) method, saponin by Obadoni and Ochuko, (2001) method and flavonoids Boham and Kocipai, (1994) method.

Animals

A total of 55 healthy Wistar strain albino rats of both sexes weighing between 150 – 200g were obtained from the Animal house, Department of Pharmacology, Ahmadu Bello University, Zaria. They were kept in well aerated laboratory cages in the animal house and were acclimatized for two weeks. They were fed with Grower's mash from Vital Feeds Company Plc. Jos and water was provided *ad libitum*. Ethical clearance was obtained from the Health Committee on Ethical Clearance of the Institution.

Formulation of high-fat-high-cholesterol diet

The feed used was commercial Grower's mash (Vital Feeds, Jos) and it contains 7%fat, 15%protein, 10%fiber, 1.0%calcium, 0.35%phosphorus, and 66.65%carbohydrate. The high-fat-diet (HFD) was formulated by adding Baker's fat (Unilever Nigeria plc) and cholesterol (LAB-TECH-CHEMICAL, Australia) to aforementioned feed to obtain the following composition; 22.71%fat, 12.23%protein, 8.15%fiber, 0.82%calcium, 0.29%phosphorus, 54.32%carbohydrate and 1.5%cholesterol.

The median lethal dose (LD_{50}) of the plant fraction was conducted using the method described by Lorke, (1983).

Experimental design

The rats were divided into 11 groups of 5 rats each and were treated for four weeks as follows: Group 1 rats were fed on normal feed *ad libitum* and acted as normal control. Group 2 rats were given high fat- high -cholesterol diet (HFD) with no further treatment (HFD control). Group 3 rats were fed with HFD *ad libitum* and given daily 200µg/kg body weight of standard hypolipidemic drug (Atorvastatin). Groups 4-6 were given HFD along with daily oral 125mg/kg, 250mg/kg and 500mg/kg doses of the methanolic fraction respectively. Groups 8-0 were given HFD along with daily oral 125mg/kg, 250mg/kg and 500mg/kg doses of the petroleum ether fraction respectively. Group 7 and 11 rats were fed normal rat and given daily 500mg/kg body weight of methanolic and petroleum ether fraction, respectively.

Sample collection

At the end of four weeks of treatment, the animals were anaesthetized with chloroform vapour, and blood samples were drawn from the heart into plain tubes, this was allowed to clot and the serum separated by centrifugation. Liver and the kidney homogenates were used for antioxidant and lipid peroxidation analysis.

Analyses

Aspartate and alanine-aminotransferases, alkaline-phosphatase, albumin, total and direct bilirubin, creatinine and urea in the serum Total cholesterol, high density lipoprotein-cholesterol (HDL-c) as well as triacylglycerols (TG) in the serum were done using commercial reagent kits. Total lipids and phospholipids concentration in blood were assayed using methods described by Stroev and Makarova, (1989). The serum level of low-density-lipoprotein-cholesterol (LDL-c) was calculated according to the protocol of Friedewald, (1972), using the equation: LDL-c =TC-TG/5–HDL-C. Atherogenic ratios were calculated using the formulae: TC/HDL-C and LDL-C/HDL-C. Superoxide dismutase was determined by method of Fridovich, (1978) and catalase was determined using method described by Sinha, (1972). Reduced glutathione was determined using Ellman, (1959) method and lipid peroxidation was assessed by assaying malondialdehyde as described by Ohkawa et al, (1979).

Statistical analysis

Data were expressed as mean ± S.D. Statistical analyses were performed with one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test using SPSS 17 statistical package. The value of $p<0.05$ was considered as significant.

RESULTS

The phytochemicals quantified are presented in Table 1 and the methanolic fraction had significantly ($p<0.05$) higher concentrations of saponins, flavonoids and phenols than the petroleum-ether fraction. The two fractions were not toxic at a dose of 5000mg/kg body weight.

The daily feed-intake of the rats fed the HFD was not statistically different from that of those fed the normal feed; however the HFD group recorded a significantly ($p<0.05$) higher increase in body weight (Table 2). Though the administration of the methanolic or petroleum ether fractions to HFD rats had no effect on the daily feed-intake it had the effect of significantly ($p<0.05$) reducing the magnitude of increase in body weight caused by the HFD. The standard drug (Atorvastatin); used as positive control had similar effect as the fractions. There was no significant ($p>0.05$) difference in the relative liver and kidney weights of all treatment groups.

The HFD caused significant ($p<0.05$) increases in the levels of serum total cholesterol, LDL-cholesterol, triacylglycerols, total lipids, phospholipids and atherogenic ratios; but significantly ($p<0.05$) reduced serum HDL-cholesterol levels (Table 3). Administration of the

methanolic extract significantly ($p<0.05$) prevented the diet-induced increases in the lipid parameters mentioned above in a dose-dependent manner; while it led to significant ($p<0.05$) increase in the HDL-C in a similar manner. The petroleum ether fraction had similar effects on all these parameters in HFD rats. The results indicated that the fractions were significantly ($p<0.05$) better at correcting the diet-induced dyslipidemia than the standard drug used, at the doses used (Table 2).

Administration of either of the two fractions at the three doses used, to the HFD rats prevented the HFD-induced significant ($p<0.05$) increases in liver and kidney TBARS (Table 3). Although the HFD had no significant ($p>0.05$) effect on the levels of liver and kidney reduced gluthathione (GSH); administration of the two higher doses of methanolic fraction to HFD rats significantly ($p<0.05$) boosted the levels of GSH in the kidney above normal values. The three doses of the petroleum ether fraction had a similar effect on kidney GSH of HFD rats. The liver GSH was however, not affected by the petroleum ether fraction.

There was a general significant ($p<0.05$) decreases in liver and kidney catalase and SOD activities in the HFD control rats (Table 3). Administration of the methanolic fraction to HFD rats restored the catalase activity in the liver to values recorded in rats fed normal feed; in the kidney, however, only the highest dos had a similar effect. The diet-induced decreases in liver and kidney SOD were prevented by only the 500mg/kg dose of the methanolic fraction. The petroleum ether fraction had a similar effect on liver catalase activity at the 250mg/kg and 500mg/kg doses only and similarly affected the kidney catalse activity only at the 500mg/kg dose. The HFD-induced fall in liver and kidney SOD activity was completely prevented by all the three doses of petroleum ether fraction used. The effects of the two fractions on oxidative stress markers were comparable to that of the standard drug used.

The levels of serum AST, DB and TB were all significantly ($p<0.05$) increased above normal in the HFD control, while the serum ALT, albumin and creatinine were unaffected and serum urea and ALP levels fall significantly ($p<0.05$) below normal values (Table 3). Administration of the methanolic or petroleum ether fractions to HFD rats not only prevented the diet-induced rise in serum AST, it brought the levels to values significantly ($p<0.05$) below those recorded in rats feed a normal diet. The diet-induced increases in Direct Bilirubin and Total Bilirubin were prevented by the two fractions in HFD rats. When either of the two fractions were given to the HFD rats they caused further significant ($p<0.05$) decreases in serum urea compared to the HFD controls.

DISCUSSION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal values of some plants lie in some chemical substances that produce definite physiological actions in the human body. The most important of these bioactive constituents are alkaloids, tannis, flavonoids and phenolic compounds. Flavonoids are known for their diverse biological activities including hypolipidemic activity (Afanas'ev et al, (1995). Flavonoids and phenols found in the methanolic and petroleum ether fractions could therefore be considered favourable in increasing HDL-C concentration and decreasing LDL-C levels in fractionts-treated HFD rats.

In this study, the extracts of *P. americana* seeds also reduced the magnitude of increase in the body weight of the HFD rats. Abnormally high dietary cholesterol levels lead to hypercholesterolemia, which is strongly associated with cardiovascular disease because it promotes atherosclerosis (Durrington, 2003). In this study, methanolic and petroleum ether fractions of *Persea americana* significantly reduced serum concentrations of total cholesterol, LDL-c and triacylglycerols in lipid

induced hyperlipidemic rats. It also caused a significant increase in HDL-c concentration. Kolawole et al, (2012) reported that methanolic extract of *Persea americana* leaf decreased the level of total cholesterol, triacylglycerols, low density lipoprotein while there was an increased in high density lipoprotein cholesterol in hyperlipidemic rats. Atherogenic ratio is an important prognostic marker for cardiovascular disease. The risk of cardiovascular diseases increases considerably when the ratio is high (Laboratoires Fournier, 1981). In this study, the TC/HDL-c and LDL-c/HDL-c ratio were high in the HFD control group. This was significantly reduced in a dose dependent way in the fractions treated HFD rats.

Also, there was a significant increase in lipid peroxidation in the hyperlipidemic animals. The levels of TBARS in liver tissues of HFD control rats were significantly elevated when compared to the level of TBARS in normal control animals. The administration of *Persea americana* seeds fractions caused significant reduction in TBARS level. The catalase and superoxide activities were reduced in HFD control group in this study, but when the fractions were administered, the catalase activity was increased, and the SOD activity was also increased in the groups given the highest dosages of the fractions. The reduced gluthathione also showed an increase in the fractions administered groups. This suggests that the fractions may possess *in vivo* antioxidant effects. Asaolu et al, (2010) had reported *in vitro* antioxidant properties of methanolic extract of *Persea americana* leaves. This is most likely the case because isolation of bioactive phytoconstituents from the leaves of *Persea americana* has produced compounds with antioxidant properties such as lutein, rutin, quercetin and apigenin (Owolabi et al, 2010).

The enzymatic activity of alanine aminotransferase (ALT) aspartate aminotransferases (AST) and alkaline phosphatase (ALP) in the serum were studied to evaluate liver function. AST of the HFD control group was significantly increased when compared to the normal control. But the extracts caused a decrease in the activity of this enzyme. The standard drug used also, caused similar effect. Although there was no statistical difference in the ALT activity of the HFD control group and the normal control group, the group given HFD and 500mg/kg body weight of petroleum ether fraction showed a significant decrease in the activity. There was decrease in ALP concentrations in all the groups. This shows that fractions may possess hepatoprotective properties.

Atorvastatin which was used as positive control in this study works by inhibiting 3-hydroxy-3-methylglutaryl coenzyme- A reductase (HMG-CoA reductase) an enzyme found in liver tissue that plays a key role in production of cholesterol in the body. HMG-CoA reductase reduces serum triglyceride levels through the modulation of apolipoprotein lipase. Rats treated with atorvastatin showed marked reduction in LDL-C and triacylglycerols and increase in HDL-C level as compare with high-fat-high-cholesterol diet group.

The active principle in the fractions used may have acted by reducing the biosynthesis of cholesterol by inhibiting the activity of HMG-CoA reductase. The fraction-induced hypocholesterolemia observed in this study may also be attributed to the presence of saponins in *Persea americana* seeds. Saponins lower blood cholesterol by binding with cholesterol in the intestinal lumen thereby preventing its absorption (Kamal, 2009). It may also bind with the bile acids causing a reduction to the enterohepatic circulation of the bile acids and increase in fecal excretion of cholesterol (Sidhu and Oakenful, 1986).

CONCLUSION

The petroleum ether and methanolic fractions of *Persea americana* contains phytochemicals such as flavonoids, tannins, phenols, alkaloids and

saponins. High-fat-high-cholesterol diet induced hyperlipidemia, hypercholesterolemia and oxidative stress in wistar rats. The administration of petroleum ether and methanolic fractions of *Persea americana* seeds was shown to reduce the level of hyperlipidemia and oxidative stress in HFD wistar rats. The fractions had led to improvement of the antherogenic ratio of hyperlipidemic wistar rats. The result of the present study suggests that fractions of *Persea americana* seeds may be used in management of lipid related disorders.

Declaration on conflict of interest: There is no conflict of interest declared.

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Table 1: Phytochemical Contents (g/100g) of the Fractions of the Seeds of *Persea Americana* Seeds

Extract	Saponins	Tannins	Flavonoids	Phenols	Alkaloids
Petroleum	4.30±0.58[a]	17.60±0.80[a]	12.80±2.43[a]	10.83±1.04[a]	5.60±0.40[a]
Methanolic	10.30±0.58[b]	21.07±2.76[a]	74.30±5.20[b]	27.83±2.47[b]	7.60±0.60[a]

Data are expressed as means ± standard deviation in triplicates. Values with different superscripts down the columns are significantly ($p<0.05$) different.

Table 2: Effects of Petroleum Ether and Methanolic Fractions of *Persea americana* Seeds on Relative Organs Weight (Row), Daily Feed Intake, Serum Levels of Lipids and Atherogenic Ratio of Diet-Induced Hyperlipidemic Wistar Rats

	NC	HFD	HFD+A	HFD+125M	HFD+250M	HFD+500M	ND+500M	HFD+125P	HFD+250P	HFD+500P	ND+500P
ROW-OF LIVER	4.3±0.5^{ab}	4.4±0.5^{ab}	4.2±0.4^{ab}	4.2±0.5^{ab}	4.6±0.5^{b}	4.1±0.3^{ab}	4.3±0.6^{ab}	3.7±0.3^{a}	4.3±0.5^{ab}	4.9±0.5^{b}	3.8±1.2^{a}
ROW-OF KIDNEY	1.2± 0.1^{a}	1.4±0.2^{ab}	1.3±0.8^{a}	1.2±0.1^{a}	1.2±0.2^{a}	1.2±0.1^{a}	1.3±0.1^{ab}	1.3±0.1^{ab}	1.4±0.2^{ab}	1.7±0.4^{b}	1.7±0.4^{b}
DAILY-FEED INTAKE (g/100g/day)	13.4±3.2^{a}	13.6±0.4^{a}	13.1±1.0^{a}	14.1±1.4^{a}	16.0±1.0^{ab}	14.5±1.0^{a}	17.9±2.4^{ab}	13.5±0.9^{a}	16.1±1.5^{ab}	20.8±2.3^{c}	15.8±2.5^{a}
TC(mg/dl)	197.7±15.8^{a}	268.6±30.4^{b}	179.3±27.5^{ac}	179.4±26.8^{ac}	153.9±12.3^{d}	149.2±2.2^{d}	129.4±11.2^{d}	160.3±20.1^{cd}	138.5±24.5^{d}	122.6±7.0^{d}	93.7±8.0^{e}
TG(mg/dl)	14.6±0.7^{a}	71.7±4.3^{b}	29.4±4.3^{c}	32.1±8.8^{c}	14.5±3.9^{a}	39.9±4.7^{d}	33.6±8.7^{d}	23.3±3.6^{c}	21.6±7.1^{c}	16.1±2.0^{a}	10.3±3.9^{e}
HDL-C(mg/dl)	45.4±3.5^{a}	20.7±0.7^{b}	26.5±6.5^{b}	19.7±4.3^{b}	46.6±3.7^{a}	50.4±7.9^{a}	75.4±9.7^{c}	33.9± 2.7^{d}	44.5±2.1^{a}	47.7±4.0^{a}	72.8±4.7^{c}
LDL-C(mg/dl)	146.8±16.0^{a}	233.4±30.5^{b}	144.3±31.4^{a}	153.3±26.0^{a}	107.0±12.6^{c}	92.1±8.8^{cd}	56.2±11.2^{d}	115.6±22.6^{c}	79.3±11.3^{d}	71.7±5.8^{d}	12.5±4.0^{e}
Total Lipids(g/l)	5.9±0.1^{a}	13.1±3.0^{b}	5.6±0.7^{ac}	6.0±0.3^{a}	5.3±0.2^{ac}	4.8±0.3^{ac}	5.1±0.3^{ac}	6.03 ±1.0^{a}	5.1±0.2^{ac}	4.2±0.2^{c}	4.5±0.6^{ac}
Phospholipids(g/l)	4.3±0.8^{a}	5.7±0.9^{b}	4.1±1.0^{a}	3.3±0.5^{c}	3.7±0.8^{ac}	3.6±0.7^{a}	3.3±0.46^{c}	4.4±0.6^{ab}	3.5±0.4^{c}	2.8±0.4^{cd}	2.2±0.4^{d}
TC/HDL-C	4.4±0.3^{a}	13.0±1.9^{b}	6.13±1.3^{c}	8.5±0.8^{d}	3.7±0.9^{ae}	3.0±0.5^{e}	1.8±0.5^{fg}	4.8±0.8^{a}	3.1±0.7^{e}	2.6±0.1^{f}	1.3±0.1^{g}
LDL-C/ HDL-C	3.2±0.4^{a}	10.0±3.5^{b}	5.9±2.4^{c}	8.1±2.4^{b}	2.6±0.9^{a}	1.9±0.5^{ad}	0.7±0.5^{ad}	3.2±1.1^{a}	2.0±0.7^{a}	1.5±0.2^{ad}	0.2±0.1^{d}

Table 3: Effects of Petroleum Ether and Methanolic Fractions of *Persea americana* Seeds on some Markers of Oxidative Stress in the Liver(L) and Kidney(K), and Liver and Kidney Function Markers of Diet-Induced-Hyperlipidemic Rats of Diet-Induced Hyperlipidemic Wistar Rats

	NC	HFD	HFD +A	HFD + 125 M	HFD+ 250 M	HFD + 500 M	ND+ 500 M	HFD+ 125 P	HFD+ 250P	HFD+ 500P	ND+ 500P
TBARS-L (µmolMDA/gtissue)	2.14±0.37^{a}	4.73±1.55^{b}	1.66±0.26^{a}	1.34±0.55^{a}	1.45±0.22^{a}	1.91±0.27^{b}	1.69±0.16^{a}	1.89±0.47^{a}	2.62±0.35^{c}	2.11±0.22^{ac}	2.29±0.17^{ac}
TBARS-K (µmolMDA/gtissue)	13.68±4.24^{a}	24.98±5.89^{b}	15.49±5.23^{b}	12.69±3.44^{a}	7.55±1.79^{ac}	8.85±1.07^{a}	7.29±2.92^{ac}	13.65±5.09^{a}	10.29±6.12^{a}	7.26±3.60^{ac}	7.63±0.83^{a}
GSH-L(mg/gtissue)	3.94± 0.09^{a}	4.04±0.13^{ab}	3.92±0.04^{a}	3.92± 0.04^{a}	3.86±0.10^{a}	3.98±0.04^{ab}	3.84± 0.05^{a}	3.86±0.05^{a}	4.10±0.12^{b}	4.14±0.11^{b}	4.04±0.05^{ab}
GSH-K(mg/gtissue)	5.64±0.05^{a}	5.88± 0.16^{a}	5.84± 0.05^{a}	5.80± 0.07^{a}	6.26±0.13^{b}	6.50±0.87^{b}	6.20±0.00^{b}	6.28±0.13^{b}	6.18± 0.04^{b}	6.78± 0.78^{c}	6.02±0.11^{ab}
CAT.L(moles of H_2O_2 /min /gtissue)	0.04±0.02^{a}	0.02±0.001^{b}	0.038±0.01^{a}	0.05±0.01^{a}	0.04±0.02^{a}	0.07±0.01^{c}	0.06±0.01^{c}	0.03±0.01^{ab}	0.08±0.01^{c}	0.06±0.01^{c}	0.06±0.01^{c}
CAT.-K(moles of H2O2 min/gtissue)	0.08±0.00^{a}	0.04±0.01b	0.09±0.03^{a}	0.06±0.01^{b}	0.07±0.03^{b}	0.09±0.03^{a}	0.10±0.02^{a}	0.05±0.02^{b}	0.08±0.01^{ab}	0.11±0.01c	0.11±0.01c
SOD-L(unit/g)	152.0±18.2^{a}	139.9±8.8^{b}	154.4±15.5^{a}	112.8±9.6^{b}	124.5±25.4^{b}	155.2±12.7^{a}	197.6±6.9^{c}	157.1±8.5^{a}	166.6±24.8^{a}	194.5±9.5^{c}	172.9±16.7^{ac}
SOD-K(unit/g)	242.1±12.8^{a}	228.2±37.0^{b}	54.1±12.3^{c}	237.0±41.6^{ab}	260.7±19.8^{a}	289.3±44.9^{d}	247.1±23.5^{a}	270.2±11.7^{ad}	266.1±20.8^{a}	297.6±48.9^{d}	257.8±16.4^{a}
ALP(nmol/min)	213.6±36.0^{a}	127.0±29.8^{bc}	80.6±13.4^{d}	181.5±34.5^{ae}	58.0±18.61^{d}	82.1±11.4^{d}	106.6±74.0^{c}	131.4±18.1^{bc}	115.9±45.1^{bc}	353.3±44.4^{f}	112.5±32.8^{bc}
ALT(U/L)	37.1±2.7^{a}	35.2±4.6^{a}	34.8±2.2^{a}	26.4±3.1^{b}	29.4±3.3^{b}	45.5±3.5^{c}	50.1±5.0^{d}	42.3±4.3^{c}	37.1±4.2^{a}	26.3±3.1^{b}	17.8±2.7^{e}
AST(U/L)	51.0± 4.1^{ab}	55.8± 6.5^{b}	48.4± 10.^{5ab}	33.6±7.1^{cd}	26.8±5.4^{c}	34.7±2.3^{d}	40.8±4.7^{e}	40.8±3.7^{e}	50.1±2.7^{ab}	27.8±3.9^{cd}	45.7±3.5^{a}

ALBUMIN(g/dl)	4.6±0.3^{a}	4.5± 0.9^{a}	4.5±0.6^{a}	4.3± 0.8^{a}	4.6±0.4^{a}	4.9±0.4^{a}	4.2± 0.9^{a}	4.8±0.5^{a}	4.5±0.1^{a}	4.2±0.7^{a}	4.1±0.8^{a}
D.B(mg/dl)	1.2± 0.2^{a}	2.8±0.4^{b}	1.0±0.2^{a}	2.1± 0.9^{c}	3.3±0.4^{d}	2.4± 0.3^{bc}	1.8±0.4^{e}	1.3±0.1^{ae}	2.4±0.8^{bc}	0.4±0.1^{f}	1.1±0.2^{a}
T.B(mg/dl)	1.8±0.1^{ae}	2.7±0.1^{b}	2.1± 0.2^{ac}	2.2±0.7^{d}	2.8±0.3^{b}	2.2±0.2^{cd}	2.1±0.5^{a}	1.6±0.1^{e}	2.6±0.3^{d}	2.1±0.1^{a}	2.1±0.1^{a}
UREA(mg/dl)	45.56±5.19^{a}	30.46±4.98^{b}	12.78±4.57^{c}	8.40±1.72^{ce}	31.03±3.83^{b}	25.42±2.26^{d}	24.73±4.00^{d}	8.38±2.67^{ce}	9.75±1.12^{c}	12.55±2.42^{c}	4.84±0.61^{e}
CREATININE (mg/dl)	0.36±0.07^{a}	0.65±0.11^{ab}	0.97±0.21^{c}	0.42± 0.08^{a}	0.55± 0.16^{ab}	1.42±0.28^{d}	1.53±0.24^{d}	1.66±0.56^{d}	0.50±0.07^{a}	0.83±0.23^{b}	0.67±0.05^{ab}

Values with different superscripts along the same row are significantly ($p<0.05$) different.

NC= Normal control, HFD= High-fat high-cholesterol diet, A=Atorvastatin, ND= Normal diet, M= mg/kgbody weight of methanolic fractions, P= mg/kgbody weight of petroleum ether extracts. Values are mean ± S.D. (n=5).