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Research Article

Effect of EDTA on Biochemical Markers of Artherosclerosis in Palm Kernel Oil Diet-Induced Hyperlipidaemic Albino Rats

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Abstract

Ethylene diaminetetraacetic acid (EDTA) is a synthetic amino acid used orally and intravenously to cleanse, detoxify, and remove heavy metals from the body. Atherosclerosis is a complex disease characterized by the accumulation of lipids within the arterial walls that goes on to form plaques causing narrowing, hardening and/or complete blockage of the arteries. This study therefore seeks to determine the protective effect of EDTA on atherosclerosis in diet induced hyperlipidemic rats using markers of lipid profile, inflammation, total antioxidant status (TAS) and histopathological changes. 50 albino rats were randomly divided into five groups (n = 10). Group I was fed with rat chow and water only. Groups II to V were induced with hyperlipidaemia by diet for six weeks. Thereafter, the level of hyperlipidaemia was confirmed in blood samples compared with group I. Group II was terminated to check for plaque formation by histology, while group III, IV and V were treated with 0.5g/kg, 1.0g/kg, and 1.5g/kg dosage of EDTA salt respectively and monitored for changes. The result showed a significant increase in total cholesterol, triglyceride, LDL-C, hsCRP, and calcium level ($p< 0.05$); and significant decrease in HDL-C level and TAS of the groups II to V ($p< 0.05$). Group III to V showed a significant decrease in the total cholesterol, triglyceride, LDL-C, hsCRP, and calcium level ($p<0.05$) and a significant increase in the HDL-C and TAS ($p< 0.05$) when compared with their pre-treatment values. A dose dependent change from baseline was observed for all parameters except for Total Cholesterol which was inverse and LDL-C where maximum change was obtained at a concentration of 1.5g/kg EDTA treatment. This present study supports the hypothesis that EDTA chelation significantly attenuates markers of atherosclerosis in diet induced hyperlipidemic rats.

Key Words: Hypercholesterolemia, Atherosclerosis, EDTA chelation, Rats

INTRODUCTION

Atherosclerosis remains the major cause of death and premature disability of humans in developed societies (Saikia and Lama, 2011). Current prediction estimates that by year 2020, cardiovascular diseases notably atherosclerosis, will become the leading global cause of total disease burden (Saikia and Lama, 2011). Atherosclerosis is a complex disease characterized by the accumulation of lipids within the arterial walls that eventually go on to form plaques which can cause the narrowing, hardening and/or complete blockage of the arteries. It is a disease of the arterial wall that occurs at susceptible sites in the major conduit arteries. It is initiated by lipid retention, oxidation, and modification, which provoke chronic inflammation, ultimately causing thrombosis or stenosis.

There are a lot of risk factors associated with atherosclerosis which may intensify or provoke its onset due to their effects on inflammation and lipids. These risk factors include lipids retention, hypertension, diabetes mellitus, tobacco smoking, stroke, and genetic predisposition. Hypertension follows closely behind lipids on a list of classical risk factors for atherosclerosis. Increasing evidence supports the view that, like atherosclerosis itself, inflammation may participate in hypertension providing a pathophysiological link between these two diseases. Diabetes is yet another risk factor for atherosclerosis of growing importance. The hyperglycemia associated with diabetes can lead to modification of macromolecules, for example, by forming advanced glycation end products (AGE). By binding surface receptors such as RAGE (receptor for AGE), these AGE-modified proteins can augment the production of pro-inflammatory cytokines and other inflammatory pathways in vascular endothelial cells. Libby *et al* (2002). Beyond the hyperglycemia, the diabetic state promotes oxidative stress mediated by reactive oxygen species and carbonyl groups. As in the case of hypertension, inflammation links diabetes to atherosclerosis. Obesity another important cause of atherosclerosis does not only predispose to insulin resistance and diabetes, it also contributes to atherogenic dyslipidemia (Libby *et al*., 2002). High levels of free fatty acids originating from visceral fat reach the liver through the portal circulation and stimulate synthesis of the triglyceride-rich lipoprotein VLDL by hepatocytes. The resulting elevation in VLDL can lower HDL cholesterol by augmenting exchange from HDL to VLDL by cholesteryl ester transfer protein. In this way obesity itself promotes inflammation and potentiates atherogenesis independent of effects on insulin resistance or lipoproteins.

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Chelation is a capture of positively-charged metal ions by a large molecule. A chelating agent is a molecule with at least two negatively charged groups that allow it to form complexes with metal ions with multiple positive charges, such as lead (Trevor, 2007). The chelate thus formed is non-toxic and can be excreted in the urine, initially at up to 50 times the normal rate (Konsett, 2005). The most widely used chelating molecule is Ethylene diamine tetraacetic acid (EDTA), which has the capacity to chelate almost every positive ion in the periodic table. It has a very strong list of preferences, which are called stability constants (formation constant). EDTA is a synthetic amino acid used orally and intravenously to cleanse, detoxify, and remove heavy metals from the body. EDTA chelation is a therapy by which repeated administrations of a weak synthetic amino acid (EDTA) gradually reduce atherosclerotic plaque and other mineral deposits throughout the cardiovascular system by literally dissolving them away. It was approved for use by the Food and Drug Administration (FDA) in 1950. Physicians and alternative medicine practitioners also use EDTA to treat cardiovascular disease to improve circulation, remove plaque and improve oxygen flow to the brain since it was approved by the FDA in 1950 (Lamas *et al*., 2013; Roussel *et al*., 2009; Krishnaiah *et al*., 2007). Researchers first started to notice EDTA in the days during and after World War II when men who worked in battery factories or painted ships with lead-based paint began coming down with lead poisoning from their high exposure in these jobs. EDTA was however found to be extremely effective for removing the lead from the men's bodies as an apparent reduction in symptoms of heart disease in many of these men was observed. When a molecule of EDTA gets near a toxic mineral, such as lead or mercury, it grasps the destructive particle and binds tightly with it, pulling it out of the membrane or body tissue, it was embedded in. Since EDTA is an artificial amino acid, and since the body regards it as a foreign substance, the body eliminates the entire particle (the heavy particle coated with EDTA). Both the EDTA and the toxic substance are excreted by the kidneys (Gordon Gary 1997). EDTA chelation has also been discovered to modestly reduce the risk of adverse cardiovascular outcomes (Gervasio, 2013). This is due to its ability to chelate ectopic or metastatic calcium from atherosclerotic plaques and inhibit cell-mediated LDL oxidation (Arafa *et al*., 2014; Natarajan *et al*., 2003). Some alternative medicine practitioners believe EDTA acts as an antioxidant, preventing free radicals from injuring blood vessel walls, therefore reducing atherosclerosis.

The use of EDTA chelation therapy for the treatment of atherosclerosis is however controversial. Some scientific evidence does not support claims that the therapy is safe. There are concerns that it may produce toxic effects, including kidney damage, irregular heartbeat, and swelling of the veins. It may also cause nausea, vomiting, diarrhea, and temporary lowering of blood pressure. Since the therapy removes minerals from the body, there is a risk of developing low calcium levels (hypocalcemia) and bone damage. Chelation therapy may also impair the immune system and decrease the body's ability to produce insulin.

The development of atherosclerosis is a progressive one, through continuous evolutions of arterial wall lesions which is centered on accumulation of cholesterol-rich lipids and accompanying inflammatory response. These changes have been described in the histopathology of human and experimental animals as plagues (Williams, 2009). The present study aimed at investigating the effects of EDTA on atherosclerosis in diet-induced hyperlipidemic rats using markers of lipid profile, inflammation and histopathological changes.

MATERIALS AND METHODS

Subjects: Fifty albino rats obtained from the animal house of the College of Medicine, University of Ibadan, Oyo state, Nigeria; were used. The rats were kept in cages in a room maintained at 26–29 °C with a 12-hour light-dark cycle to acclimatize, and was allowed free access to food and water ad libitum. The protocol conforms to the guidelines of the National Institute of Health (NIH) publication 85–23, 1985, for laboratory animal care and the University of Ibadan animal use and ethical committee.

Experimental Design: Rats were randomly divided into five groups (n = 10). Group I (control) was fed with rat chow and water only. Group II to V were fed with palm kernel oil at 10ml/kg body weight per day (Mona *et al*., 2006) for 6 weeks in addition to the normal rat chow and water ad libitum.

At the end of the six weeks, blood samples were drawn from the animals to check for hyperlipidemia by comparing groups II to V with the control group (group I). After hyperlipidemia has been confirmed in groups II to V, group II was terminated to check for plaque formation while group III to V were treated with different doses of EDTA salt. Group III received 0.5g disodium EDTA/kg for two weeks, group IV received 1.0g disodium EDTA/kg for two weeks and group V received 1.5g disodium EDTA/kg for one week.

Collection and Storage of Samples: At the end of the experiment, the rats were fasted for 12 h before collection of blood samples. While a total of fifty (50) rats were obtained for this study and divided into five groups of ten (10) rats each, the total number of rats remaining was forty-two (42) in number. The group IV and V treated with EDTA 1.0g/kg and 1.5g/kg reduced to eight (8) and four (4) respectively.

Each rat was anesthetized with chloroform and blood was withdrawn from the orbital sinus of the animals, transferred to a centrifuge tube and allowed to clot. The serum was separated and kept at -20ºC for biochemical analysis. After the blood collection, the animals were sacrificed; the aorta was removed as quickly as possible, fixed in 10 % formalin and prepared for histological examination.

Determination of Total Cholesterol: Total cholesterol was determined by enzymatic endpoint method as described Allen *et al*., (1974). 10µL of standard, control and unknown samples was pipetted into clean glass test tube, 1ml of cholesterol CHOD pap reagents pipette into all test tubes, mixed and then incubated at 370c for 5 minutes. The absorbance of the samples was measured against reagent blank within 60 minutes at 500nm.

Determination of Triglycerides: Triglyceride was determined by spectrophotometric method, based on GPO-PAP methodology (Schettler and Nussel, 1975). All reagents and specimen we brought to room temperature. 10µl of standard, control and unknown sample were pipetted into grease free glass test tube, 1ml of triglyceride GPO-PAP reagent was added, mixed and incubated at 370c for 5minutes. The absorbance of the sample and standard was then measured against reagent blank within 60 minutes at 500nm.

Determination of HDL-Cholesterol
Standard and specimen to be analysed were precipitated by pipetting 200µl of standard and specimens into a test tube and adding 500µl of diluted precipitant. They were mixed and allowed to sit for 10minutes at room temperature. Thereafter, they were centrifuged for 10 minutes at 4000 revolution per minute. The clear supernatant was separated within two hours and the cholesterol content was determined by the CHOD-PAP method of cholesterol. 100µl of supernatant was pipetted into clean grease free test tube; 1ml of cholesterol CHOD-PAP reagent was added, mixed and incubated at 370c for 5 minutes. Finally, the absorbance of the sample/standard was measured against reagent blank at 500nm.

Determination of LDL-Cholesterol
LDL-cholesterol was calculated using the Friedewald equation. Friedewald *et al*., (1972).
LDL-Cholesterol conc. (mg/dL) = (TC-(HDL-C+TG))/5

Determination of High Sensitivity C-Reactive Protein (hs-CRP)
The quantitative determination of hs-CRP concentration was done using enzyme-linked immunosorbent assay.Ridker (2001).

Determination of Serum Calcium
Serum calcium level was determined using colorimetric method. 25µl of standard and sample was pipetted into a test tube. 1ml of working reagent (equal volumes of reagent 1 and 2) was added and mixed. The absorbance of the sample and standard was read against reagent blank after 5 to 50 minutes.

Determination of Total antioxidant:
Total antioxidant level was determined using the method developed by Koracevic*et al*., 2001. Each sample (A1) had its own control (A0, sample blank) in which Fe-EDTA mixture and H2O2 were added after 20% acetic acid. For each series of analysis, a negative control (K1 and K0) was prepared (at least in triplicate), containing the same reagents as A1 or A0, except that serum was replaced with phosphate buffer. Standards containing 1mmol/litre uric acid (UA1 and UA0) are used for calibration. Incubation was done for 10 minutes at 1000C (in a boiling water bath) then it was cooled in an ice bath. Thereafter, absorbance was measured at 532nm against deionised water

Histopathological studies: Basic procedures involving tissue sample fixation, dehydration, clearing, embedding, blocking, sectioning, dewaxing, staining and counter staining with hematoxylin and eosin respectively was carried out to prepare permanent slide for each of the tissue for microscopic examination.

Statistical Analysis: All results were expressed as mean ± SD. The statistical package for the social sciences (SPSS) program, version 22 was used to compare significance between groups using the Student's t-test. Differences were considered significance when $P<0.05$.

RESULTS

Table 1 shows the mean results of all the biochemical parameters of the control and hyperlipidemic rat group after six weeks. There was a significant increase in the serum level of total cholesterol, triglyceride and LDL-C in the hyperlipidemic group (p=0.002, 0.000, 0.002 respectively) and a significant decrease in HDL-C level (p= 0.000). There was a significant difference in both the marker of inflammation (HsCRP) and calcium level (p= 0.006 and p=0.001 respectively). An increase in HsCRP level was observed in the hyperlipidemic group when compared with the control group. A significant decrease was also observed across the groups in the total antioxidant activity (p = 0.000).

Table 2 shows a comparison of the biochemical parameters observed pre and post treatment for the group treated with 0.5g/kg EDTA salt. A significant decrease was observed in the total cholesterol, triglyceride, LDL-C, and serum calcium level, p-value (0.000, 0.000, 0.000, and 0.000) respectively while a significant increase was observed in the HDL-C and total antioxidant activity level with a p-value of 0.000 after the treatment. Percentage change in this group of rat was 30.19%, 12.38%, 73.13%, 3.76%, 25.26% 17.73% and 27.72% in total cholesterol, triglyceride, HDL-C, LDL-C, Hs-CRP, calcium, and total antioxidant respectively above the baseline.

Table 1:
Comparison of biochemical parameters between the hyperlipidemic albino rats and controls (mean ± SD)

Parameter	Hyperlipidemic rats N=40	Control N=10	t value	p-value
Total cholesterol (mg/dl)	143.07±6.17	102.03±1.81	-3.291	0.002
Triglyceride (mg/dl)	104.03±4.64	65.63±1.46	-4.098	0.000*
HDL-C (mg/dl)	35.48±1.34	49.58±1.29	5.104	0.000*
LDL-C (mg/dl)	115.17±5.86	75.99±3.90	-3.277	0.002*
Hs-CRP (mmol/l)	1.43±0.04	1.21±0.12	-2.875	0.006*
Serum Calcium (mg/dl)	7.60±0.34	5.27±0.17	-3.407	0.001*
Total Antioxidant (mmol/l)	0.83±0.03	1.18±0.04	6.194	0.000*

*p-value is significance at $p<0.05$

Table 2:
Comparison of biochemical parameters pre- and post-treatment with 0.5g/kg EDTA (mean ± SD)

Parameter	Post-treatment (N=10)	Pre-treatment (N=10)	t value	p-value
Total cholesterol (mg/dl)	144.71±3.96	207.29±4.17	27.236	0.000*
Triglyceride (mg/dl)	102.50±1.79	116.98±7.63	6.946	0.000*
HDL-C (mg/dl)	55.61±2.99	32.12±1.53	-16.631	0.000*
LDL-C (mg/dl)	113.08±3.59	177.50±5.20	27.312	0.000*
Hs-CRP (mmol/l)	1.45±0.24	1.94±0.11	9.686	0.000*
Serum Calcium (mg/dl)	8.54±0.42	10.38±0.99	6.201	0.000*
Total Antioxidant (mmol/l)	1.29±0.56	1.01±0.08	-7099	0.000*

*p-value is significance at p<0.05

Table 3:
Comparison of biochemical parameters pre- and post-treatment with 1.0g/kg EDTA (mean ± SD)

Parameter	Post-treatment (N=8)	Pre-treatment (N=8)	t-value	p-value
Total cholesterol(mg/dl)	96.35±2.84	114.92±4.59	10.883	0.000*
Triglyceride (mg/dl)	69.91±5.05	92.13±10.33	5.051	0.001*
HDL-C (mg/dl)	42.14±3.04	25.42±2.04	-22.401	0.000*
LDL-C (mg/dl)	73.94±4.11	91.41±6.47	7.181	0.000*
Hs-CRP (mmol/l)	1.53±0.38	2.08±0.33	2.588	0.036*
Serum Calcium (mg/dl)	4.33±0.49	5.82±0.25	9.138	0.000*
Total Antioxidant (mmol/l)	1.34±0.06	0.87±0.11	-10.051	0.000*

*p-value is significance at p<0.05

Table 4: Comparison of biochemical parameters pre- and post-treatment with 1.5g/kg EDTA (mean ± SD)

Parameter	Post-treatment (N=6)	Pre-treatment (N=6)	T-value	P-value
Total cholesterol (mg/dl)	117.84±1.65	136.62±2.67	11.326	0.000*
Triglyceride (mg/dl)	76.73±4.79	132.39±19.58	7.162	0.001*
HDL-C (mg/dl)	85.18±3.19	38.86±2.33	-21.101	0.000*
LDL-C (mg/dl)	85.46±1.89	102.37±1.54	24.491	0.000*
Hs-CRP (mmol/l)	1.47±0.21	2.75±0.09	23.600	0.000*
Serum Calcium (mg/dl)	5.75±2.02	8.12±1.63	7.993	0.000*
Total Antioxidant (mmol/l)	1.39±0.26	0.79±0.22	-4.620	0.006*

*p-value is significance at p<0.05

The biochemical parameters changes observed pre and post treatment with 1.0g/kg EDTA salt are shown in Table 3. There was a significant decrease in the total cholesterol, triglyceride, LDL-C, CRP and serum calcium level with a p-value of 0.000, 0.001, 0.000, 0.036, and 0.000 respectively while a significant increase was observed in the HDL-C and total antioxidant activity level with a p-value of 0.000 at the end of the treatment. A percentage shift of 16.16%, 24.12%, 65.78%, 19.11%, 26.44%, 25.60% and 54.02% was induced in the serum level of total cholesterol, triglyceride, HDL-C, LDL-C, Hs-CRP, calcium and total antioxidant respectively against the baseline

The results observed pre and post treatment for the group treated with 1.5g/kg EDTA salt are shown in Table 4. A significant decrease was observed in the total cholesterol, triglyceride, LDL-C, and serum calcium level with a p-value of (0.000, 0.001, 0.000, and 0.000) respectively while a significant increase was observed in the HDL-C and total antioxidant activity level with a p-value of 0.000 and 0.006.Treatment with 1.5g/kg EDTA induced a percentage change of 13.75%, 42.04%, 119.20%, 16.52%, 46.55%, 29.18%, and 75.95% from the baseline of total cholesterol, triglyceride, HDL-C, LDL-C, Hs-CRP, calcium and total antioxidant respectively.

A dose dependent change from baseline was observed for all parameters except for Total Cholesterol which was inverse and LDC where maximum change was obtained at a concentration of 1.5g/kg EDTA treatment. Table 5.

Table 5:
Percentage change in in study parameters with EDTA dosage from baseline

Parameters	0.5g/kgEDTA (% change)	1.0g/Kg EDTA (% change)	1.5g/Kg EDTA dose (% change)
Total Chol	30.19	16.6	13.75
TG	12.38	24.12	42.04
HDL C	73.13	65.78	119.20*
LDL C	3.76	19.11	16.52
Hs-CRP	25.26	26.44	46.55
Calcium	17.73	25.60	29.18
TAS	27.72	54.02	75.95*

*Percentage increase in concentration

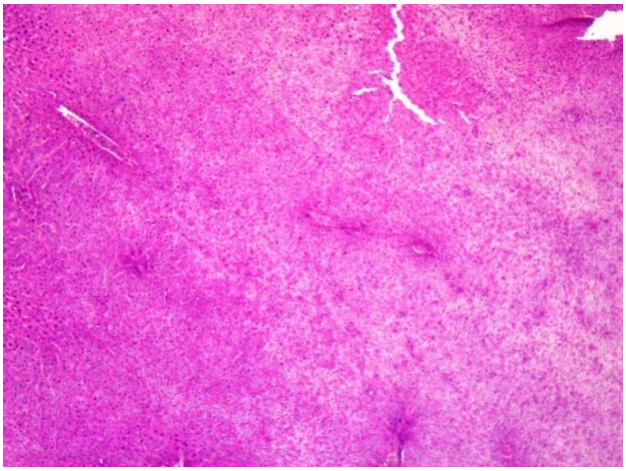

Plate 1:
Photomicrograph showing no development of plaque (H&E ×100) in the aorta of albino rat (Control group).

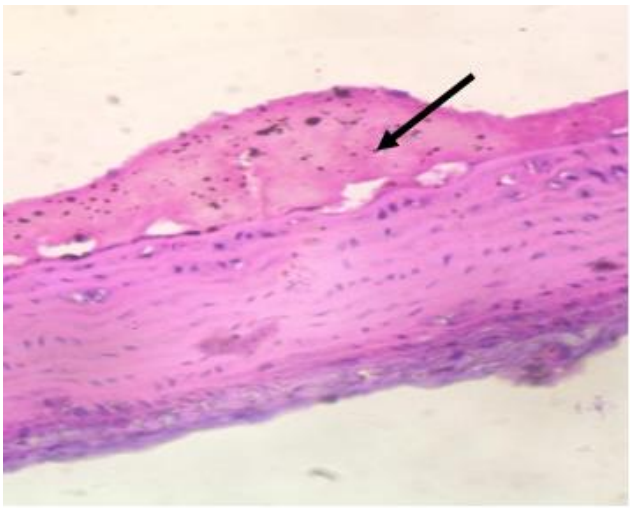

Plate 2:
Photomicrograph showing moderate development of plaques (black arrow) H&E ×100 in the aorta of hyperlipidemic rat

DISCUSSION

Atherosclerosis is a multifactorial disease of the large and medium-sized muscular arteries and the leading cause of morbidity and mortality in industrialized countries. (Braunwald, 1997). It is characterized by endothelial dysfunction, vascular inflammation, and build-up of lipids, cholesterol (Shiao *et al*., 2008), calcium and cellular debris within the intima of the vessel wall (Anuradha and Sugumar, 2009). Protection against arterial endothelial injuries such as the development of fatty streak plaques and in-vessel-wall cholesterol accumulation may prevent atherosclerosis. Hyperlipidemia and oxidative stress have been reported to play a critical role in endothelial dysfunction and most chronic diseases such as atherosclerosis (Leopold and Loscalzo, 2008).

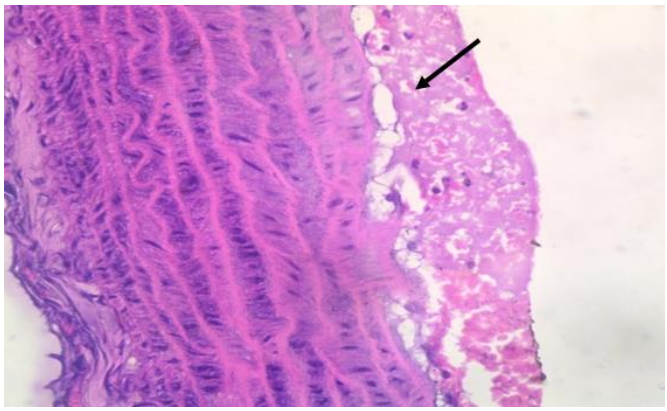

Plate 3:
Photomicrograph showing moderate development of plaques (black arrow) in the aorta of hyperlipidemic rat

This study revealed a marked disturbance in the lipid profile of rats fed palm kernel oil as shown by the increase in the serum levels of total cholesterol, triglyceride and LDL, along with a decrease in the serum level of HDL in comparison with the control group. This is in agreement with work done by other workers (Arafa *et al*., 2014; Ahmed *et al*., 2011; Ajayi and Ajayi, 2009; Azonov *et al*., 2008). This increase could be due to the involvement of cholesterol ester hydrolase (CEH) and cholesterol ester synthase (CES) which balance cholesterol ester synthetase in the blood. With an increased esterifying activity, cholesterol will be predominantly in its ester form as LDL which can lead to an accumulation; leaving the plasma LDL levels high (Varikasuvu *et al*., 2013). It has been reported that cholesterol transport to extra hepatic tissues is primarily by LDL lipoprotein while HDL lipoprotein plays an important role in reversing the cholesterol transport process (Yugarani *et al*., 1992). It has also been demonstrated that increased total cholesterol is associated with the production of oxidized LDL which is involved in endothelial injury, vascular calcification and increased aortic thickness (Arafa*et al*., 2014; Rousell *et al*., 2009). This study also revealed a significant increase in serum hsCRP level which is a marker of inflammation. Sign of inflammation has been reported to occur hand-in-hand with incipient lipid accumulation in variety of animal models of atherosclerosis. This could be explained by the oxidation hypothesis which ndicates that LDL retained in the intima, in part by binding to proteoglycan undergoes oxidative modification (Williams and Tabbas, 1998; Berliner *et al*., 1997). According to the findings of Mohammed *et al*., (2013), there is a significant association between serum or plasma concentrations of hsCRP and the prevalence of atherosclerotic disease. A significant increase in serum calcium level and decrease in total antioxidant status of the hyperlipidemic rats was seen in this study. The reduced antioxidant status in the hyperlipidemic rat could be due to the increased inflammation caused probably by production of oxidized LDL which is involved in endothelial injury.

A significant decrease was observed in the total cholesterol, triglyceride, LDL, and serum calcium levels while a significant increase was observed in the total antioxidant status of the albino rats treated with EDTA. This observation is in agreement with Lamas *et al*., (2013) and Arafa *et al*., (2014). EDTA improves lipid profile; relieves atherosclerosis and cardiovascular diseases via their ability to chelate ectopic or metastatic calcium from atherosclerotic plaques as well as inhibit cell-mediated LDL oxidation (Natarajan *et al*., 2003). EDTA can greatly reduce the excessive production of free radicals by binding those ionic metals, making them chemically inert or its ability to chelate calcium and rapidly removing them from the body (Kempaiah and Srinivasan, 2003). It has also been reported that EDTA inhibits cell-mediated LDL oxidation, and chelates transitional metals which are important catalyst for lipid peroxidation, LDL oxidation and free radical formation Roussel *et al*., (2009). This probably led to the increased total antioxidant status in the rats treated with EDTA.

Histopathological study of the aorta of the hyperlipidemic rats revealed atherosclerotic plaque while the aorta of rats in the control group and the groups treated with EDTA did not show any plaque. The extent of the plaque displayed could be projected to increase had the hyperlipidemic diet continued. This is consistent with the findings of Olubukola *et al*., (2012). This observation had been explained by Gurr *et al*., (1989) who demonstrated that hypercholesterolemia is accompanied by lipid deposition in vessels resulting in foam cell, plaque formation and vascular calcification.

EDTA chelation was most effective in the groups treated with 1.5g/kg EDTA salt, followed by those treated with 1.0g/kg, while those treated with 0.5g/kg had the least effect hence dose dependent affectation. However, the marked increase in HDL level of the rats treated with 0.5g/kg EDTA could be activity related, they were noticed to be more active compared to the other groups treated with EDTA and their mean weight was lower than those of the other groups. The groups treated with 1.5g/kg were the less active, they appeared to be the weakest and they showed an increased faecal and urinary loss than those of other groups just after a week of treatment. It is probable that a dosage of 1.5g/kg was a bit toxic to the rats coupled with the precipitous hypocalcaemia. At the end of seven days of treatment, the number of rats present in the groups treated with 1.5g/kg reduced to four; therefore, the treatment was stopped. The other two groups however thrived well and were able to complete their two weeks of treatment although those treated with 1.0g/kg were also reduced to eight. No death was recorded in the groups treated with 0.5g/kg EDTA salt. The increase faecal and urinary loss observed in the group treated with 1.5g/kg dose is in agreement with the findings of Milioni *et al*., (2005), Mohammed *et al*., (2013), and Arafa *et al*., (2014) referring to the properties of EDTA as a chelating agent to minerals, they further observed significantly increased urinary losses of minerals following EDTA chelating therapy. Thus it could be said that treatment with 0.5g/kg of EDTA was the safest as well as effect in treatment of hyperlipidaemia in this study.

In conclusion, our work confirmed EDTA to be effective at reducing hyperlipidemia at a dose dependent rate. It thus supports the hypothesis that EDTA chelation has a significant positive effect on atherosclerosis. It appears that hyperlipidemia seems to be proportional to the length of feeding with palm kernel oil. Therefore, the first line of management of hyperlipidemia is to change or stop the implicating diet which agrees with lifestyle modification prescribed in hyperlipidaemia management.

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