Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(8); 1372-1376

ISSN: 0975-4873

Research Article

Evaluation of Anti-Cholesterol Activity of Ethyl Acetate and N-Hexane Extracts of *Tetracarpidium conophorum* (Mull. Arg.) Hutch and Dalziel (African Walnut) Towards Hypercholesterolemic Rats

Ezealisiji K M[1*], Stanley C N[2], Ekanem E S[1]

[1]*Department of Pharmaceutical and Medicinal Chemistry, University of Port Harcourt, Nigeria.*
[2]*Department of Pharmaceutical Microbiology and Biotechnology University of Port Harcourt Nigeria*

Available Online:10th August, 2016

ABSTRACT

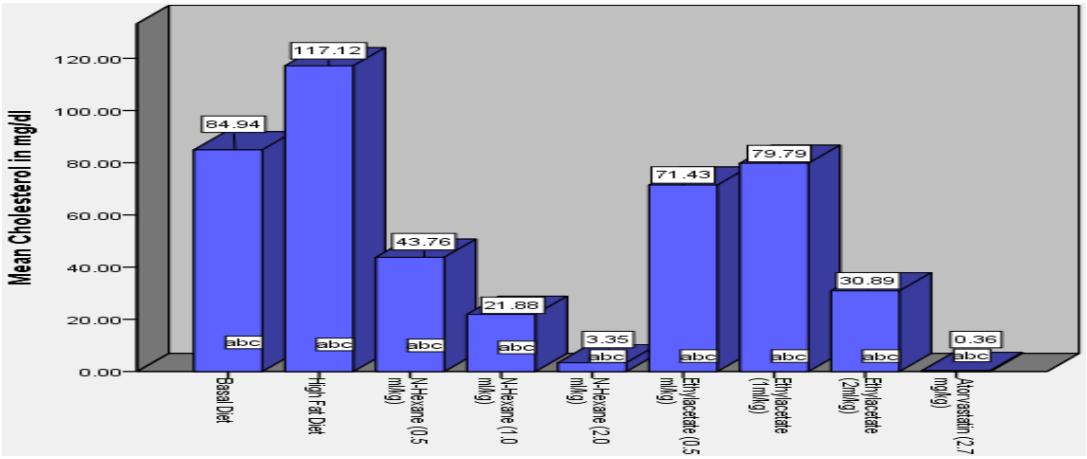
The main risk factor of atherosclerosis which is directly related to coronary heart disease is Hypercholesterolemia and this is the most common cause of death in the developing world. Ethno-medical claim on *Tetracarpidum conophorum* is that it is used in the treatment of hypertensive patients. Also, previous studies have shown that the seed of *Tetracarpidum conophorum* has antioxidant properties. The present research was aimed at determining the anticholesterol activity of n-hexane extract and ethyl acetate fractions of *Tetracarpidum conophorum* compared to atorvastatin in decreasing the plasma level of total cholesterol, LDL and triglyceride and increasing the HDL level on hypercholesterolemic rats. Twenty-nine male Wistar rats were divided into nine groups. Group I was fed with standard diet as negative control group and all the other groups were fed with high-fat diet and were also given fructose solution. High-fat diet and fructose solution increased the level of total cholesterol, LDL-cholesterol and triglyceride and decreased the level of HDL-cholesterol significantly compared to the negative control group. The treatment groups were given 3 various doses of (0.5, 1.0, 2.0 ml/kgBW) of crude extract, ethyl acetate and n-hexane fractions respectively and artovastatin 2.7 mg/kgBW daily for ten days. The data were analyzed by one way Analysis of Variance (ANOVA) and Duncan's Post Hoc Test. The best results were shown by 2.0 ml/kgBW of n-hexane extract fraction in decreasing total cholesterol, LDL-cholesterol and triglyceride as well as increasing HDL-cholesterol when compared with the standard cholesterol lowering agent (artovastatin). Ethyl acetate fraction (2.0 ml/kg) showed significant effect in increasing HDL- cholesterol and decreasing LDL- cholesterol.

Keywords: Anticholesterol; hypercholesterolemia; *Tetracarpidium conophorum*; African walnut.

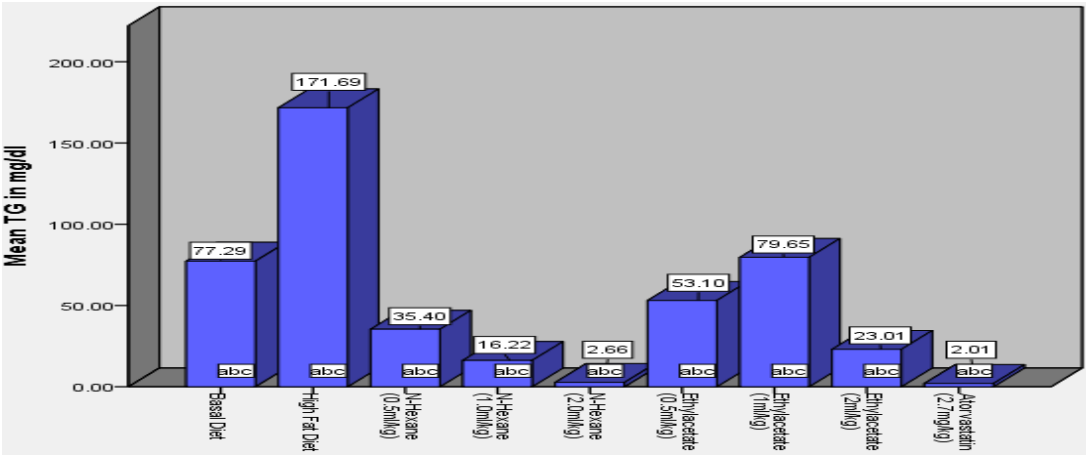
INTRODUCTION

Cardiovascular diseases are the second largest cause of mortality worldwide. Progression of atherosclerosis, which is usually accompanied by the production of free radicals are shown to be caused by elevated levels of cholesterol. The development of coronary heart disease (CHD) have been identified to be associated with the blood lipids such as total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides, since these risk factors play an important role in determining atherogenesis and the subsequent pace of atherosclerosis. According to National Heart, Lung, and Blood Institute (NIH), CHD is a disease in which a waxy substance called Plague builds up inside the coronary arteries. These arteries supply oxygen-rich blood to your heart muscle. This plague when build up in the arteries, results in a condition called atherosclerosis. Hyperlipidemia is the primary precondition for atherosclerosis manifested in premature cardiovascular disability and death as confirmed from epidemiological and clinicopathological studies. Hyperlipidemia is caused by elevated levels of fat in diets, especially saturated fat and cholesterol. The International Atherosclerosis Project, discovered that CHD and stroke are related to plague damage, hence the degree of atherosclerosis was directly proportional to the prevalence of CHD and stroke, and that lipid levels were directly related to plaque damage. The higher the serum cholesterol, the greater the formation of plague[1]. Drugs such as clofibrate, niacin, cholestyramine, statin and gemfibrozil are among the various drugs employed in lowering blood lipids and were successful in reducing serum cholesterol levels, they produced unpleasant and distressing side effects. This was established from the etiological preeminence of hyperlipidemia in CHD[1]. The previous study which examined the antioxidant activity of crude extract and fractions of African walnut in vitro, shows that crude extract of the dried leaves had the best antioxidant activity; the broad range of this extract shows the potential of the plant as a source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress and consequent health benefits[2]. In vitro studies demonstrated that flavonoids are good free radical scavengers. About half of the ingested flavonoids are absorbed into the bloodstream through the gastrointestinal tract lining and half are metabolized to other compounds by gastrointestinal microflora. However, there is variation

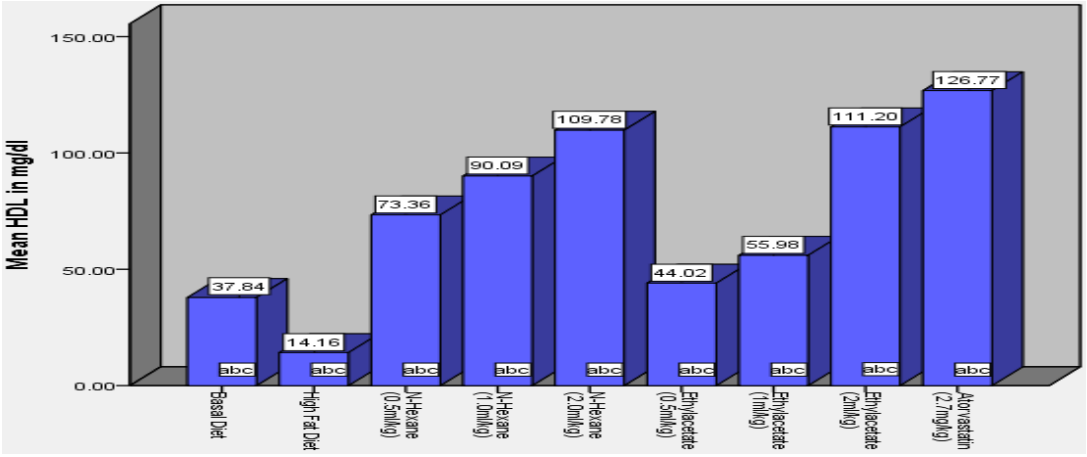
**Author for Correspondence: kenneth.ezealisiji@uniport.edu.ng.*



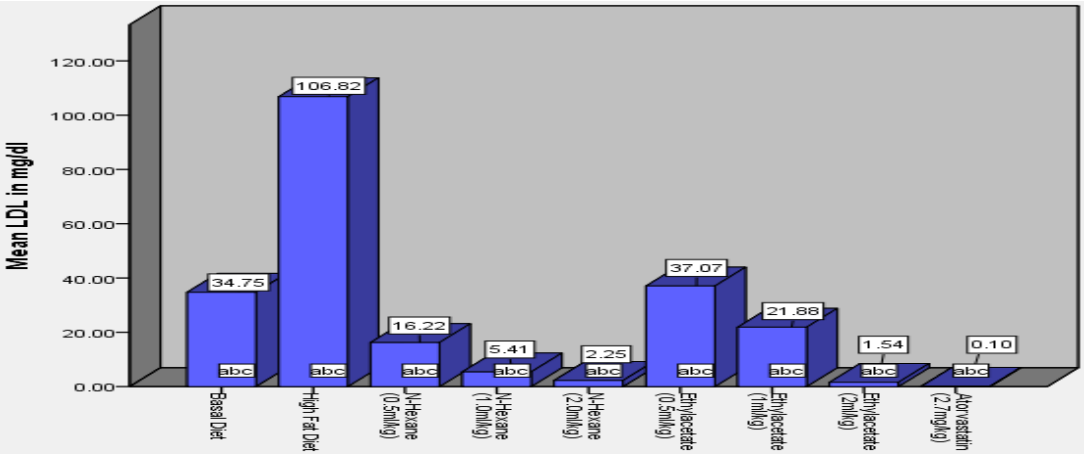
The plasma total cholesterol in hypercholesterolemic rat treated with n-hexane and ethyl acetate fraction of *Tetracarpidium conophorum.* Result are expressed as mean ± SD of three animals per group, Duncan's post Hoc tests are showed by letter a,b,c are significantly different among treatment groups at confidence internal 95%



The plasma triglyceride level in hypercholesterolemic rat treated with n-hexane and ethyl acetate fraction of *Tetracarpidium conophorum.* Result are expressed as mean ± SD of three animals per group, Duncan's post Hoc tests are showed by letter a,b,c are significantly different among treatment groups at confidence internal 95%



The plasma HDL-cholesterol level in hypercholesterolemic rat treated with n-hexane and ethyl acetate fraction of *Tetracarpidium conophorum.* Result are expressed as mean ± SD of three animals per group, Duncan's post Hoc tests are showed by letter a,b,c are significantly different among treatment groups at confidence internal 95%



The plasma LDL-cholesterol level in hypercholesterolemic rat treated with n-hexane and ethyl acetate fraction of *Tetracarpidium conophorum.* Result are expressed as mean ± SD of three animals per group, Duncan's post Hoc tests are showed by letter a,b,c are significantly different among treatment groups at confidence internal 95%.

depending on the flavonoid[3,4]. Free radicals are chemical species that have a singly occupied orbital. They possess unpaired valence electrons, hence are highly reactive towards other substances as well as themselves. Formation of lipid peroxidation within plasma and organellar membranes is as a result of free radicals in the presence of oxygen. Initiation of free radicals could be blocked by antioxidants and terminate radical damage and inhibit lipid peroxidation which was one of the factors implicated in atherosclerosis. Natural antioxidants are safe, causes less adverse reactions and widely used[4,5]. Since the clinical efficacies of *Tetracapidium conophorum* in lowering the lipids profile is not established, it is important to study the anticholesterol activity of n-hexane, ethyl acetate fractions of *Tetracapidium conophorum* compared to atorvastatin in decreasing the plasma level of cholesterol total, LDL and triglyceride and increasing the HDL level on hypercholesterolemic rats.

MATERIALS AND METHODS

Phytoconstituent Extraction

The unshelled seeds of *Tetracarpidium conophorum* were cut into smaller pieces to enhance drying. Drying was done under shade for one week before the seeds were pulverized using a mechanical grinder. 600 g of the pulverized seeds was defatted using 2.5 L of n-Hexane for 72h and filtered. To the marc, 1.5 L of ethyl acetate was added and macerated for 72 h. This was filtered using a sieve and further separated by means of a separating funnel. The different extracts (ethyl acetate and n-Hexane) were concentrated using rotor evaporated and were further subjected to evaporation using a water bath. A 23.115g (equivalent to 25ml) of ethyl acetate extract and 120 ml (equivalent to 110.952g) of n-Hexane oil extract were recovered and their extractive values were calculated using the following formula:

$$\frac{\text{Weight of extract}}{\text{Weight of pulverized seed}} \times 100 \qquad (1)$$

Animals and Diets

Twenty-nine adult male Wistar rats obtained from Animal house, Department of Agriculture, Choba, University of PortHarcourt were housed in standard cages and provided with food and water ad libitum. The rats were adapted for 7 days until the body weight were 125-175 g. Hypercholesterolemia were induced with high fat diet (5000 g of standard diet mixed with 550 g duck egg yolk, 500 g palm oil, 1250 g beef liver, 750 g beef brain and hot water). The difference between the standard diet and high fat diet is shown in Table 1. In spite of high fat diet, the rats were given fructose liquid 60% (100 g/400 ml aquadest) 1 mL/rat/day. The high fat diet and fructose liquid were given for 2 weeks until the body weight were 150 – 200 g. The rats were divided into 9 groups (n=3) for different treatments. The first group of animals was given standard diet (negative control). The second group of rats was given high fat diet (positive control). The third, fourth and fifth groups of rats were treated with high fat diet plus n-hexane fraction 0.5 ml/kgBW, 1.0 ml/kgBW and 2.0 ml/kgBW daily. The sixth, seventh and eighth groups of rats were treated with high fat diet plus crude extract 0.5 ml/kgBW, 1.0 ml/kgBW and 2.0 ml/kgBW daily. Group nine was treated with high fat diet plus atorvastatin 2.5 mg/kgBW daily. All the treatments were given for 10 days.

Sample Preparation for Lipid Profile Test

After the treatments, 1.5 mL blood from the orbital vein were collected in tubes containing EDTA (heparinized tubes). The samples were centrifuged at 3000 rpm for 10 min and the plasma were used for measuring the total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride level. The total plasma cholesterol and triglyceride were measured according to the instruction manual accompanying the diagnostic kits from Lively Stones Medical Diagnostic Laboratories. High density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol were measured according to the instruction manuals accompanying the diagnostic kits from Lively Stones Medical Diagnostic Laboratories.

Statistical Analysis

Mean and standard deviation (M ± SD) were calculated and 95% confidence interval (CI) of means was used. To compare between groups, analysis of variance (ANOVA) were calculated. P-values of less than 0.05 were considered

Table 1: The nutrient value of standard and high fat diet.

Item	High fat diet	Standard diet
Water (%)	14.20	6.20
Ash (%)	7.18	4.86
Crude protein (%)	20.08	24.12
Crude fiber (%)	10.02	14.02
Crude fat (%)	30.50	8.09
Carbohydrate (%)	42.42	60.04

as statistically significant. Furthermore, to know the best treatment, Duncan's post-Hoc test 95% confidence interval was used. Statistical analysis was carried out using SPSS 16.0 program.

RESULTS AND DISCUSSION

The present research showed that high fat diet and fructose liquid significantly increased the plasma total cholesterol, LDL-cholesterol and triglyceride, however, the HDL-cholesterol was decreased compared to basal diet. After treatment with various dose of ethyl acetate and n-hexane fractions of *Tetracarpidium conophorum*, and 2.7 mg/kgBW atorvastatin for 10 days, the effect on plasma total cholesterol is as shown in Figure 13. Statistical analysis with ANOVA showed that the treated groups were significantly different (p = 0.000) and Duncan post-Hoc test showed that 2.0ml/kgBW/day of n-Hexane fraction of *Tetracarpidium conophorum*, gave the best result in decreasing the plasma total cholesterol. This is better compared to atorvastatin (patent anticholesterol agent). The effect of treatment observed for LDL-cholesterol is shown in Figure 2. The statistical analysis showed that there was significant difference (p = 0.000) and 2.0ml/ kgBW/day of n-hexane fraction of *Tetracarpidium conophorum* gave the best result in decreasing the LDL-cholesterol compared to atorvastatin. Ethyl acetate fraction at concentration of 2.0ml/kgBW/day showed similar activity to atorvastatin. The effects of the treatments on HDL-cholesterol is shown in Figure 3. There were significant differences with p=0.000 and 2.0ml/kgBW/day n-hexane extract of *Tetracarpidium conophorum* increased the HDL-cholesterol when compared to atorvastatin and other treatments. Ethyl acetate fractions at concentration of 2.0ml/kgBW/day showed similar activity in increasing the HDL-cholesterol level when compared to artovastatin. Figure 4 shows the effect of treatments on triglyceride concentration. Statistical analysis with ANOVA showed a highly significant (p = 0.000) difference and Duncan's post-Hoc test showed that n-hexane fraction 2.0 ml/ kgBW/day of *Tetracarpidium conophorum* had similar activity in decreasing the triglyceride concentration when compared to atorvastatin. The seeds of *Tetracarpidium conophorum* are high in protein, carbohydrates, lipids, fiber and minerals. They are also rich in novel alkaloids, saponins, and sterols. The seeds of *Tetracarpidium conophorum* contains essential fatty acids such as alpha linolenic acid (ω-3 fatty acids), fatty acids such as n-Hexadecanoic acid (palmitic acid) and vitamins such as methyl nicotinate, which all play vital role in lowering cholesterol levels. Phytosterols, which are usually referred to as plant sterol and stanol esters, are a group of naturally occurring compounds found in plant cell membranes. However, because phytosterols are structurally similar to the body's cholesterol, when they are consumed they compete with cholesterol for absorption in the digestive system. As a result, cholesterol absorption is blocked, and blood cholesterol levels reduced. The most common phyosterols in human diet are campesterol, sitosterol and stigmasterol. It is known that phytosterols have a greater affinity for micelles than cholesterol because of their greater hydrophobicity, thereby reducing intestinal cholesterol absorption, and consequently reduce hepatic and plasma cholesterol concentrations. Saponins are also capable of precipitating cholesterol from micelles and interfering with enterohepatic circulation of bile acids making it unavailable for intestinal absorption and hence reduce plasma cholesterol levels. The anticholesterolemia effect of ethyl acetate and n-hexane extract of *Tetracarpidium conophorum* could be due to its high content of flavonoid and high antioxidant activity. Isoflavones could protect the circulating and membrane lipids by sparing endogenous antioxidant[6]. An antioxidants such as flavonoid either blocks the initiation of free radical formation or inactivate (e.g. scavenge) free radicals and terminates radical damage[5]. Lipid peroxidation may occur due to free radicals presence in oxygen. The lipid-free radical interactions yield peroxides, which are unstable and reactive which resulting in extensive membrane, organellar and cellular damage. Free radical could be captured by a scavenger, such as flavonoid, a potent free radical scavengers, even greater than vitamin E[4]. For example, superoxide is unstable and decays (dismutates) spontaneously into oxygen and hydrogen peroxide in the presence of water. However, several non-enzymatic and enzymatic systems contribute to inactivation of free radical reactions. A series of enzymes act as free-radical scavenging systems and break down hydrogen peroxide and superoxide anion. Superoxide dismutase (SOD), which is found in many cell types, can convert superoxide to hydrogen peroxide. Flavonoid as an antioxidant in the ethyl acetate and n-hexane fraction of African walnut could increase SOD activity in hypercholesterolemic rats. Walnuts are rich in linoleic and alpha linolenic acids (ω-3 fatty acids) and in other health-related compounds such as high-biological-value proteins (e.g. arginine) fibre, vitamins, tannins, folates and polyphenols which may provide additional antiatherogenic properties[7]. The omega-3 fatty acids are believed to lower lipids by inhibiting the synthesis of vLDL in the liver. This results in smaller, less-dense vLDL and LDL particles and therefore, a reduced plague producing lipid profile. In 2001, the Adult Treatment Panel (ATP111) of the National Cholesterol Education Program recommended that omega-3 fatty acids be used as adjunct to pharmacological therapy for lowering triglycerides. Walnuts contain polyunsaturated fatty acids, which may protect against cardio vascular disease (CVD) and may enhance tocopherol absorption[8]. n-Hexane and ethyl acetate fraction of *Tetracarpidium conophorum* significantly decreased the total cholesterol, LDL cholesterol and triglycerides and increased the HDL cholesterol in hypercholesterolemic rats.

CONCLUSION

The present study has shown that the extracts of *T. conophorum* had significant anticholesterol activity which could be attributed to the oleic acids (ω-9 fatty acids) and alpha- linolenic acids (ω-3 fatty acids) which are believed to lower lipid levels.

ACKNOWLEDGEMENT

The Authors are grateful to Dr. Ukwueze S E, the Head of Department of Pharmaceutical and Medicinal Chemistry, University of Port Harcourt, Nigeria for using his laboratory.

REFERENCES

1. Temple, N.J. & Burkitt, D.P. 1994. Western Diseases: Their Dietary Prevention and Reversibility. Totowa, New Jersey: Humana Press 170-172.
2. O. U. Amaeze *et.al* 2011. Evaluation of Antioxidant Activity of *Tetracarpidium conophorum* (Mull. Arg) Hutch & Dalziel Leaves.
3. Koshy, A.S., Anila, L. & Vijayalaksmi, N.R. 2001. Flavonoids from Garcinia combagia lower lipid levels in hypercholesterlemic rats. Food Chemistry 72: 289-294.
4. Boik J. 1996. Cancer and Natural Medicine. USA: Oregon Medical Press. 150-156.
5. Kumar V., Abbas A.K. & Fausto N. 2005. Robbins and Cotran Pathologic Basis of Disease, 7th ed. Philadelphia: Elsevier saunders 16-18.
6. Tsai, P.J. & Huang, P.C. 1999. Effects of isoflavones containing soy protein isolate compared with fish protein on serum lipids and susceptibility of low density lipoprotein and liver lipids to in vitro oxidation in hamsters. J.Nutr. Biochem, 10: 631-637.
7. Nus, M., Ruperto, M. & Sa´nchez-Muniz, F.J. Nuts, cardio and cerebrovascular risks. A Spanish perspective. Archivos Latinoamericanos de Nutrición, 2004, 54, 137-148.
8. Jeanes, Y., Hall, W., Ellard, S., Lee, E. & Lodge, J. The absorption of vitamin E is influenced by the amount of fat in a meal and the food matrix. British Journal of Nutrition, 2004, 92, 575-579.