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Separate and co-administration of *Amaranthus spinosus* and vitamin C modulates cardiovascular disease risk in high fat diet-fed experimental rats

Abayomi S. Faponle[1], Adeleke Atunnise[1], Bukunola O. Adegbesan[1], Olugbenga O. Ogunlabi[1], Kuburat T. Odufuwa[1] and Emmanuel O. Ajani[2]*

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Ikenne Campus, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.
[2]Phytomedicine, Toxicology and Drug Development Laboratory, Department of Biosciences and Biotechnology, Kwara state University, Malete, P. M B. 1530, Ilorin, Nigeria.

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Report indicates that global death from cardiovascular diseases is more than any other diseases hence research is being intensified to provide scientific data in support of the use of traditional plants for its management. In this study, the efficacy of *Amaranthus spinosus* on lipid disorder occasioned by dietary regimen supplemented with lard and its role in oxidative stress was compared with vitamin C. Forty adult male rats randomized into 5 groups of 8 each were used. Group 1 was the control, while groups 2, 3, 4 and 5 were placed on lard supplemented diet. Leaf extract of *A. spinosus* was administered to rats in groups 3 while group 4 was co-administered with *A. spinosus* and vitamin C. Group 5 was administered with vitamin C alone. The extract was administered at a dose of 250 mg/kg while vitamin C was administered at a dose of 10 UI/kg. All administrations were performed orally as a single dose continuously for 28 days. High fat diet increased malondialdehyde concentration but reduced the concentrations of glutathione (GSH) and the activities of catalase and superoxide dismutase in the heart. It also increased plasma cholesterol, triglyceride, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol but lowered high density lipoprotein (HDL) cholesterol concentration. Although, no significant alterations were observed in the cholesterol and triglyceride levels of the heart, there was a significant increase in the atherogenic indices of plasma. Separate and combined administration of *A. spinosus* and vitamin C reverses these unfavorable alterations. The effect of separate administration of *A. spinosus* was also observed in the study to compare effectively with its combined administration with vitamin C. Based on this study, *A. spinosus* may be useful as a base medicine for the management of cardiovascular diseases (CVD) related disorder.

Key words: Atherogenic index, *Amaranthus spinosus,* cardiovascular risk, herbal medicine, high-fat diet, lipoprotein.

INTRODUCTION

Cardiovascular diseases (CVDs) are a class of pathologies involving the heart or blood vessels (arteries, capillaries, and veins). They refer to any disease that affects the cardiovascular system, mainly cardiac diseases, vascular diseases of the brain and kidney, and peripheral arterial disease (Ursula et al., 2014). Reported data (Sydney et al., 2012) indicated that an estimated 17.5 million people died from CVDs in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke and the number is expected to grow to 23.6 million by 2030 (World Health Organization (WHO), 2015). This report is supported by data from previous studies (Ignarro et al., 2007; Burta et al., 2008; Norozi et al., 2011).

Obesity, high blood pressure, insulin resistance, and aging are associated with the development of CVDs (De Marchi et al., 2013). Other important factors are diet, lifestyle, environmental, genetic and epigenetic interactions (Haslam and James, 2002). These factors reflect complex pathological processes in which oxidative stress caused by reactive oxygen species (ROS) plays a pivotal role. Oxidative stress represents an imbalance between reactive oxygen species (ROS) production and the cellular antioxidant defense system. In stress conditions, ROS levels increase, and because of their high reactivity, participate in a variety of chemical reactions. They are involved in cell damage, necrosis and apoptosis via oxidation of lipids, proteins and DNA (Elahi et al., 2009) and provoke also endothelial dysfunction, infiltration and activation of inflammatory cells (Hulsmans et al., 2012). Oxidative stress has been noted to play a central role in the pathogenesis of atherosclerosis and thus a critical feature in atherogenesis (Lee et al., 2012). An increased generation of ROS in the vascular wall and a reduction of nitric oxide (NO) bioavailability lead to endothelial dysfunction in atherogenesis (Lee et al., 2012; Channon and Guzik, 2012). ROS cause damage to cellular structures within the vascular wall, and trigger several redox-sensitive transcriptional pathways, shifting the cell towards a proatherogenic transcriptomic profile.

Increasing evidence indicates that certain natural products can influence the aetiology, progression and treatment of CVD (Osadolor et al., 2005; Amadou et al., 2009; Zuchi et al., 2010; Sahebkar, 2013) and this may occur by modifying risk factors such as obesity, dyslipidaemia as well as factors involved in systemic inflammation, oxidative stress and thrombosis (Parikh et al., 2005; Napoli et al., 2007). The role of dietary fat in health has been under intensive research during the past decades (Ursula et al., 2014). Many observational studies reported that the total amount of dietary fat has only a minor or no effect on the risk of lifestyle diseases such as CVD, type 2 diabetes mellitus (T2DM), and cancer or the level of the risk factors of these diseases, or markers (abdominal adiposity, blood pressure (BP), serum lipid profile, and insulin sensitivity) (Food and Agriculture Organization (FAO), 2010). However, the quality of fat has been shown to have a significant effect on serum lipid profile and blood pressure (BP) (Uusitupa et al., 2013) as well as endothelial function and low-grade inflammation, and these has been shown to affect the risk of CVD (Graham et al., 2007; FAO, 2010; Astrup et al., 2011; Lopez-Garcia et al., 2004; Uusitupa et al., 2013). Current studies are focused at investigating certain neutraciticals with potential to reduce cardiovascular disease risk by improving plasma lipid profile (Cinzia et al., 2010; Norata et al., 2013; Sahebkar et al., 2014).

Amaranthus spinosus L. (prickly amaranthus or water leaf) is known among the Yoruba tribe of Nigeria as “efo tete” or “tete eleegun”. It is a medicinal plant under the family of Amaranthaceae and an annual or perennial herb native to Tropical America (Bagepalli et al., 2010). The plant grows in cultivated areas as well as in waste places. The leaves are stacked and alternate (Debiprasad et al., 2013). Medicinal uses of *A. spinosus* as reported in the literatures (Bagepalli et al., 2010; Debriprasad et al., 2013) include: diuretic, stomach disorder, peptic ulcer and anemia (leaf infusion); gonorrhea, eczema and menorrhea (root paste). In some traditional homes in Nigeria, the plant is being used as analgesic, antipyretic, laxative, diuretic, anti-snake venum and antidiabetic.

Although the plant is very popular in the traditional medicine in Nigeria as anti-diabetic and anti- lipidemic agent suggesting that it may be a good agent in the management of cardiovascular diseases, but scientific evidence is lacking in support of this use. The aim of this study therefore is to provide scientific data to validate the use of the plant in the management of cardiovascular disorders and oxidative stress and compare its efficacy with that of vitamin C.

MATERIALS AND METHODS

Preparation of plant extract

Fresh leaves of *A. spinosus* were collected from a local garden in Ikenne, Nigeria in July, 2013. The plant materials were

*Corresponding author. E-mail: emmanuel.ajani@kwasu.edu.ng or ajaniimman@gmail.com. Tel: +2348055533192.

authenticated at the Department of Plant Science, Faculty of Science, Olabisi Onabanjo University, Nigeria. A voucher number Ars 013 NF was assigned and voucher specimen was thereafter deposited at the Herbarium. Twenty (20) grams of the powderedsample was macerated with 100 ml 70% methanol, filtered, concentrated using rotary evaporator (Yamato Scientific RE301A- W, Tokyo) and lyophilized with Hull brand (SP Scientific Series, USA) freeze-drier. Stock solution was prepared by dissolving the dried extracts in distilled water and was stored at -20°C until used.

Animal handling

Forty male Wistar strain rats, weighing between 150 and 220 g self-reed at the Animal holding, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ikenne, Nigeria were used in the study. The animals were kept under ambient condition and were allowed to acclimatize for a week while being fed with standard rat chow (obtained from Animal Care Nig. Ltd) and water *ad libitum.* Experimental animals were handled appropriately as outlined by the guidelines of Experimental Animals Ethics Code of the Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Nigeria. The protocol conforms to the guidelines of the National Institute of Health for laboratory animal care and use (NIH, 2011), and in accordance with the principles of good laboratory procedure (WHO, 1998). The rats were randomly assigned in to 5 groups of 8 rats each. The rats were then treated as follows:

Group 1: Normal control (standard rat chow),
Group 2: Test control (high dietary fat),
Group 3: Test I (high dietary fat and administered with *A. spinosus*, extract),
Group 4: Test II (high-fat fat and administered with *A. spinosus* extract + vitamin c),
Group 5: Test III (high dietary fat and administered with vitamin c).

High fat diet was prepared by supplementing the normal rat chow with 15% lard (Chi et al., 1982). All the rats were fed with their respective diet *ad libitum*. The extract and vitamin C were administered orally using oral intubator at a dose of 250 mg kg^{-1} and 10 UI kg^{-1}, respectively. All administrations were carried out once daily for 28 consecutive days.

Preparation of organ homogenate and blood sample

After the administration of last dose, the rats were fasted for 12 h and then anesthetized in a closed jar of cotton-soaked diethyl ether. Blood was withdrawn from the rats by cardiac puncture after which they were sacrificed by cervical dislocation. The blood samples were collected in heparinized bottles and the heart excised, weighed and stored in buffered petri-dishes before being homogenized in phosphate buffer (pH 7.3) (Paul et al., 2013). Afterwards, blood samples and heart homogenates were centrifuged and supernatant collected for biochemical analyses.

Biochemical assay

Assay for catalase and superoxide dismutase activities was according to the previously described methods of Sinha (1972) and Marklund and Marklund (1974), respectively. Lipid peroxidation was assessed by determining the malondialdehyde as decribed by Varshney and Kale (1990). Reduced glutathione was measured by the method of Beutler et al. (1963). Heart lipid was extracted using the method of Folch et al. (1957). Randox^{TM} diagnostic kits (Randox Laboratories, U.K.) was then used for estimation of the lipid content. Cholesterol and triglyceride were determined enzymatically (Belcher et al., 1991). HDL-cholesterol (HDL-C) was estimated by the HDL precipitant method (Bachorik and Albers, 1996). VLDL-cholesterol (VLDL-C) and LDL-cholesterol (LDL-C) were calculated using Friedewald et al. (1972) formula. Atherogenic index of plasma (AIP) was calculated using the formula of Abot et al. (1998) and coronary risk ratio (CRR) was obtained by the method of Allard et al. (1994).

VLDL-C = Serum triglyceride/2.2,
LDL-C = Total serum cholesterol-Total serum triglycerides/5-Total serum HDL-C,
AIP = Total serum triglycerides/Total serum HDL-C,
CRR = Total cholesterol/HDL-C.

Statistical analysis

Statistical package for social sciences (SPSS) *v*20.0 software package was used for data processing. Data analyses were done with one-way analysis of variance (ANOVA) and level of significance tested at $p \leq 0.05$ with Duncan multiple range test (DMRT).

RESULTS

When compared with the normal control, the result of the lipid peroxidation and antioxidant status (Table 1) showed that increased dietary fat significantly ($p \leq 0.05$) raised the level of malondialdehyde (MDA) in the rat and lowered significantly the level of reduced GSH and catalase activity. Separate administration of *A. spinosus* and vitamin C and when co-administered into rats placed on high dietary fat prevented increase in the level of heart MDA and significantly raised the level of reduced GSH and catalase activity. Although the catalase activity of rats placed on vitamin C (61.59 ± 2.09) was not different from that of those treated with *A. spinosus* (61.09 ± 1.47) and that which was co- administered with vitamin C and *A. spinosus* (65.94 ± 2.17), the level of reduced GSH of rats placed on vitamin C alone (25.97 ± 1.18) was observed to be lower than that of rats placed on *A. spinosus* (29.02 ± 1.58) and that of rats co-administered with vitamin C and *A. spinosus* (28.71 ± 1.18). No significant ($p \geq 0.05$) difference was observed in the superoxide dismutase (SOD) activity of the treated groups when compared among each other and the activities were also not significantly different from that of the control groups. No significant alteration was observed in the total cholesterol and triglyceride level of the heart both with high fat diet and when vitamin C and *A. spinosus* were either separately administered or co-administered to rats placed on high fat diet (Table 2).

Result of plasma lipid profile (Table 3) indicates that

Table 1. Effects of treatment on heart oxidative status.

Group	Treatment	MDA (nmol MDA/g tissue)	Catalase (µg/mg protein) ×10^3	SOD (ng/mg protein) × 10^3	Reduced GSH (µg/g tissue) × 10^3
1 (Normal control)	SRF	5.21 ± 0.19^a	54.61 ± 4.45^a	3.30 ± 0.01^{a,b}	28.13± 1.16^a
2 (Test Control)	HF	6.56 ± 0.08^b	25.15 ± 2.90^b	3.33 ± 0.00^b	22.20 ± 0.46^b
3 (Test 1)	HF + AS	5.21 ± 0.08^a	61.09 ± 1.47^c	3.25 ± 0.02^a	29.02 ± 1.58^a
4 (Test 2)	HF +As + VC	5.26 ± 0.12^a	65.94 ± 2.17^c	3.30 ± 0.01^{a,b}	28.71 ± 1.18^a
5 (Test 3)	HF + VC	4.56 ± 0.04^c	61.59 ± 2.09^c	3.32 ± 0.01^b	25.97 ± 0.85^c

Results presented are mean ± SEM (n=8). Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan's multiple range test (DMRT). Value in the same column with similar superscript are not significantly different (p>0.05) from each other. SRF = Standard Rat Feed, HF = High fat diet, AS= *Amaranthus spinosus*, VC = Vitamin C, MDA = Malondialadehyde, SOD = Superoxidedismutase and GSH = Reduced glutathione.

Table 2. Effect of treatment on the heart cholesterol and protein level.

Group	Treatment	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Total protein (mg/dl)
1 (Normal control)	SRF	89.70 ± 3.47^a	54.50 ± 0.93^a	5.88 ± 0.33^a
2 (Test control)	HF	88.70 ± 3.47^a	56.17 ± 2.55^a	5.46 ± 0.09^a
3 (Test 1)	HF + AS	86.90 ± 3.05^a	51.79 ± 3.58^a	5.20 ± 0.19^a
4 (Test 2)	HF + As + VC	82.00 ± 4.64^a	57.46 ± 3.27^a	5.17 ± 0.18^a
5 (Test 3)	HF + VC	87.60 ± 6.99^{a,}	57.17 ± 1.08^a	5.22 ± 0.12^a

Results presented are mean ± SEM (n=8). Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan's multiple range test (DMRT). Value in the same column with similar superscript are not significantly different (p>0.05) from each other. SRF = Standard Rat Feed, HF = High fat diet, AS= *Amaranthus spinosus*, VC = Vitamin C, MDA = Malondialadehyde, SOD = Superoxidedismutase, GSH = Reduced glutathione.

increased dietary fat significantly raised both the total cholesterol concentration (224.56 ± 9.03 mmol/L) and plasma triglyceride concentration (101.30 ± 6.68 mmol/L) significantly ($p \leq 0.05$) above the normal control (133.94 ± 0.95 mmol/L) concentration. Separate treatment with *A. spinosus* and vitamin C and their co-administration prevented this increase. The result also indicates that when separately administered, the concentration of plasma cholesterol of rats treated with vitamin c (158.18 ± 4.08 mmol/L) was significantly higher than that of the rat treated with *A. spinosus* (138.21 ± 0.42 mmol/L), but when co-administered, the plasma cholesterol concentration (126.81 ± 1.24 mmol/L) was lower than that of the rat treated with *A. spinosus* alone. No significant difference was observed in the plasma triglyceride concentration of rat treated with *A. spinosus* alone (67.48 ± 0.96 mmol/L) and that which was co-administered with *A. spinosus* and vitamin C (66.08 ± 4.03 mmol/L). The observed triglyceride concentration in both groups though lower than that of the group placed on high dietary fat without subsequent treatment (101.30 ± 6.68 mmol/L) was significantly higher ($p \leq 0.05$) than the normal control value (58.96 ± 0.36mmol/L). Separate administration of vitamin C also reduced the plasma triglyceride level below that of rats placed on high fat diet without treatment, however, the observed value of 92.16 ± 3.70 mmol/L was still higher than that of the group treated with *A. spinosus*.

Table 3 also indicates that high fat diet significantly ($p \leq 0.05$) increased the plasma LDL-cholesterol above the normal control value but lowered the plasma HDL-Cholesterol significantly ($p \leq 0.05$). There was no significant difference in LDL- concentration for all treatments. The observed plasma HDL-cholesterol concentration of rat separately treated with vitamin C and *A. spinosus* (73.29 ± 0.56 and 86.88 ± 5.41 mmol/L, respectively) were not different from each other and were also not different from that of rats co-administered with *A. spinosus* and vitamin C (74.75 ± 1.26 mmol/L) and the normal control value (79.19 ± 0.77 mmol/L). However, the observed values in all these groups of rats were significantly ($p \geq 0.05$) higher than that of the rats placed on high fat diet but without treatment (30.87 ± 0.04 mmol/L). The result of VLDL- cholesterol concentration followed the same pattern, however the observed VLDL-cholesterol concentration of 23.14 ± 1.03 mmol/L in rats treated with vitamin C alone was significantly higher than that of the rats treated with *A. spinosus* (13.51 ± 0.05

Table 3. Effect of treatment on plasma lipid profile.

Group	Treatment	Cholesterol (mmol/L)	Triglyceride (mmol/L)	LDL-cholesterol (mmol/L)	HDL-cholesterol (mmol/L)	VLDL-cholesterol (mmol/L)
1 (Normal control)	SRF	133.94 ± 0.95[a]	58.96 ± 0.36[a]	31.95 ± 1.48[a]	79.19 ± 0.77[a]	13.10 ± 0.07[a]
2 (Test control)	HF	224.56 ± 9.03[b]	101.30 ± 6.68[b]	42.43 ± 1.21[b]	30.87 ± 0.04[b]	22.09 ± 1.01[b]
3 (Test 1)	HF + AS	138.21 ± 0.42[a]	67.48 ± 0.96[c]	33.84 ± 5.51[b]	86.88 ± 5.41[a]	13.51 ± 0.05[a]
4 (Test 2)	HF + As + VC	126.81 ± 1.24[c]	66.08 ± 4.03[c]	38.85 ± 0.82[b]	74.75 ± 1.26[a]	13.29 ± 0.61[a]
5 (Test 3)	HF + VC	158.18 ± 4.08[d,]	92.16 ± 3.70[b]	38.46 ± 4.23[b]	73.29 ± 0.56[a]	23.14 ± 1.03[b]

Results presented are mean ± SEM (n=8). Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan's multiple range test (DMRT). Value in the same column with similar superscript are not significantly different (p>0.05) from each other. SRF = Standard Rat Feed, HF = High fat diet, AS = *Amaranthus spinosus*, VC = Vitamin C, MDA = Malondialadehyde, SOD = Superoxidedismutase, GSH = Reduced glutathione.

mmol/L) alone and that of rats co-administered with *A. spinosus* and vitamin C (13.29 ± 0.61 mmol/L).

Table 4 shows that high fat diet significantly ($p \geq 0.05$) increased the atherogenic indices (atherogenic risk index of plasma, AIP and coronary risk ratio, CRR). However, in rats placed on high fat diet and separately treated with *A. spinosus,* the AIP (0.81 ± 0.01) and CRR (1.60 ± 0.03) were not significantly different ($p \geq 0.05$) from those of the normal control value (0.77 ± 0.01 and 1.68 ± 0.01, respectively). Similarly, the observed AIP (0.89 ± 0.05) and CRR (1.70 ± 1.10) in rats co administered with *A spinosus* and vitamin C, were not different from that of the normal control value. Although the AIP and CRR was reduced during treatment with vitamin C alone (when compared with the normal control value), the observed values were significantly higher than those observed in the normal control group.

DISCUSSION

Cardiovascular disease is currently one of the world's leading causes of death (Ikewuchi and Ikewuchi, 2009). One of the major risk factors for the development of cardiovascular disease is dyslipidemia (American Diabetic Association, 2004; Shen, 2007). Dyslipidemia usually involve elevated plasma levels of triglycerides, total cholesterol, LDL and VLDL-cholesterol and a low level of HDL-cholesterol (Howard, 1987; Lekin and Lipsky, 2003; American Diabetic Association, 2004). Our result indicates that lard is a dietary fat capable of predisposing to cardiovascular disease. This is indicated by increased plasma cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol and a reduce HDL-cholesterol observed in the study when rats were placed on lard supplemented diet. Lard is an animal fat which contains saturated fatty acid. Over the years, studies had reported an association between intake of dietary saturated fatty acids (SFA) and serum cholesterol levels (Mensink et al., 2003; Lukas and George, 2014) Therefore, any nutritional and pharmacologic intervention that improves or normalizes abnormal lipid metabolism may be useful in reducing the risk of cardiovascular diseases. Data from our study thus support this assertion.

Presently, several drugs are available for the management of dyslipidemia. However, there is renewed interest in the use of herbal products (Mudhaffar, 2013; Assmann et al., 2008). This is partly due to the perceived safety of herbal drug and poverty level particularly among the rural populace. Report from the present study indicates that *A. spinosus* leaves contain phytochemicals capable of ameliorating symptoms of cardio-vascular disease. Rats treated with the extract while subsisting on high fat diet showed a reduced level of triglyceride, plasma total cholesterol, plasma LDL-cholesterol and VLDL-cholesterol and an increased level of plasma HDL-cholesterol when compared with those not treated with the extract. A high plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases (Dobiasova, 2004; McBride, 2007) and is often associated with hypertension, abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus (Ostlund and Lin, 2007; McBride, 2007).

Our study demonstrated a reduction in triglyceride level occasioned by *A. spinosus* in rats

Table 4. Effect of Treatment on Atherogenic and Coronary Risk Indices.

Group	Treatment	AI	CRR
1 (Normal control)	SRF	0.77 ± 0.01	1.68 ± 0.01
2 (Test control)	HF	3.45 ± 0.05	7.45 ± 0.12
3 (Test 1)	HF + AS	0.81 ± 0.01	1.60 ± 0.03
4 (Test 2)	HF + AS + VC	0.89 ± 0.05	1.70 ± 0.10
5 (Test 3)	HF + VC	1.26 ± 0.06	2.16 ± 0.05

Results presented are mean ± SEM (n=8). Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan's multiple range test (DMRT). Value in the same column with similar superscript are not significantly different (p>0.05) from each other. SRF = Standard Rat Feed, HF = High fat diet, AS = *Amaranthus spinosus*, VC = Vitamin C, MDA = Malondialadehyde, SOD = Superoxidedismutase, GSH = Reduced glutathione, AI = Atherogenic Index, CRR: Coronary Risk Index.

placed on high fat diet. We also observed that along with triglyceride and total cholesterol, *A. spinosus* also caused significant reduction in plasma LDL-cholesterol and VLDL-cholesterol and an increase in LDL-cholesterol, suggesting that it is a potent agent in reducing risk of cardiovascular disease. This is in conformity with reports from previous study which indicated that an elevated LDL-cholesterol concentration in plasma is atherogenic (Rang et al., 2005; Lichtennstein et al., 2006; Martirosyan et al., 2007), whereas**,** a high HDL-cholesterol level is cardioprotective (Miller and Miller, 1977; Assmann et al., 2008).

Atherogenic indices are powerful indicators of the risk of heart disease and have been successfully used as an additional index when assessing cardiovascular (CV) risk factors: the higher the value, the higher the risk of developing cardiovascular disease and *vice versa* (Frohlich and Dobiasova, 2003; Dobiasova, 2004; Brehm et al., 2004; Takasaki, 2005). AIP values of −0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high CV risk (Dobiasova, 2006). Observation from our study indicates that high fat diet increases atherogenic indices (AI and CRR) suggesting that high fat diet predisposes to cardiovascular diseases. *A. spinosus* was observed to significantly reduce atherogenic indices (CRR and AIP). The effect produced by separate administration of the extract was not different from that observed with co-administration of the extract and vitamin C. Although separate administration of vitamin c was also observed in our study to also reduce atherogenic indices, the effect was however not as pronounced as it was with *A. spinosus*. In an attempt to be able to investigate the role of oxidative stress in the development of CVD and the possible mechanism by which *A. spinosus* ameliorates the disorder, we assessed the oxidative status of the heart during treatment. Result from the study indicate that rats placed on high fat diet showed increased MDA level, decreased catalase activity and decrease reduced glutathione concentration. Administration of *A. spinosus* during treatment however prevented increase in malondialdehyde level and also boosted the antioxidant status. Thus, this suggests that the extract is capable of reducing oxidative stress induced by high dietary fat.

The imbalance between ROS production and the cellular antioxidant defense system resulting from oxidative stress has been implicated in cell damage, necrosis and apoptosis via oxidation of lipids, proteins and DNA (Elahi et al., 2009) and provoke also endothelial dysfunction, infiltration and activation of inflammatory cells (Hulsmans et al., 2012). Studies have reported that oxidative stress has a central role in the pathogenesis of atherosclerosis and that it is indeed a critical feature in atherogenesis (Liao et al., 1994; Elahi et al., 2009; Lee et al., 2012). An increased generation of ROS in the vascular wall and a reduction of nitric oxide (NO) bioavailability lead to endothelial dysfunction in atherogenesis (Lee et al., 2012). ROS cause damage to cellular structures within the vascular wall, and trigger several redox-sensitive transcriptional pathways, shifting the cell towards a proatherogenic transcriptomic profile. Animal models of atherosclerosis demonstrate the involvement of ROS in atherosclerosis by the accumulation of lipid peroxidation products (Liao et al., 1994). This is supported by the report from our study when we observed increased MDA level with increased fat diet. ROS and reactive nitrogen species (RNS) produced by the endothelium promote oxidative modification of low density lipoprotein in the phase that precedes the transfer into the subendothelial space of the arterial wall, where they initiate atherosclerosis (Stocker and Keaney, 2004). Data from our study thus suggest that *A. spinosus* contains bioactive component which has antioxidative potential and thus is able to mop up these ROS and prevent subsequent damage to the cellular macromolecules.

Studies on the stem bark extract of *A. spinosus* identified the betalains isolated from the bark of the plant

as amaranthin, isoamaranthine, hydroxycinnamates, rutin, quercetin and kaempferol glycosides (Stintzing et al., 2004; Ashok et al., 2008). Betalains are well known for their antioxidant, anticancer, antiparasitosis and antiviral properties (Hussain et al., 2008). Our study thus suggests the antioxidant properties of *A. spinosus* as a basis for its cardioprotective effect. This agrees with previous reports on cardiovascular protective effect of *Vaccinium meridionale* (Yasmin et al., 2013), *Phyllanthus emblica* and *Piper rostratum* (Wattanpitayakul et al., 2005) and methanolic extract of *Amaranthus viridis* **(**Saravanan et al., 2013).

Conclusion

Our study showed antihyperlipidaemic effect of *A. spinosus*, indicating a possible protective mechanism of the plant against the development of atherosclerosis and coronary heart disease in high fat fed rat. The study also lends support to the antioxidative potential of the plant and suggests that this is the mechanism by which it serves as a cardioprotective agent. Provided animal to human extrapolation is allowed, our result suggests that the plant may be recommended as base medicine for use in managing cardiovascular diseases and in the development of drug against cardiac related disorder.

Conflicts of interest

Authors have none to declare.

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