Contents lists available at ScienceDirect IF: 0.926

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60344-8

Hypolipidemic, antioxidant and anti-atherosclerogenic effects of aqueous extract of *Zanthoxylum heitzii* stem bark in diet-induced hypercholesterolemic rats

Fidele Ntchapda[1*], Kakesse Maguirgue[1], Hamadjida Adjia[2], Paul Faustin Seke Etet[3], Théophile Dimo[4]

[1]*Department of Biological Sciences, Faculty of Science, University of Ngaoundéré, P.O. Box 454, Ngaoundéré, Cameroon*
[2]*Department of neuroscience, Faculty of Medicine, University of Montréal, 2960, Chemin de la Tour, Pavillon Paul-G. Desmarais, Montréal, Québec Canada, H3T 1J4*
[3]*Department of Basic Health Sciences, College of Applied Medical Sciences, Qassim University, Buraydah 51452, Saudi Arabia*
[4]*Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé 1, P.O. Box 812, Yaoundé, Cameroon*

ARTICLE INFO

Article history:
Received 15 February 2015
Received in revised form 20 March 2015
Accepted 15 April 2015
Available online 20 May 2015

Keywords:
Zanthoxylum heitzii
Plant extract
Antioxidant
Anti-atherosclerogenic
Hypocholesterolemic
Hypolipidemic

ABSTRACT

Objective: To evaluate anti-dyslipidemic, antioxidant and anti-atherosclerogenic properties of this extract in diet-induced hypercholesterolemic rat, a model of metabolic syndrome-induced atherosclerosis and associated cardiovascular diseases. **Methods:** Normocholesterolemic (NC) male rats were divided into six groups (n=10) and fed a high-cholesterol (HC) diet for 30 days (5 groups), or normal rat chow (normal control group). Rats given a HC diet also received distilled water (disease control), the potent hypocholesterolemic agent with anti-atherosclerotic activity atorvastatin (2 mg/kg, positive control), or one of the three doses of *Zanthoxylum heitzii* stem bark aqueous extract tested (225, 300 and 375 mg/kg) concomitantly for four months. Signs of general toxicity, body temperature and weight, and water and food intake were monitored in live animals. After sacrifice, lipid profiles and oxidative stress markers were assessed in the blood and liver, aorta, and feces, and histopathological analysis of aorta was performed. **Results:** Plant extract prevented the elevation of aortic total cholesterol and triglycerides, and hepatic low density lipoprotein, very low density lipoprotein, and total cholesterol. Lipid peroxidation (TBARS) was decreased and aortic atherosclerotic plaque formation prevented. **Conclusions:** These observations strongly suggest that stem bark aqueous extract of *Zanthoxylum heitzii* has anti-atherosclerogenic properties, at least partly mediated by antioxidant and hypolipidemic effects.

1. Introduction

Hyperlipidemia and oxidative stress are major risk factors for atherosclerosis, and all three are among the most important risk factors for cardiovascular diseases and conditions[1,2]. Strategies used to prevent and treat atherosclerosis, and to reduce the incidence and severity of associated cardiovascular diseases, mainly include fighting against hyperlipidemia using dietary approaches such as diet rich in fibers[3,4], and/or anti-dyslipidemic drugs like atorvastatin[5-7].

A huge body of population based and experimental evidence shows that high levels of plasma low density lipoprotein (LDL) cholesterol and total cholesterol considerably increase the risk for developing atherosclerosis and associated arterial hypertension[1,2,8-10]. Other changes in lipid parameters associated with atherosclerosis include decreases in high density lipoprotein (HDL) cholesterol and increases in triglycerides.

A number of anti-dyslipidemic drugs was developed to slow

*Corresponding author: Dr. Ntchapda Fidèle, PhD, Department of Biological Sciences, Faculty of Sciences, University of Ngaoundere, PO Box 454 Ngaoundere, Cameroon.
Tel: +237 677 92 18 69, +237 242 15 84 84
E-mail: ntchapda71@yahoo.fr

or prevent atherogenesis, and eventually treat atherosclerosis. Unfortunately, these drugs may have serious undesired effects[11,12], and thus, new therapeutics against atherosclerosis are needed in the field.

Traditional healers have used different species of the *Zanthoxylum* for treatment of a wide range of disorders. Among *Zanthoxylum* species, aqueous extracts of the shrub of humid rain forest *Zanthoxylum heitzii* (*Z. heitzii*) (Rutaceae) is used against syphilis and other urogenital infections, malaria, cancer, cardiopathies, and hypertension[13,14]. The chemical fractions have been determined with insecticidal activity in bark extract of *Z. heitzii* against Anopheles gambiae adults. dihydronitidine and trans-pellitorine have been elucided as identified compounds in this plant. Dihydronitidine has antiplasmodial, antileishmanial and anti-cancer activity, but first time reported as insecticidal. Sesamin and trans-pellitorine has insecticidal activities[15]. Two new natural lignans, meso-2,3-bis(3,4,5-trimethoxybenzyl)-1,4-butanediol and 4-acetoxy-2,3-bis(3,4,5-trimethoxybenzyl)-1-butanol, four known lignans, two alkaloids and triterpenes were isolated from the bark of *Z. heitzii* and identified on the basis of their spectral data[16,17].

Although extracts of stem bark[15] and fruits[18] of *Z. heitzii* were reported antioxidant effects, no data on the effect of this medicinal plant on cardiovascular diseases and conditions are available.

The present study was aimed at evaluating the anti-dyslipidemic, antioxidant, and anti-atherogenic effects of the stem bark aqueous extract of *Z. heitzii* as potential mechanisms driving the putative protective and therapeutic effects of these extracts.

2. Materials and methods

2.1. Biological material

2.1.1. Animals

Sixty normocholesterolemic (NC) male Wistar rats [(220±10) g] were purchased from Yaounde (Cameroon) Pasteur Institute and acclimated to the Laboratory of Medicinal Plants, Health and Galenic Formulation of the Department of Biological Sciences, University of Ngaoundere (Cameroon). Animals were housed under controlled room temperature [(24±2) ℃] and had *ad libitum* access to food [National Veterinary Laboratory (LANAVET), Garoua, Cameroon] and tap water. Animals were monitored for signs of general toxicity, under the supervision of a veterinary. All experimental procedures were approved by the institutional ethics committee.

2.1.2. Plant material processing

Z. heitzii bark samples were collected during flowering period (January) in a rice zone of Tandjilé (North Cameroon) and authenticated by both the laboratory of Botany, Department of Biological Sciences, University of Ngaoundere andthe National Herbarium of Cameroon (specimen Nº 60695/HNC).

To prepare the aqueous extract, *Z. heitzii* bark samples were dried and crushed, andthe powder obtained was macerated in distilled water for 12-h (1 kg/1 L).The macerate was filtered using Whatman filter paper (N° 3), and the filtrate was concentrated using a rotary evaporator (40 ℃, 24-h). After various repetitions of this process 10.04 g of concentrated crude extract (oily paste) was obtained. The extract was stored at -20 ℃ until use.

2.1.3. Phytochemical study

Preliminary phytochemical tests were performed at the Institute of Medicinal Plants for Medicinal research (IMPM, Yaounde, Cameroon) to unravel the gross chemical composition of the plant extract studied.

2.2. Experimental procedures

NC rats were randomly divided in 6 groups. For four weeks, five of these groups were fed a hypercholesterolemic (HC) diet (normal rat chow +1% cholesterol)[19,20], whereas normal control rats (destined to remain NC) were receiving normal rat chow. Groups exposed to HC diet were also given distilled water (disease control group), the hypocholesterolemic agent with anti-atherosclerotic activity atorvastatin (2 mg/kg, positive control)[21], or one of three doses of the aqueous extract of the stem bark of *Z. heitzii* (225, 300 and 375 mg/kg), once daily at 0.5 mL/100 g body weight. Five rats over 10 were sacrificed at the end of the four weeks of treatment, and the remaining five, four additional weeks after last day of treatment. Blood samples, aorta, liver, and fresh feces were collected and processed for biochemical tests and H&E histopathological studies.

2.2.1. Body temperature monitoring

Body temperature was monitored, with daily measurement 5 h after treatment. To measure body temperature, a rat rectal thermometer was placed at a distance of approximately 2 mm from the anus.

2.2.2. Blood and homogenate processing

Blood freshly collected in heparinized tubes was centrifuged (3 000 rev/min for 10 min), and the supernatant (plasma) was used for the enzymatic determination of total cholesterol, HDL cholesterol, triglycerides and malondialdehyde. Catalase, hydroperoxide, and protein levels were determined in hemolysates obtained from blood pellets, and from liver homogenates.

2.2.3. Histopathological studies

Aorta samples were fixed in 10% formal calcium, paraffin embedded, serially cut with a microtome (5 μm), processed for hematoxylin and eosin staining. Planimetry studies of cross sections of aorta were made at ×20 magnification using camera lucida drawings.

2.2.4. Statistical analysis

Data obtained from the different experimental groups were compared by one-way ANOVA followed by LSD test for post hoc analysis, using Origin software (OriginLab, Northampton, MA, USA). Test groups were compared to normal, disease, and positive control groups. Differences with $P<0.05$ were considered significant. Data are presented as mean ± SEM.

3. Results

3.1. General observations

3.1.1. Plant extract tolerance

No sign of general toxicity was observed in animals at the dose used either in preliminary studies in few animals or in this study. Notably, animal fur was normal, no massive porphyrin deposit was observed around the nose or eye, and normal exploratory behavior components were maintained (including grooming, rearing, and sniffing activities), with apparently normal defecation and no sign of increased aggressiveness (*eg*. No increase in vocalization, biting, or metal cage bar chewing).

Average daily water and food intake are shown in Figure 1A, B. Unlike all the three test groups (plant extract-treated) and positive controls (atorvastatin-treated), HC rats did not show a fast increase in water intake (y = 0.73x + 37.8, R^2 = 0.61). Water intake displayed a dose-dependent faster increase in test groups [225 mg/kg (y = 5.19x + 34.7, R^2= 0.97), 300 mg/kg (y = 5.63x + 38.5, R^2= 0.99), 375 mg/kg (y = 7.2x + 44.6, R^2= 0.88)] compared to positive controls (y = 1.25x + 35.3, R^2= 0.52); and this parameter was significantly increased in all these groups at fourth week of treatment (P<0.05 compared to first) (Figure 1A). On the other hand, food intake increased comparably in all groups [HC rats (y = 2.44x + 18.4,R^2= 0.99), extract doses 225mg/kg (y = 3.23x + 14.43, R^2= 0.99), 300 mg/kg (y = 2.04x + 17.65, R^2= 0.99), 375 mg/kg (y = 2.36x + 16.94, R^2= 0.97), and atorvastatin (y = 1.66x + 19.13, R^2= 0.93)] (Figure 1B).

During the four weeks of treatment extract-treated animals showed a dose-dependent loss of body weight [extract doses 225 mg/kg (y = -3.14x + 264.6, R^2= 0.98), 300 mg/kg (y = -4.37x + 256.2, R^2= 0.95), and 375 mg/kg (y = -8.62x + 271.1, R^2= 0.99)] (Figure 1C). Test groups resumed gaining weight after the end of the treatment, with an increase of 11.7% four weeks after the last treatment (data not shown).

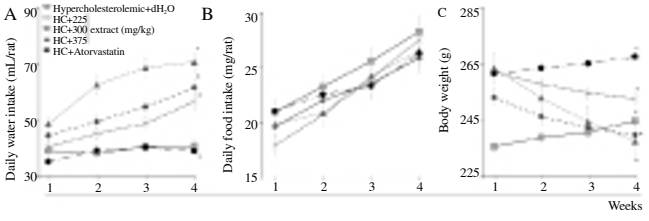

Figure 1. Body weight, food and water intake.

A: Water intake progression during four weeks of daily treatment of hypercholesterolemic (HC) rats with *Z. heitzii* extract (test groups), distilled water and atorvastatin (negative and positive control groups). Note the faster increase in test groups with increasing dose.

B: Food intake progression in HC rats' from test groups, negative and positive control groups. Note the comparable increase in all groups.

C: Body weight progression in HC rats' from test groups, negative and positive control groups. Note the increase in control groups and the decrease in test groups compared to baseline values. * P< 0.05.

In addition, no significant change was observed in organ weight, including the liver, kidney, heart and the testis (Table 1).

Effects of the stem bark extract of *Z. heitzii* on body temperature (as mentioned above, measured daily 5-h after treatment) are presented in Table 2. No significant change was observed in the body temperature of any experimental group compared to baseline values (pre-treatment) or after inter-group comparisons, during the treatment period (Table 2) and 4 weeks after (data not shown), although a slight decrease was observed at the end of treatment with the higher doses of plant extract tested and atorvastatin.

3.1.2. Phytochemical study

Phytochemical screening performed on crude stem bark extract of *Z. heitzii* revealed the presence of various families of primary and secondary metabolites with potential biological activity, mainly fatty acids, flavonoids, alkaloids, sterols, triterpenes, saponins, tannins, coumarins and phenolic compounds.

3.2. Effect of Z. heitzii extract on blood lipid parameters

The effects of *Z. heitzii* extract on blood lipid parameters are shown in Table 3 and Figure 2A. Compared to those given normal rat chow (NC rats), animals given a diet enriched in cholesterol four weeks and only distilled water (HC rats) showed significant (P<0.05) increases in total cholesterol (69.55%), in triglycerides (59.18%), in very low density lipoprotein (VLDL) cholesterol (71.89%), in LDL cholesterol (45.36%), and a decrease in HDL cholesterol (43.24%). These alterations were prevented in HC rats treated with atorvastatin, as expected, but also by the three doses of extract in a dose-dependent fashion (Table 3). The ratios of LDL to HDL cholesterol and of total cholesterol to HDL cholesterol were significantly increased in HC rats (P<0.05) compared to NC rats (Figure 2). Such alteration in these ratios was prevented in HC rats by atorvastatin treatment, but also by treatments with extracts of *Z. heitzii*, in a dose-dependent fashion (Figure 2A).

Four weeks after replacement of HC diet with normal rat chow and concomitant end of treatments, physiological levels of blood lipid parameters were still observed in animals treated with either atorvastatin or a dose of extract (Table 3, Figure 2A).

3.3. Effect of Z. heitzii extract on lipid parameters in liver, aorta, and/or feces

The effect of *Z. heitzii* extract on levels of total cholesterol, triglycerides and lipid peroxidation byproducts thiobarbituric acid reactive substances (TBARS) in liver, aorta, and/or feces homogenates are shown in Table 4. Compared with NC rats, these markers of dyslipidemia were significantly increased in HC rats (P<0.01) as follows: total cholesterol (liver 37.33%; aorta 33.02%; feces 54.9%), triglycerides (liver 39.17%, aorta 41.42%), and TBARs (liver 30.85%, aorta 35.64%). For all these parameters, values observed were comparable with NC rats (not significantly different from NC group) and significantly different (P<0.05) from HC rats (Table 4).

3.4. Effect of the aqueous extract of Z. heitzii on oxidative stress markers in liver homogenates and blood

The effect of the aqueous extract of *Z. heitzii* on various markers of oxidative stress markers in liver homogenates, hemolysates, and plasma was dose-dependent as shown in Figures 2B-E. Protein level was decreased in liver homogenates (y = -7.86x + 67.6, R^2= 0.93) and increased in hemolysates (y = 3.02x + 43.8, R^2= 0.96) of HC rats treated with the extract (Figure 2B). Changes in liver homogenates were significant at all doses tested (P<0.01 at higher doses of extract), whereas hemolysate changes were significant only at higher doses of extract (P<0.05) (Figure 2B).

Catalase level was increased in the liver (y = 0.05x - 0.05, R^2= 0.856 2) of HC rats treated with the extract (Figure 2C). Such increase was significant at the highest dose tested (P<0.05). Catalase level was also increased in hemolysates of these animals (y = 0.04x - 0.005, R^2= 0.81). However, this increase was significant at all doses of extract tested (P<0.001 at highest dose) (Figure 2C).

Malondialdehyde (MDA) level was decreased in the liver (y = -0.77x + 11.19, R^2= 0.94) and in blood plasma (y = -4.57x + 26.9, R^2= 0.97) of HC rats treated with the extract (Figure 2D). These decreased were significant at higher doses tested (P<0.05) and at all doses of extract (P<0.001), respectively (Figure 2D).

Hydroperoxide (ROOH) level was increased in the liver (y = 0.01x + 0.02, R^2= 0.99, with P<0.05 at the highest dose tested), and in plasma (y = -0.28x + 1.8, R^2= 0.80) (significant at all doses of extract, with P<0.001 at the highest dose) (Figure 2E).

3.5. Effect of Z. heitzii extract on rat aorta morphometric parameters

The observation of H&E sections of HC rats revealed more marked atherosclerotic plaque formation in negative control animals than in animals receiving *Z. heitzii* extract (Figure 3).

Aorta morphometric parameters studied were thickness and surface area. A four-fold (respectively five) increase in thickness (respectively surface area) was observed in HC rats compared with NC rats (Figure 2F). Although *Z. heitzii* significantly slowed the aorta thickness increase (P<0.05 against HC rats, and not significantly different from NC rats), plant extract only displayed a

Table 1

Effect of *Z. heitzii* extract administration for four weeks on organ weight.

Experimental group		Liver	Heart	Kidney	Testis
Hypercholesterolemic rats +dH_2O		3.26±0.32	0.31±1.09	0.32±0.14	0.54±0.16
Z. heitzii-treated	225 mg/kg	3.05±0.03	0.30±0.11	0.30±0.11	0.52±0.14
	300 mg/kg	3.09±0.17	0.31±0.12	0.31±0.14	0.52±0.09
	375 mg/kg	3.12±0.40	0.31±0.13	0.31±0.13	0.53±0.12
Atorvastatin		3.15±0.18	0.29±0.10	0.31±0.11	0.51±0.06
Normocholesterolemic rats		3.10±0.16	0.30±0.11	0.30±0.12	0.52±0.08

Values are mean ± SEM. n = 5. No significant change was observed.

Table 2

Effect of *Z. heitzii* extract administration for four weeks on body temperature.

Time (days)	Extract of *Z. heitzii* (mg/kg)				Atorvastatin(2 mg/kg)
	Control	225	300	375	
1	37.20 ± 0.23	37.20 ± 0.12	36.50 ± 0.11	35.40 ± 0.15	36.30 ± 0.16
2	37.40 ± 0.17	37.30 ± 0.15	37.40 ± 0.13	36.50 ± 0.14	37.20 ± 0.23
3	36.40 ± 0.18	37.50 ± 0.21	35.50 ± 0.15	35.50 ± 0.16	37.40 ± 0.17
4	36.20 ± 0.15	36.40 ± 0.18	37.50 ± 0.17	36.60 ± 0.22	36.40 ± 0.18
5	37.40 ± 0.11	37.50 ± 0.14	36.40 ± 0.13	36.20 ± 0.15	36.20 ± 0.15
6	36.40 ± 0.14	36.40 ± 0.16	35.50 ± 0.18	37.30 ± 0.11	37.30 ± 0.11
7	36.40 ± 0.15	37.40 ± 0.14	35.30 ± 0.17	37.20 ± 0.17	37.20 ± 0.17
30	37.40 ± 0.17	37.40 ± 0.11	36.40 ± 0.14	36.20 ± 0.21	36.20 ± 0.21

dH_2O: distilled water. Values are mean ± SEM., n = 5. No significant change was observed.

Table 3

Effect of aqueous extract of *Z. heitzii* on blood lipid parameters.

Experimental groups		Lipid parameters (mg/dL)				
		TC	TG	VLDL-c	HDL-c	LDL-c
HC rats +dH_2O		266±9	196±13	217±6	32±2	194±11
HC +4 weeks treatment	225 mg/kg	213±13*	175±4*	169±12*	60±2*	130±11
	300 mg/kg	183±11*	169±4*	140±11*	61±2*	90±12*
	375 mg/kg	174±16*	156±1*	135±15*	66±2*	83±16*
HC 4weeks after treatment	225 mg/kg	212±12*	174±2*	168±3*	61±1.5*	130±9
	300 mg/kg	182±10*	160±3*	142±1*	62±1.2*	85±7.6*
	375 mg/kg	172±15*	155±1*	133±2*	65±1.3*	84±7.4*
HC +Atorvastatin		172±16*	146±4*	136±9*	66±4*	76±10*
NC rats		185±17*	116±18*	156±8*	74±2*	88±14*

Data are mean ± SEM, n=5. One-way ANOVA+LSD test against hypercholesterolemic (HC) rats: *P<0.05, HDL-c: high density lipoprotein cholesterol. LDL-c: low density lipoprotein cholesterol. NC: normocholesterolemic. T: treatment. TC: total cholesterol. TG: triglyceride. VLDL-c: very low density lipoprotein cholesterol.

slight mitigation of HC treatment-induced surface area increase (not significantly different from HC and significantly different from NC rats), at all doses tested (Figure 2F).

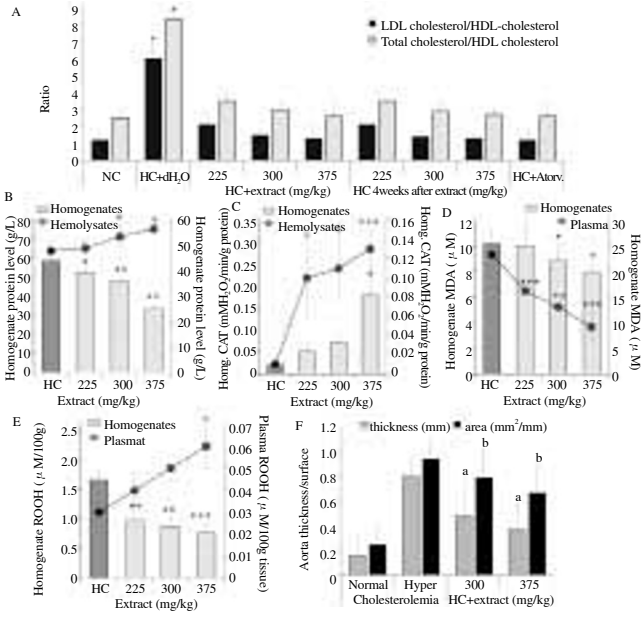

Figure 2. Lipid parameters and oxidative stress.

A: Effect of *Z. heitzii* extract on the ratios of LDL to HDL cholesterol and of total cholesterol to HDL cholesterol. Note the significant increase in hypercholesterolemic (HC) rats compared to normocholesterolemic (NC) rats, and the protective effects of the extract at all doses tested.

B-D: Effect of *Z. heitzii* extract on various oxidative stress markers in liver homogenates and blood. Levels of proteins were decreased in liver homogenates and increased in hemolysates (B), whereas catalase (CAT) level was significantly increased in the liver and in hemolysates (C) of HC rats treated with the extract. Malondialdehyde (MDA) level was significantly decreased (D), while hydroperoxide (ROOH) level was significantly increased (E), in the liver and in blood plasma of HC rats treated with the extract. Note that all these effects are dose-dependent. ANOVA+LSD test against hypercholesterolemic (HC) rats: *P<0.05, **P<0.01, ***P< 0.001.

E: Morphometric analysis of aorta. Note the decrease in thickness and surface area in HC rats treated with the extract. (i) against HC rats, [a] P < 0.05; (ii) against NC rats [b] P<0.05.

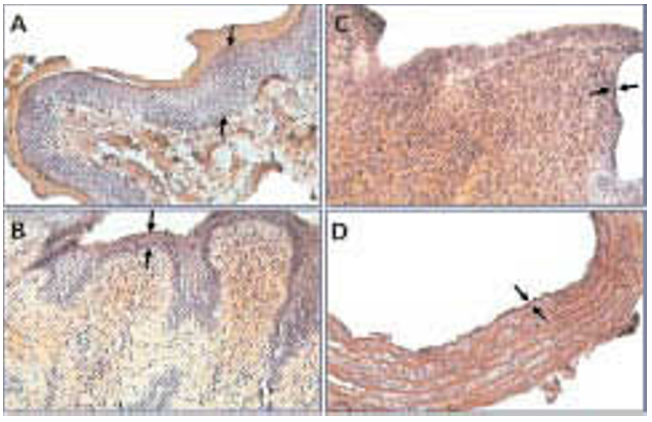

Figure 3. Aortic intima cross-sections of representative cases.

A: Hypercholesterolemic (HC) rat receiving distilled water (negative control rat, magnification: ×400). B: HC rat treated with *Z. heitzii* extract at dose 300 mg/kg (magnification: ×400). C: HC rat treated with *Z. heitzii* extract at dose 375 mg/kg (magnification: ×100). D: Normocholesterolemic (NC) rat (magnification: ×100). Arrows indicate atherosclerotic plaque formations. Note the extensive atherosclerotic plaques covering almost the whole upper part of the excised aorta in negative control rat (A), and the less important extent of plaques in the animal receiving the extract at dose 300 mg/kg (B). Note also that the comparable extent of plaques in HC rat treated with *Z. heitzii* extract at dose 375 mg/kg (C) and in NC rat (D).

4. Discussion

In the present study, crude stem bark extract of *Z. heitzii* significantly improved various risk factors of atherogenesis in rats chronically exposed to hypercholesterolemic (HC) diet (HC rats), including blood, aorta, liver and feces lipid profiles, blood and liver levels of markers of oxidative stress. The histopathological study of cross-sections of aortic intima revealed extensive atherosclerotic plaques covering almost the whole upper part of the excised aorta in negative control HC rats, whose formation was mitigated in HC rats given the extract, suggesting that the plant may possess anti-

Table 4

Effect of *Z. heitzii* extract on levels of total cholesterol, triglycerides, and TBARS in the liver, aorta, and/or feces.

Experimental groups		Samples	Total cholesterol(mg/g)	Triglycerides(mg/g)	TBARs(nmol/g)
HC rats+dH$_2$O		Liver	21.43±1.24	18.46±1.21	36.47±3.11
		Aorta	18.87±1.15	16.32±1.44	34.15±3.44
		Fecal	78.12±4.26	n/a	n/a
HC +*Z. heitzii* (mg/kg)	225	Liver	11.42±1.25**	12.32±1.33**	21.18±1.22**
		Aorta	12.32±0.23**	12.43±1.17*	19.28±2.47**
		Fecal	104.33±3.73**	n/a	n/a
	300	Liver	12.33±1.11**	13.22±0.89**	22.69±1.46**
		Aorta	12.12±0.16**	11.67±1.14*	19.26±2.12**
		Fecal	101.77±3.34**	n/a	n/a
	375	Liver	13.41±1.31**	14.32±1.32**	21.39±1.67**
		Aorta	12.11±0.67**	13.32±1.24*	20.39±2.21**
		Fecal	102.33±3.61**	n/a	n/a
NC rats		Liver	8.00±0.16**	7.23±0.63**	11.25±1.13**
		Aorta	6.23±0.27**	6.76±0.67**	12.17±1.17**
		Fecal	42.89±2.23	n/a	n/a

Data are mean±SEM, n=5. One-way ANOVA+LSD test against hypercholesterolemic (HC) rats: *P<0.05, ** P<0.01. n/a: not analyzed.

atherosclerogenic properties. This hypothesis was also sustained by the observation of less marked changes in morphometric parameters of aortas of animals given the extract concomitantly with HC diet exposure.

Dyslipidemia, the most important risk factor for atherosclerosis, is marked by an increase in the plasmatic levels of total cholesterol, LDL cholesterol and triglycerides, together with a reduction in HDL cholesterol[22,23]. In our study, plasmatic levels of these markers in rats concomitantly exposed to HC diet and *Z. heitzii* extract for four weeks were comparable with NC rats (normal control group) and atorvastatin-treated (positive control group) levels, suggesting that *Z. heitzii* extract significantly prevented dyslipidemia in rats exposed to HC diet, indicating suggesting hypotriglyceridemic and hypocholesterolemic effects. Considering that abnormal lipid profiles constituting the hallmark of HC-induced metabolic syndrome were also prevented in the liver by the extract concomitantly with a marked increase in total cholesterol excreted, we hypothesize that hypolipidemic activity of the extract may be mediated by reducing/inhibiting cholesterol intestinal absorption and increasing reverse cholesterol transport, as observed with agents inducing comparable hypolipidemic effects together with antioxidant effects such as ezetimibe[24,25] and bile acid sequestrant cholestyramine[26,27]. The extract decreased total cholesterol levels in the liver, aorta and feces, but unlike the mentioned classical anti-dyslipidemic agents[9,10], at the doses tested the extract failed to keep plasma total cholesterol at control levels. Considering that the extract significantly slowed the increase in this parameter compared with HC rats, it can be hypothesized that active principles of the extract may use different mechanisms of action than ezetimibe to induce their hypolipidemic effects, explaining differences in anti-dyslipidemic potential. Thus, further studies addressing mechanisms of action of the extract may prove useful for developing new classes of hypolipidemic and anti-dyslipidemic drugs with less cardiovascular side effects associated with total cholesterol drastic decrease[1,2,28].

In addition, and notably, *Z. heitzii* extract prevented HC diet-associated pathological changes in various oxidative stress markers[29,30] in the liver and blood, and mitigated lipid peroxidation. Notably, catalase level increase in the liver and in hemolysates, hydroperoxide increase and malondialdehyde decrease in the liver and in blood plasma, and increases in liver and aorta levels of TBARs were prevented in HC-diet exposed rats treated with the extract, in a dose-dependent fashion. These findings corroborate a recent *in vitro* study reporting antioxidant effects of fruit extract of *Z. heitzii*[15]. Oxidative stress being a major risk factor for atherogenesis[31,32], it appears that besides the aforementioned hypolipidemic effects, oxidative stress mitigation may represent another mechanism by which *Z. heitzii* extract may mediate anti-atherogenic effects. Also considering that oxidative stress, dyslipidemia, and atherosclerosis are three of the most important risk factors for cardiovascular diseases and conditions[1,2], the findings of this study suggest that *Z. heitzii* extract may have preventive and therapeutic effects in these pathologies. Furthermore, *Z. heitzii* fruit extract was also reported antisickling[15] and antifilarial effects[33]. The lack of major signs of toxicity following administration of the extract for four weeks suggests that it was well-tolerated[34-36], further encouraging characterizing the medicinal properties of *Z. heitzii*.

The present study assessed hypolipidemic, antioxidant, and anti-atherosclerogenic properties in aqueous extract of *Z. heitzii* bark, used in African traditional medicine against arterial hypertension. Findings suggested hypocholesterolemic effects probably mediated by inhibition of intestinal absorption of cholesterol and increase of fecal excretion of sterols, as well as hypotriglyceridemic and antioxidant effects, which explain at least partly the anti-atherosclerogenic effects also observed in the study. However, plasma total cholesterol was not markedly affected, suggesting that future studies addressing active principles of this plant may lead to the development of hypolipidemic and anti-dyslipidemic agents with less marked side effects associated with drastic decrease in total blood cholesterol.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors thank the Laboratory of the Medicinal Plants, Health and Galenic Formulation of the Department of Biological Sciences. and ALLARAMADJI Ndohortongar of N'Djaména hospital Le Bon Samaritain, for their assistance in this project.

References

[1] Li C, Zhang W, Zhou F, Chen C, Zhou L, Li Y, et al. Cholesteryl ester transfer protein inhibitors in the treatment of dyslipidemia: a systematic review and meta-analysis. *PLoS One* 2013; **8**: e77049.

[2] Schwingshackl L, Hoffmann G. Comparison of the long-term effects of high-fat v. low-fat diet consumption on cardiometabolic risk factors in subjects with abnormal glucose metabolism: a systematic review and meta-analysis. *Br J Nutr* 2014; **111**: 2047-2058.

[3] Gorinstein S, Caspi A, Libman I, Katrich E, Lerner HT, Trakhtenberg S. Preventive effects of diets supplemented with sweetie fruits in hypercholesterolemic patients suffering from coronary artery disease. *Prev Med* 2004; **38**: 841-847.

[4] Azorin-Ortuno M, Yanez-Gascon MJ, Pallares FJ, Rivera J, Gonzalez-Sarrias A, Larrosa M, et al. A dietary resveratrol-rich grape extract prevents the developing of atherosclerotic lesions in the aorta of pigs fed an atherogenic diet. *J Agric Food Chem* 2012; **60**: 5609-5620.

[5] Assmann G, Schulte H. Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAM experience). Prospective Cardiovascular Munster study. *Am J Cardiol* 1992; **70**: 733-737.

[6] Buyukhatipoglu H, Sezen Y, Yildiz A, Guntekin U, Bas M, Polat M,

et al. Effects of statin use on total oxidant and antoxidant capacity and ceruloplasmin activity. *Clin Invest Med* 2010; **33**: E313-E320.

[7] Bello A, Aliero AA, Saidu Y, Muhammad S. Hypoglycaemic and hypolipidaemic effects of *Leptadenia hastata* (Pers.) decne in alloxan induced diabetic rats. *Nigerian J Basic Appl Sci* 2011; **19**: 187-192.

[8] Sans S, Kesteloot H, Kromhout D. The burden of cardiovascular diseases mortality in Europe. Task Force of the European Society of Cardiology on Cardiovascular Mortality and Morbidity Statistics in Europe. *Eur Heart J* 1997; **18**: 1231-1248.

[9] Amano Y, Shimada M, Miura S, Adachi R, Tozawa R. Antidyslipidemic effects of a farnesoid X receptor antagonist in primates. *Life Sci* 2014; **106**: 25-31.

[10]Leyes P, Martinez E, Larrousse M, Cofan M, Trabal J, Perez-Heras AM, et al. Effects of ezetimibe on cholesterol metabolism in HIV-infected patients with protease inhibitor-associated dyslipidemia: a single-arm intervention trial. *BMC Infect Dis* 2014; **14**: 497.

[11]Lankin VZ, Tikhaze AK, Kukharchuk VV, Konovalova GG, Pisarenko OI, Kaminnyi AI, et al. Antioxidants decreases the intensification of low density lipoprotein *in vivo* peroxidation during therapy with statins. *Mol Cell Biochem* 2003; **249**: 129-140.

[12]Tenenbaum A, Fisman EZ. Fibrates are an essential part of modern anti-dyslipidemic arsenal: spotlight on atherogenic dyslipidemia and residual risk reduction. *Cardiovasc Diabetol* 2012; **11**: 125.

[13]Zirihi GN, Mambu L, Guede-Guina F, Bodo B, Grellier P. *In vitro* antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. *J Ethnopharmacol* 2005; **98**: 281-285.

[14]Sandberg F, Perera-Ivarsson P, El-Seedi HR. A Swedish collection of medicinal plants from Cameroon. *J Ethnopharmacol* 2005; **102**: 336-343.

[15]Mbaze LM, Lado JA, Wansi JD, Shiao TC, Chiozem DD, Mesaik MA, et al. Oxidative burst inhibitory and cytotoxic amides and lignans from the stem bark of *Fagara heitzii* (Rutaceae). *Phytochemistry* 2009; **70**: 1442-1447.

[16]Mikolo B, Overgaard HJ, Stangeland T, Malterud KE, Wangensteen H, Paulsen BS, et al. Adulticide activity in fractions of *Zanthoxylum heitzii* bark extract against *Anopheles gambiae* s.s. 6th European Mosquito Control Association Workshop, Budapest, Hungary Norwegian University of Life Sciences; 2011. [Online] Available at: www.umb.no

[17]Ngouela S, Tsamo E, Connolly JD. Lignans and other constituents of *Zanthoxylum heitzii*. *Int J Plant Biochem* 1994; **37**(3): 867-869.

[18]Overgaard HJ, Patcharawan S, Mikolo B, Malterud KE, Wangensteen H, Yuan-Feng Z, et al. Insecticidal activities of bark, leaf and seed extracts of *Zanthoxylum heitzii* against the African malaria vector *Anopheles gambiae*. *Molecules* 2014; **19**: 21276-21290; doi:10.3390/molecules191221276

[19]Pauline N, Cabral BN, Anatole PC, Jocelyne AM, Bruno M, Jeanne NY. The *in vitro* antisickling and antioxidant effects of aqueous extracts *Zanthoxyllum heitzii* on sickle cell disorder. *BMC Complement Altern Med* 2013; **13**: 162

[20]Itokawa Y, Inoue K, Sasagawa S, Fujiwara M. Effect of S-methylcysteine sulfoxide, S-allylcysteine sulfoxide and related sulfur-containing amino acids on lipid metabolism of experimental hypercholesterolemic rats. *J Nutr* 1973; **103**: 88-92.

[21]Mohamed AR, El-Hadidy WF, Mannaa HF. Assessment of the prophylactic role of aspirin and/or clopidogrel on experimentally induced acute myocardial infarction in hypercholesterolemic rats. *Drugs R D* 2014.

[22]Li L, Wang Y, Xu Y, Chen L, Fang Q, Yan X. Atorvastatin inhibits CD68 expression in aortic root through a GRP78-involved pathway. *Cardiovasc Drugs Ther*; 2014.

[23]Koba S, Hirano T. Dyslipidemia and atherosclerosis. *Nihon Rinsho* 2011; **69**: 138-143.

[24]Catapano AL, Farnier M, Foody JM, Toth PP, Tomassini JE, Brudi P, et al. Combination therapy in dyslipidemia: Where are we now? *Atherosclerosis* 2014; **237**: 319-335.

[25]Sudhop T, Lutjohann D, Kodal A, Igel M, Tribble DL, Shah S, et al. Inhibition of intestinal cholesterol absorption by ezetimibe in humans. *Circulation* 2002; **106**: 1943-1948.

[26]Davidson MH, Voogt J, Luchoomun J, Decaris J, Killion S, Boban D, et al. Inhibition of intestinal cholesterol absorption with ezetimibe increases components of reverse cholesterol transport in humans. *Atherosclerosis* 2013; **230**: 322-329.

[27]Terunuma S, Kumata N, Osada K. Ezetimibe impairs uptake of dietary cholesterol oxidation products and reduces alterations in hepatic cholesterol metabolism and antioxidant function in rats. *Lipids* 2013; **48**: 587-595.

[28]Nwozo SO, Orojobi BF, Adaramoye OA. Hypolipidemic and antioxidant potentials of *Xylopia aethiopica* seed extract in hypercholesterolemic rats. *J Med Food* 2011; **14**: 114-119.

[29]Psichas A, Little T, Lal S, McLaughlin J. Colestyramine slows gastric emptying of liquids and reduces appetite in healthy subjects. *Neurogastroenterol Motil* 2012; **24**: 1095-1101.

[30]Gershkovich P, Sivak O, Contreras-Whitney S, Darlington JW, Wasan KM. Assessment of cholesterol absorption inhibitors nanostructured aluminosilicate and cholestyramine using *in vitro* lipolysis model. *J Pharm Sci* 2012; **101**: 291-300.

[31]Buchwald H, Moore RB, Varco RL. Ten years clinical experience with partial ileal bypass in management of the hyperlipidemias. *Ann Surg* 1974; **180**: 384-392.

[32]Oh PS, Lee SJ, Lim KT. Hypolipidemic and antioxidative effects of the plant glycoprotein (36 kDa) from *Rhus verniciflua* stokes fruit in Triton WR-1339-induced hyperlipidemic mice. *Biosci Biotechnol Biochem* 2006; **70**: 447-456.

[33]Gupta RK, Kesari AN, Diwakar S, Tyagi A, Tandon V, Chandra R, et al. *In vivo* evaluation of anti-oxidant and anti-lipidimic potential of *Annona squamosa* aqueous extract in Type 2 diabetic models. *J Ethnopharmacol* 2008; **118**: 21-25.

[34]Mangge H, Becker K, Fuchs D, Gostner JM. Antioxidants, inflammation and cardiovascular disease. *World J Cardiol* 2014; **6**: 462-477.

[35]Tomey MI, Narula J, Kovacic JC. Advances in the understanding of plaque composition and treatment options: year in review. *J Am Coll Cardiol* 2014; **63**: 1604-1616.

[36]Mengome LE, Akue JP, Souza A, Feuya Tchoua GR, Nsi EE. *In vitro* activities of plant extracts on human *Loa loa* isolates and cytotoxicity for eukaryotic cells. *Parasitol Res* 2010; **107**: 643-650.