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Hypocholesterolemic and Protective Effects of Aqueous Extract of *Moringa oleifera* on High Fat Diet-Induced Cardiovascular Lesions in Rats

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ABSTRACT: This study came up as result of the contraindications of the synthetic hypolidemic drugs which have created lacunae in the treatment of hyperlidemia. In order to study the relationship between the plasma cholesterol level and the degrees of aortic lesions, twenty five adult rats were divided into five groups of five animals each. The animals in group A were given normal feed and 1ml distilled water daily for a period of 12 weeks. Groups B and C received fat high diet (30 % w/w of the total rat cow) and aqueous extract of *Moringa oleifera* respectively at doses of 200 mg/kg body weight for a duration of 12 weeks . Groups D received aqueous extract of *Moringa oleifera* at doses of 200 mg/kg body weight and fat high diet simultaneously for a period of 12 weeks whereas groups E received fat high diet for a period of six weeks and thereafter treated with aqueous extract of *Moringa oleifera* at doses of 200 mg/kg body weight for a period of six weeks. Administration of high fat diet to rats for the aforementioned period caused significant ($p<0.05$) increase. Histological examination of the abdominal aorta and heart reveal that the surface of the intima of the rats that were fed with high fat diet were characterized with migration of smooth muscle cells, congestion, infiltration of macrophases, foam cells appearance and lamellar calcification. Other pathohistological features that were observed include fibro fatty tissues, narrowing in the lumen, focal and vacuolated cell. Supplementation of *Moringa oleifera* leaves extract reversed the elevation in body weight, serum total cholesterol and aortic lesions. *Moringa oleifera* can be useful in the management of obesity, hypercholesterolemia and atherosclerosis.

Keywords: Hyperlidemia, *Moringa oleifera*, Aorta, Intima, Atherosclerosis

Introduction

High fat diet consumption has been regarded as risk factor in the development of atherosclerosis (Lim *et al.,* 2012; Abbott *et al.,* 1988; Marzyieh *et al.,* 2007). Several studies have shown that hypercholesterolemia is a major cause of coronary heart diseases and is generally manifested as an increase in plasma concentration of LDL and VLDL (Marzyieh *et al.,* 2007; Hexebergs *et al.*, 1993; Brown, 1996). Reducing the high levels of cholesterol in these apolipoprotein can slow the progression of atherosclerotic lesions (Jed and Fahey, 2005) .The quantity of cholesterol transported in some apolipoproteins such as chylomicrons, VLDL, IDL and LDL are regarded as pro-atherogenic cholesterol and it predisposes individual to the prevalence of cardiovascular diseases (Kane and Malloy, 1982). Triglyceride component of chylomicron are normally discharged into liver, adipose, cardiac and skeletal tissues by the activities of an enzyme known as lipoprotein lipase.

The TG-rich chylomicron and its remnant play a role in the early stage of pathogenesis arteriosclerosis (Kannel, 1995). VLDL and LDL cause atherosclerosis in a similar mechanism which entails a complex multicellular process of oxidation of cholesterol and the accumulation of oxidized cholesterol in the arterial wall. This is followed by the recruitment of monocytes and macrophages to the accumulation of oxidized cholesterol in the arterial wall which then transform to foam cells. This cascade of reactions lead to an unstable atherosclerotic plaque that ultimately burst to gives rise to myocardial infarction (Kannel, 1995; Rudenko *et al.*, 2010; Wihelm and Cooper, 2003,).

Studies have shown that many synthetic hypolipidemic drugs used to reduce the risk associated with high serum cholesterol levels are toxic (Vanderlaan and Getz, 2009). Their toxicity are in connection with the development of type 2 diabetes mellitus, abnormal liver function, diarrhea, nausea and myositis (Rudling, 2006; Hamad *et al.*, 2010) These drugs are potential inhibitor of HMG-CoA reductase, the key enzyme in the cholesterol biosynthesis pathway and its inhibition has proven to be the most efficient therapy for managing hypercholesterolemia (apart from their contraindications) since about 70% of total cholesterol in human is synthesized de novo and only 30% is supplied by dietary source (Bligh *et al.*, 1959).

Medicinal plants have gained more popularity in recent years because they are less toxic than synthetic drugs and are recently being used to fill the lacunae created by the synthetic drugs. *Moringa oleifera* is the only genus in the family of Moringaceae and is the most widely cultivated species of the genus. A number of nutritional and medicinal qualities have been ascribed to its roots, bark, leaves, flowers, fruits and seeds of the plant (Satter *et al.*, 2010; Speight, 1982). *Moringa oleifera* has been found to possess hepatoprotective, antiulcer, antihypertensive, cardioprotective, antihyperglycemic, .anti inflammatory, antiarthritic and analgesic properties (Answer *et al.*, 2007; Fakurazi *et al*., 2008; Devaraji *et al.*, 2007; Rukmani *et al.*, 1998; Rao *et al.*, 1998; Caceres *et al.*, 1992).

Other forms of usage of *Moringa oleifera* include; diuretics, anti-hypertensive, Anti ulcer, hypocholesterolemic, biomass production, animal forage, domestic cleaning agent , and wound healing properties (Answer *et al.*, 2005; Dangi *et al.,* 2002; Steven *et al.,* 2013). The correlation of hyperlidemia with the development of atherosclerotic lesion coupled with the unsatisfactory results of the synthetic hypolipidemic drug prompted us to search for plant based compound with potential hypolidemic effect.

Materials and Methods

***Plant collection and identification*:** *Moringa oleifera* leaves were harvested from area around Owan East Local Government Area of Edo state, Benin City. They were identified in the Department of Plant biology and Biotechnology, Faculty of life Sciences, University of Benin. The leaves were dried in a shaded room for one week and grinded to powdered form using mortar and pestle (mechanical form).
The powdered extract was put in a dried container and was kept in a cool dry place**.**

Preparation of Plant Extract: The powdered extract was soaked in distilled water for forty-eight hours at room temperature. The mixture was filtered using a Buchner funnel and Whatman No.1 filter paper. Dried aqueous extracts were obtained after removing the solvent by evaporation under reduced pressure using Rotary evaporator.The extract was stored in an air-tight container and kept in the refrigerator at 4°C until use.

***Experimental Animals*:** Twenty-five (25) Adult Wistar rats of both sexes, weighing 150-300 g were randomly separated into five (5) groups; Control, A, B, C, D and E, of five rats each. The rats were procured from the Animal House, Department of Pharmacology, Unversity of Benin, Benin City. The rats were allowed two weeks to acclimatize to Anatomy Departmental Animal House, where they were housed in standard animal cages. Weighed rat chow (vital grower's feed, livestock feed company, Ibadan) were given daily to the rats and they were allowed free access to drinking water. All animals were treated in accordance with the Guide for the care and use of laboratory Animals prepared by the National Academy of Sciences and Published by the National Institute of Health Guide for the use of Laboratory Animal (NIN, 2002 Production, No. 83-23), Revised 1978 .

Preparation of High Fat Diet: A mixture containing equal amounts of egg yolk (boiled) and saturated fat were then mixed with rat chow (30 %; w/w) to give high fat diet. The high diets were then used to feed rats for a period of 12 weeks.

***Experimental Design*:** The design consisted of 25 rats, divided in five groups of five rats each.

Group A: (Control) received1 ml distilled daily.
Group B: Fed with high fat diet only.
Group C: Treated with 200 mg/kg b.wt. of the aqueous extract *Moringa oleifera* only .
Group D: Fed with high fat diet and 200 mg/kg b.wt. of the aqueous extract *Moringa oleifera* simultaneously.
Group E: Fed with high fat diet for six weeks and thereafter treated 200 mg/kg b.wt.of the aqueous extract *Moringa oleifera*.

To ensure accuracy of treatment, administration of the aqueous leaf extract of *Moringa oleifera* was done using orogastic tube daily for 12 weeks.

***Method of Sacrifice and Tissue Collection*:** At the end of the duration of study, the animals were kept on overnight fasting after which they were anaesthetized using chloroform, dissected and their blood samples taken by cardiac puncture of the heart for lipid profile test. The animals were then sacrificed with their hearts and abdominal aorta harvested for tissue processing and histopathological analysis.

***Histopathological Analysis*:** The aorta and hearts were examined grossly in all the dissected rats. The portions of the liver was immediately fixed in 10% formal saline for a period of 24 hours, dehydrated in several grades (70-100 %) alcohol embedded in paraffin (58-60 °C) and sectioned at 5 μm thickness . The sections were stained with hematoxylin and eosin (Carleton 1979)

***Estimation of Lipids Profile*:** The serum total cholesterol (TC) was determined by the method of Searcy and Berquist (Searcy and Berquist,1960)

***Statistical Analysis*:** Data were expressed as (Mean ± standard deviation, SD) of six replicates and were subjected to one way analysis of variance (ANOVA) using SPSS version 10.0 and the individual comparisons were obtained by the Duncan multiple range test (DMRT). A value of $P<0.05$ was considered to indicate a significant difference between groups (Duncan 1975).

Results

In the present study, the rats fed with high fat diet for 12 weeks showed significant ($p>0.05$) increase in percentage weight gain as compared to the control group fed with normal diet (Table 1). Our results revealed that there was no significant difference in percentage weight gain in Groups C, D and E compared to control group fed with normal diet in Table 1 (Group 1). The percentage weight gain of test groups were 60.26 %, 55.77 % and 59.52 for groups C, D and E respectively and were significantly lower than group fed with high fat diet (84.43 %) (Group B). Administration of high fat diet to rats for the aforementioned period caused significant ($p>0.05$) increase in total cholesterol which led to increase in the body weight as compared to the control group.

Histological examination of the abdominal and heart aorta reveal that the surface of the intima of the rats that was fed with high fat diet were characterized with migration of smooth muscle cells, congestion, infiltration of macrophases, foam cells appearance and lamellar calcification. Other pathohistological features that were observed include fibro fatty tissues, narrowing in the lumen, focal and vacuolated cell. Supplementation of *Moringa oleifera* leaves extract reversed the elevation in body weight, serum total cholesterol and aortic lesions .

Table 1: Effect of *Moringa oleifera e*xtract of rats fed with high fat diet

Paremeter	**Group A**	**Group B**	**Group C**	**Group D**	**Group E**
TC (mg/dl)	71.67±11.5[b]	113.67±25.8[a]	64.0±9.7 [b]	78.33.9±16.4[b]	72.93±19.5[b]
Initial Wt.(g)	142.0 ±27.9	122±21.7	132.6±30.9	130±32.1	126±28.8
Final Wt.(g)	216.3±35.8	225±28.5	212.5±32.3	202.5±36.7	201±26.3
% Wt.gain	52.32 [b]	84.43[a]	60.26[b]	55.77[b]	59.52[b]

Data is expressed as Mean ±SD for the five animals each group
[a]p<0.05 Compared with control (group i)
[b]p<0.05 Compared with HFD –induced group control (group ii)
TC: total cholesterol.

The figures below show photomicrographs representing the histological results of the processed tissues.

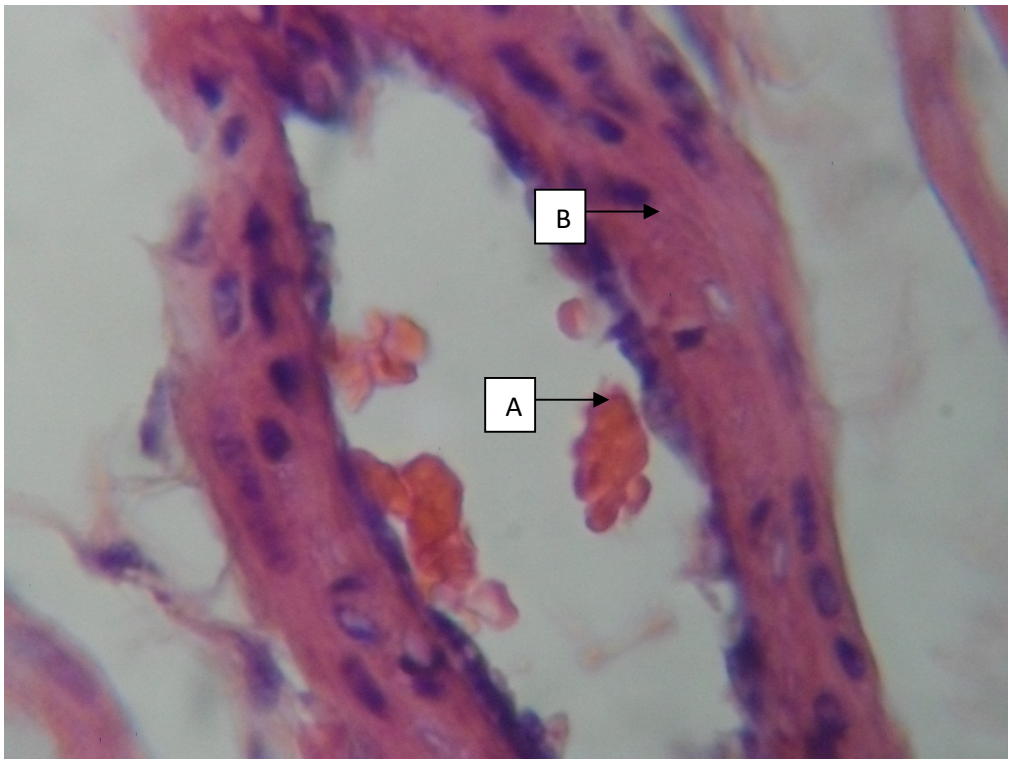

Fig 1: Rat heart showing coronary vessel of the Control group (group A) (H&E X 40). It shows a patent lumen A with an unremarkable intima B

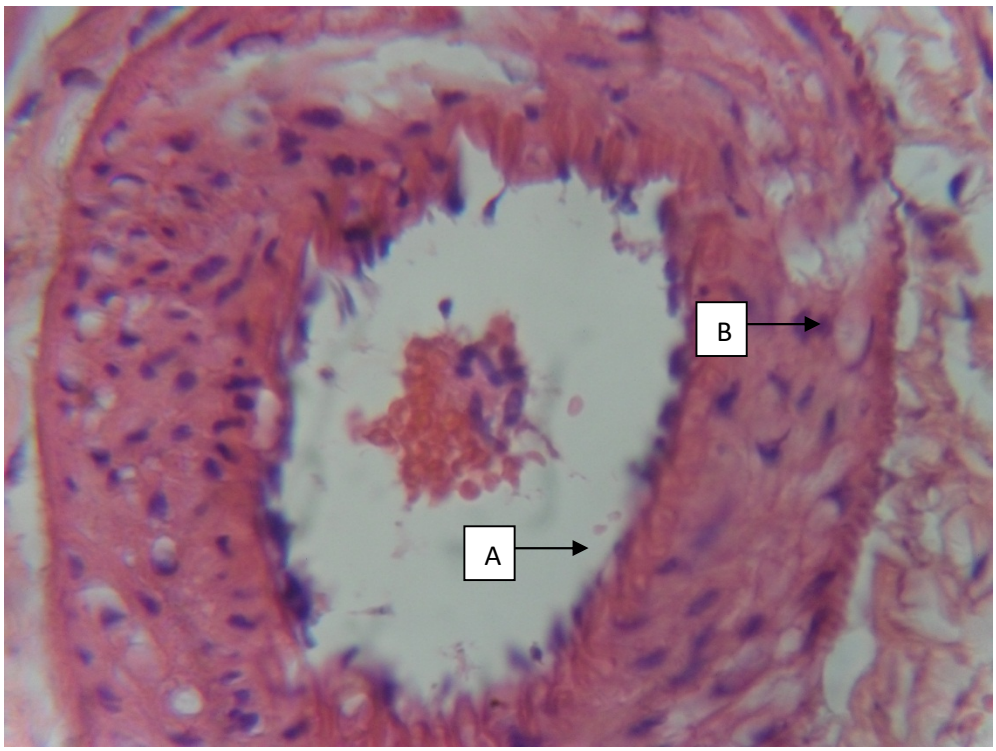

Fig 2: Abdominal aorta of the Control group (group A) (H&E X 40). It shows a patent lumen A with unremarkable intima B

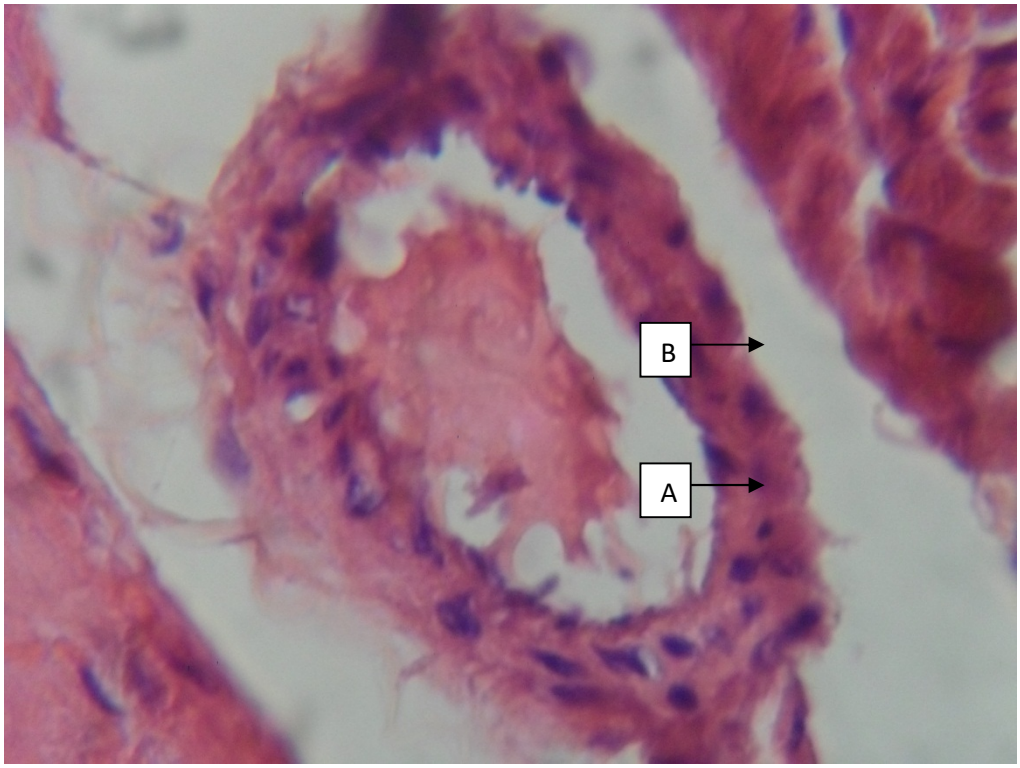

Fig 3: Rat heart showing Coronary vessel of group B (fed with high fat diet only) H&E X 40. The intima B is unremarkable with mild congestion A

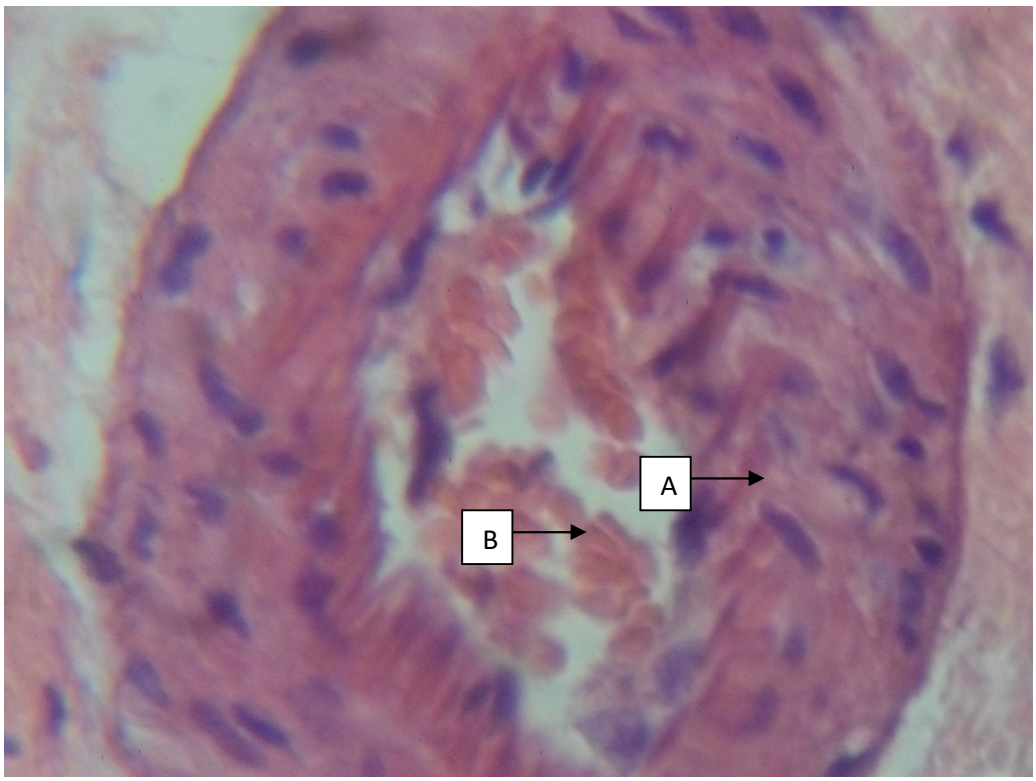

Fig 4: Abdominal aorta of the group B (H&E X 40),showing unremarkable intima A and mild congestion B (H&E x 40)

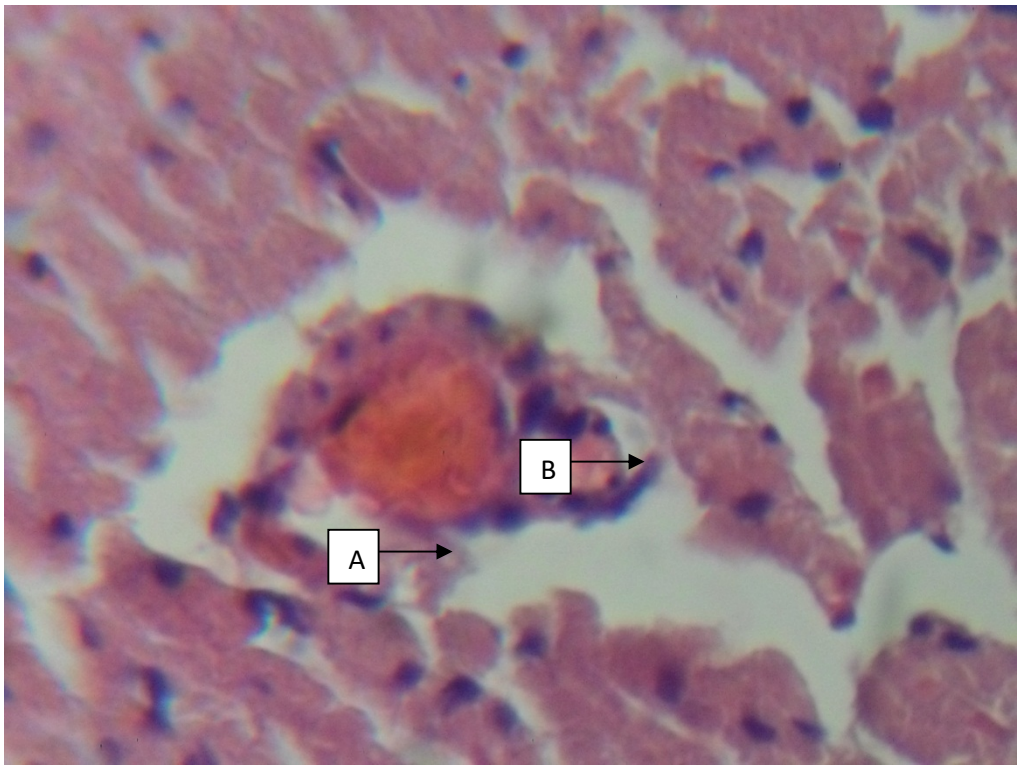

Fig 5: Rat heart showing Coronary vessel of group C (treated with *Moringa oleifera* only) H&E X 40 showing a coronary vessel with intimal damage A and deposition of fibrous and fatty tissue (H&E x 40)

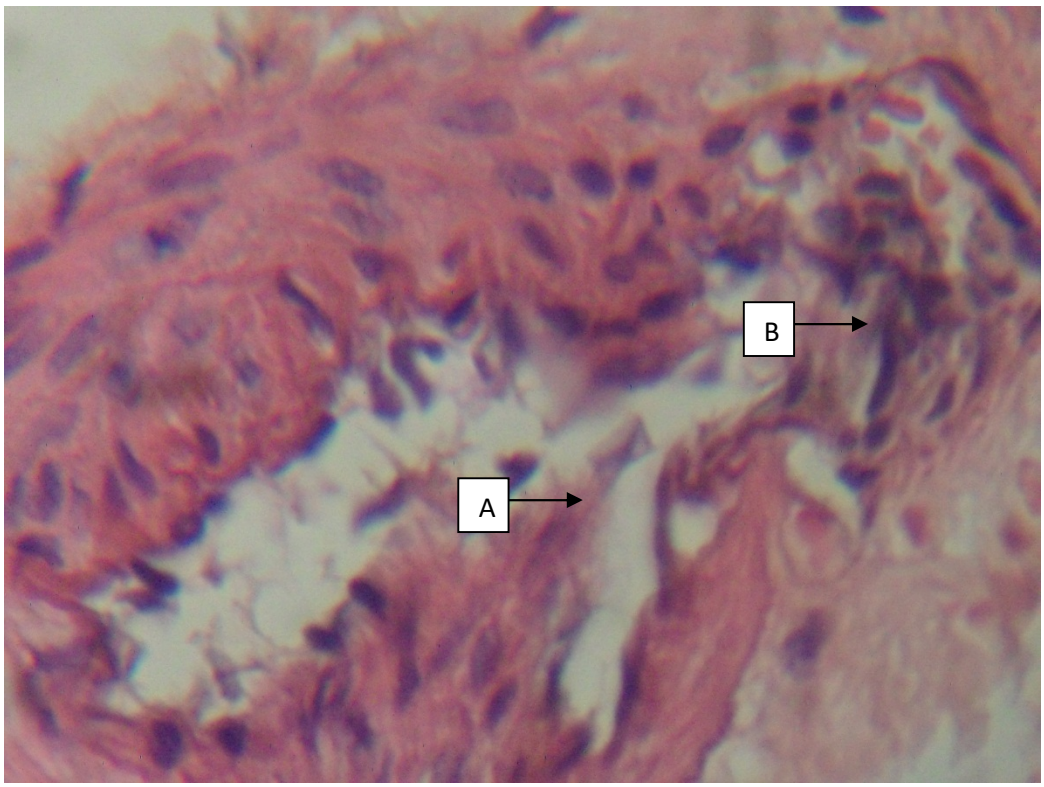

Fig 6: Abdominal aorta of the group C (H&E X 40), showing intimal damage A and deposition of fibro fatty material B (H&E x 40)

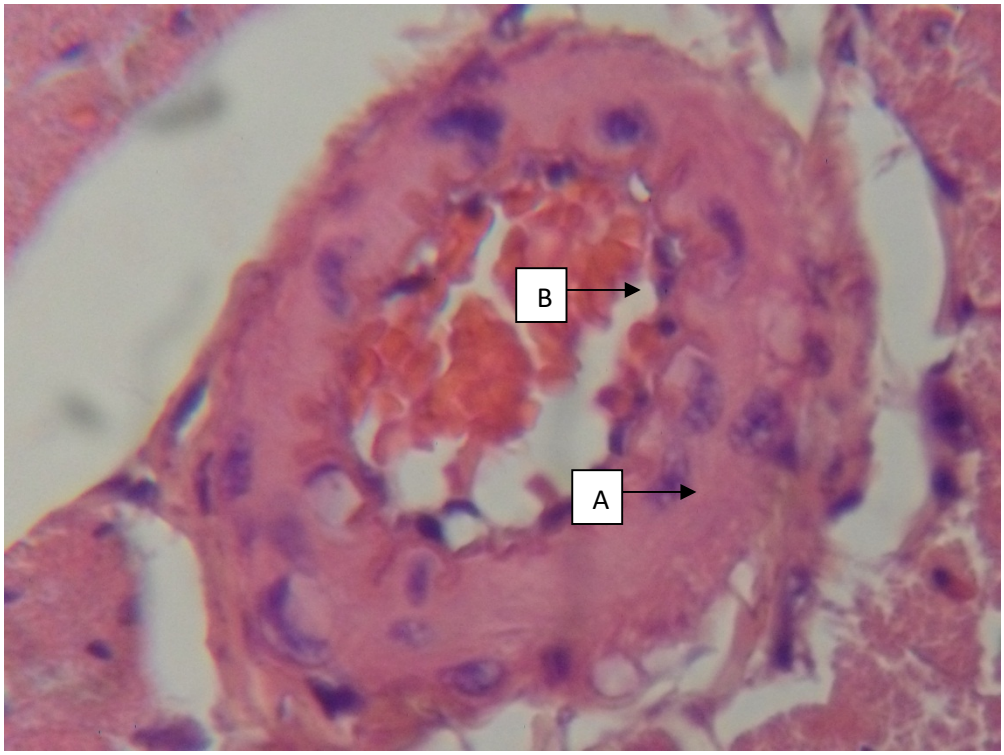

Fig 7**:** Rat heart showing Coronary vessel of group D (fed with high fat and treated with *M. oleifera*) H&E X 40, showing a coronary vessel with unremarkable intima A and mild congestion B (H&E x 40)

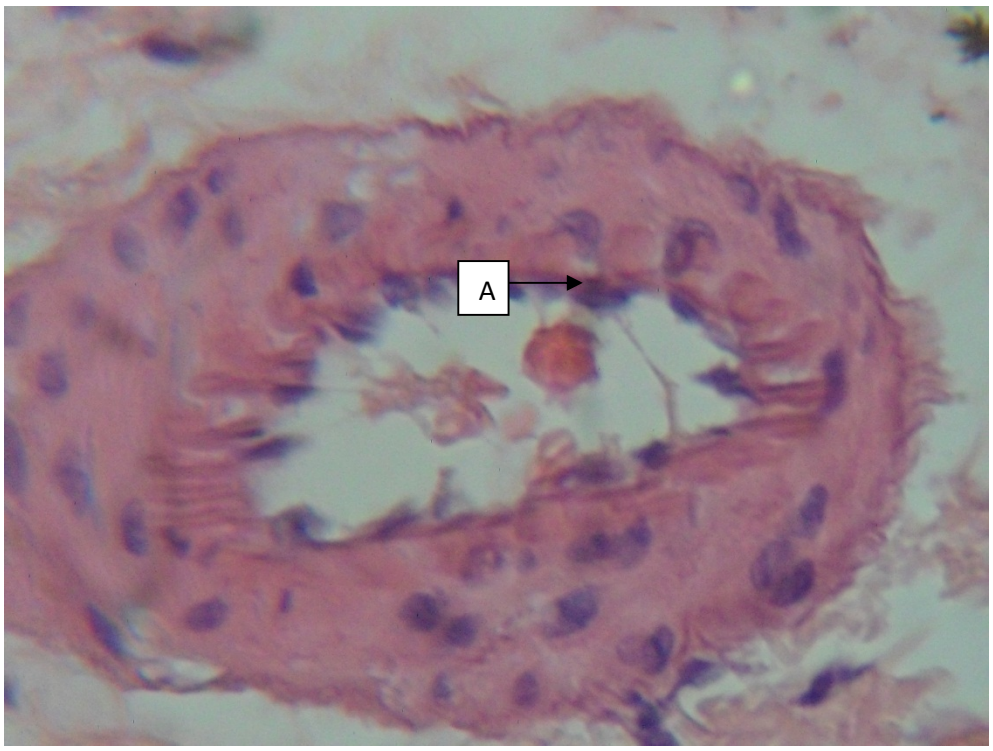

Fig 8: Abdominal aorta of the group D (H&E X 40), showing unremarkable intima A (H&E x 40)

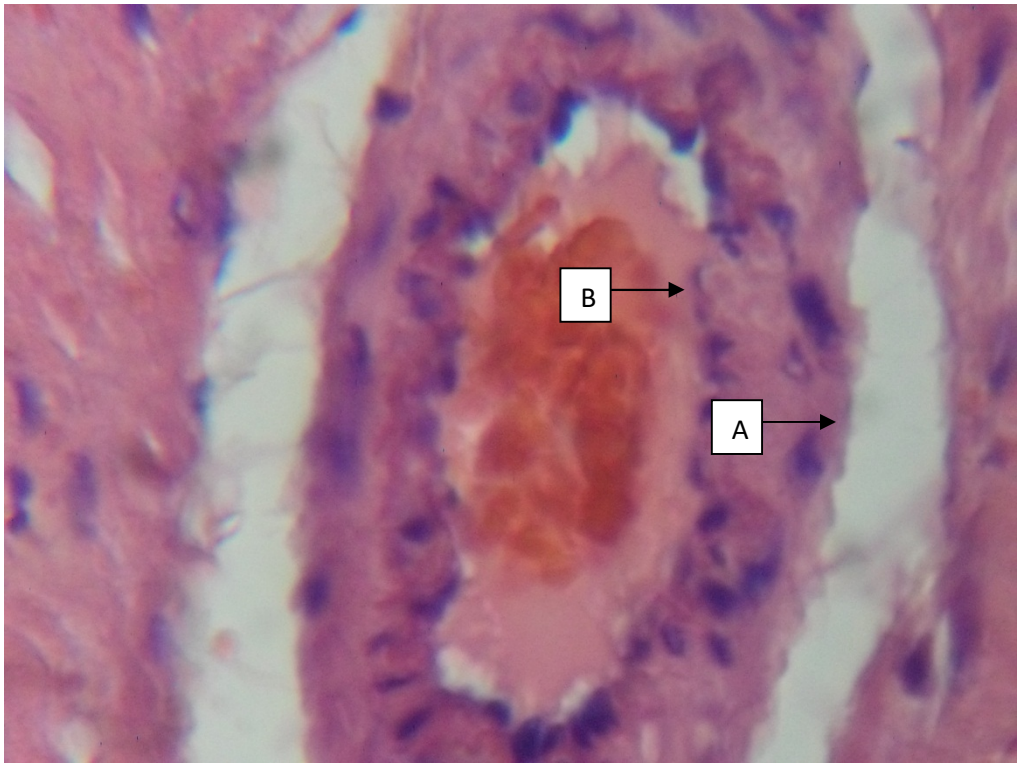

Fig 9: Rat heart showing Coronary vessel of group E(fed with high fat diet for 6weeks, and thereafter treated with *M. oleifera* for 6 weeks) H&E X40**,** showing a coronary vessel with unremarkable intima A and moderate congestion B (H&E x 40)

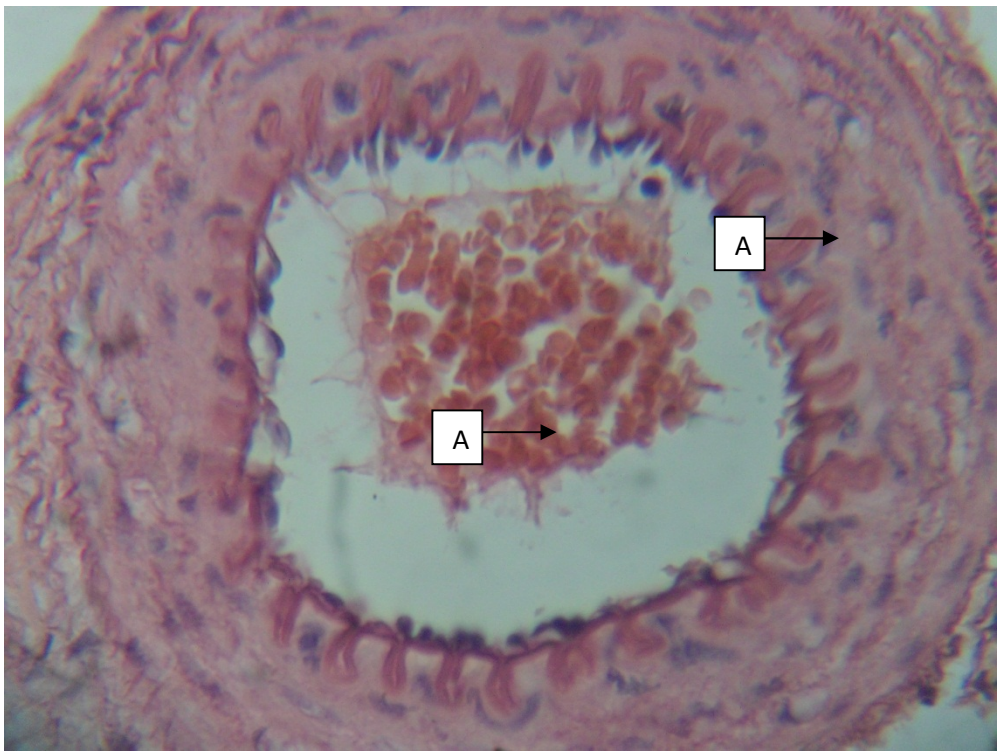

Fig 10: Abdominal aorta of the group E (H&E X 40), showing unremarkable intima A and mild congestion B (H&E x 40)

Discussion

Atherosclerosis is a chronic disease characterized by lipid deposition and inflammation in arterial wall (Naznin 2003; Raida *et al.,* 2008). The principal causes of atherosclerosis include hypercholesterolemia, hyperlipidemia, hypertension, obesity, diabetes mellitus and sedentary lifestyle. High fat diet consumption also play a significant role in causing heart failure (Merg *et al.,* 2014). The high mortality of the disease indicated that there is imperative need for a new management of the disease. High fat diet is generally used to induce hypercholesterolaemia on rat with the view of investigating the strong relationship between the plasma cholesterol level and the degrees of aortic lesions which is often marked by thickening of the intima (Odbayar *et al.,* 2006; Lee *et al.,* 2008; Lafortan and Langin, 2009).

Human studies have shown that increased consumption of high fat diet is associated with body weight gain, which can lead to hypercholesterolemia and other related metabolic diseases (Komal, 2003; Lusis, 2000) .Since increase in body weight may be reflection of the amount of food consumed by the animals, equal amount of both normal and high fat diet were given to control and test group respectively.

In the present study, the rats fed with high fat diet for 12 weeks showed significant($p>0.05$) increase in percentage weight gain accompanied by significant($p>0.05$) increased serum total cholesterol as compared to the control group fed with normal diet. Our results revealed that there was no significant difference in percentage weight gain in Groups C, D and E compared to control group fed with normal diet (Group A). The percentage weight gain of test groups were 60.26 %, 55.77 % and 59.52 % for group C, D and E respectively and were significantly lower than the group fed with high fat diet (84.43 %) (Group B). The observed effect on weight could be as result of increased synthesis of proteins or enzymes and even accumulation of fat cells in some of the tissues of the animals. This, however may be attributed to valuable phytochemicals such as tannins, saponins, volatile oils, saponin glycosides and alkaloids present in the plant material (Weber and Noels, 2011; Naznin, 2003) . The extract showed weight lowering potential and thus suggesting that it may be effective in management of obesity and other related diseases. The results are in consonance with the previous study where decrease in the body weight of rats and total cholesterol were observed (Adedapo *et al.,* 2009; Bucttner *et al.,* 2006; Milagro *et al.,* 2006; Afoso *et al.,* 2013).

Studies have demonstrated that under pathological condition, the elevated serum total cholesterol is due to the increased production of VLDL and LDL by the hepatocytes (Carleton 1975; Akhtar and Ahmad, 1995; Aikawa and Libby, 2004). This condition stimulates the accumulation of LDL cholesterol in the arterial wall, promoting endothelial cell dysfunction and the development of atherosclerosis (Zhang *et al.,* 2011; Groyer *et*

al., 2006). Their subsequent oxidation and formation of oxidized LDL in the arterial wall under oxidative stress lead to the formation of lesions that stimulate monocytes and macrophages to become foam cells, eventually leading to atheroma (Bok *et al.,*1999; Kanung *et al.,* 2007). Several reports have also showed that medicinal plants are able to mitigate the elevated serum cholesterol through a inhibition of the key enzymes in cholesterol biosynthetic pathway such 3-hydroxy-3-methyl-glutaryl-CoA, HMG-CoA reductase and acyl-CoA acetyltransferase which catalyse the acylation of cholesterol forming cholestry ester thereby decreasing the levels of cholesterol esters available for the formation of very low density lipoproteins (VLDL) (Amin and Nagy, 2009; Kane and Malloy, 1982). This will leads to diminish level of VLDL and LDL secretion by the liver. Other phytochemicals act by suppressing the expressioin of several enzymes involved in the biosynthesis of fatty acids (Groyer *et al.,* 2006; Ryan, 2003). Recent studies have shown that phytosterol and β-sitosterol present in the leaf of *Moringal oleifera* are able to mitigate plasma total cholesterol and elevate HDL (Bok *et al.,* 1999; Jackson and Beaglhole, 1995; Bligh *et al*., 1959).

Histopathological examination of aorta of normal rats reveals that tunica media, tunica intima, and tunica adventitia appear to have normal cellular architecture. In fact, there were no histological changes in the aorta of the control group as the cellular architecture are intact (Figure 1). The histopathological examination of the group that were fed with high fat diet for a period of 12 weeks was characterized with Fibro fatty tissues, focal and vacuolated cells at the tunica initima. Hemorrhage as well as narrowing in the lumen was showed in rats fed on high fat-diet (Figure ii). Treatment with the extract attenuated these histopathological manifestation as only mild congestion were seen (Figures 3-10).

Also histopathological examination of the heart showed that the firmness of vessel in the control group was better than that of the group fed with high fat diet. Also the intima surface of the control group was smooth and shiny, there was neither congestion of the intima nor migration of smooth muscle cells to the intima region whereas the surface of the intima of the rats that was fed with high fat diet were characterized with migration of smooth muscle cells, congestion , infiltration of macrophases,foam cells appearance and lamellar calcification under the endothelium. Progression of these lesions usually leads to atheromatous formations.

In conclusion, administration of high-fat diet to rats for a period of 12 weeks causes elevation in body weight and serum total cholesterol which can lead to the formation of several lesion that may progress to atheromatous formation as clearly seen in the histopathological examination. The study further demostrated that the supplementation of *Moringa oleifera* leaves extract for a period of 12 weeks reversed the elevation in body weight, serum total cholesterol and aortic lesions. *Moringa oleifera*t can be useful in the management of obesity, hypercholesterolemia and atherosclerosis.

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