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RESEARCH ARTICLE

Influence of Moringa (*Moringa oleifera*) leaf extracts on the antioxidant and angiotensin-1 converting enzyme inhibitory properties of lisinopril

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Abstract
This study investigated the in vitro effect of various combinations of aqueous extracts of moringa (*Moringa oleifera*) leaves on the ACE inhibitory and antioxidant properties of lisinopril, which is a popular synthetic ACE inhibitor. Moringa leaves were air-dried and blended into powdery form. Thereafter, the aqueous extract was prepared and freeze-dried. The following sample mixtures were prepared: L = 100% lisinopril (1 mg/ml); M = 100% moringa extract (1 mg/ml); 1L + 3M = 25% lisinopril + 75% moringa extract; L + M = 50% lisinopril + 50% moringa extract; 3L + 1M = 75% lisinopril + 25% moringa extract. The results revealed that 100% moringa and the various combinations showed higher ACE inhibition than 100% lisinopril in both rat heart and lung homogenates. Furthermore, all the samples caused the inhibition of Fe^{2+}-induced lipid peroxidation in rat heart and lung tissue homogenates, with moringa leaf extract having the highest inhibitory ability. All the samples also showed antioxidant properties (ferric reducing antioxidant property, 2,2'-azino-bis (-3-ethylbenzthiazoline-6-sulphonate [ABTS*] and 1,1-diphenyl-2-picrylhydrazyl [DPPH] free radicals scavenging abilities, and Fe^{2+} chelating ability). This study revealed that moringa leaf extracts could improve the antioxidant and ACE inhibitory properties of lisinopril. However, further in vivo experiments and clinical trials are recommended.

Keywords Angiotensin-1 converting enzyme · Antioxidant · Lipid peroxidation · Lisinopril · Moringa

Introduction

A key therapeutic approach in the management of high blood pressure and related disorders is the inhibition of angiotensin-1 converting enzyme (ACE) because the renin-angiotensin system (RAS) plays a key role in the regulation of blood pressure (Oboh and Ademosun 2011). Renin produces angiotensin I, which is cleaved by ACE to release angiotensin II. Angiotensin II is a potent vasoconstrictor, bradykinin inactivator and depressor (Erdos and Skidgel 1987). Increased angiotensin II production has also been linked to increased oxidative stress (Sprague and Khalil 2009; Virdis et al. 2011). Therefore, the use of ACE inhibitors with antioxidative properties is considered a key therapeutic approach to the management of high blood pressure (Rodriguez–Rodriguez and Simonsen 2012). Although several ACE inhibitors such as enalapril, captopril, and lisinopril are in use, natural products are also been promoted as complementary therapy for the management of high blood pressure.

Moringa (*Moringa oleifera*) leaves are used in folk medicine and alternative therapy for the management of various degenerative conditions such as diabetes, erectile dysfunction, cognitive disorder and high blood pressure (Stohs and Hartman 2015; Oboh et al. 2016; Adefegha et al. 2017). Moringa leaves are also popular in tropical regions of the world because the leaves can be consumed in many different forms. The leaves are eaten in the raw form, cooked or as infusions. Oboh et al. (2015) had earlier reported the antioxidant properties of the leaves and its use as an ACE inhibitor. The authors also linked these bioactivities to the phenolic compounds they identified such as gallic acid, catechin, chlorogenic acid, ellagic acid, epicatechin, rutin, quercitrin, isoquercitrin, quercetin, and kaempferol.

Several patients combine the use of synthetic ACE inhibitors with moringa leaves for the management of high blood

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pressure. This practice is gaining popularity even though there is dearth of information on the effect of such combinations on the ACE inhibitory properties of synthetic inhibitors.

Therefore, this study investigated the in vitro effect of various combinations of moringa leaves aqueous extracts with lisinopril, which is a popular synthetic ACE inhibitor. The synergistic or antagonistic effect of the combinations on the antioxidant properties of the extracts or lisinopril was also investigated.

Materials and methods

Sample collection and preparation

The moringa leaves were collected from Akure main market. Authentication of the samples was carried out at the Department of Crop, Soil and Pest management (CSP), Federal University of Technology, Akure, Nigeria. The moringa leaves were air-dried and blended into powdery form using Warring Commercial heavy Duty Blender (Model 37BL18; 24ØCB6). 5 g of the powdered sample was soaked in 100 ml of distilled water and left for 24 h. The mixture was filtered after 24 h and the filtrate kept in a refrigerator below 0 °C. The frozen extract solution was recovered as dried extract under pressure of approximately—450 mbar at 40 °C. The dried extract was reconstituted in distilled water (1 mg/ml) and used for subsequent analysis. Lisinopril was purchased from Teva Pharmaceutical Limited. A stock concentration of 1 mg/ml lisinopril was prepared for subsequent use. Thereafter, the following sample mixtures were prepared: L = 100% lisinopril (1 mg/ml); M = 100% moringa extract (1 mg/ml); 1L + 3M = 25% lisinopril + 75% moringa extract; L + M = 50% lisinopril + 50% moringa extract; 3L + 1M = 75% lisinopril + 25% moringa extract. All samples were kept in the refrigerator at 4 °C for subsequent analysis.

Determination of total phenolic content

Briefly, 1 mg/ml of extract was oxidized with 2.5 ml of 10% Folin-Ciocalteu's reagent (v/v) and neutralized with 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 °C and absorbance was measured at 765 nm using a UV/Visible Spectrophotometer. Total phenolic content was subsequently calculated as the gallic acid equivalent (Singleton et al. 1999).

Determination of total flavonoid content

Briefly, 0.5 ml of extract (1 mg/ml) was mixed with 0.5 ml of methanol, 50 ml of 10% $AlCl_3$, 50 ml of 1M potassium acetate and 1.4 ml of water and incubated at room temperature for 30 min. Absorbance of the reaction mixture was subsequently measured at 415 nm, and total flavonoid content was calculated and expressed as the quercetin equivalent (Meda et al. 2005).

Angiotensin I-converting enzyme (ACE) inhibition assay

Five male Wistar albino rats (150–200 g) were decapitated under mild diethyl ether anesthesia, and the hearts and lungs were removed and weighed. These tissues were subsequently homogenized in cold saline using glass homogenizer. The homogenate was centrifuged. The moringa extract and lisinopril (1 mg/ml) and homogenates were incubated at 37 °C for 15 min. The enzymatic reaction was initiated by adding 150 ml of 8.33 mM of the substrate Bz–Gly–His–Leu in 125 mM Tris–HCl buffer (pH 8.3) to the mixture. After incubation for 30 min at 37 °C, the reaction was arrested by adding 250 ml of 1 M HCl. The Gly–His bond was then cleaved and the Bz–Gly produced by the reaction was extracted with 1.5 ml ethyl acetate. Thereafter the mixture was centrifuged to separate the ethyl acetate layer; then 1 ml of the ethyl acetate layer was transferred to a clean test tube and evaporated. The residue was re-dissolved in distilled water and its absorbance was measured at 228 nm. The ACE inhibitory activity was expressed as percentage inhibition (Cushman and Cheung 1971).

Lipid peroxidation and thiobarbituric acid reactions assay

Five male Wistar albino rats (150–200 g) were decapitated under mild diethyl ether anesthesia, and the hearts and lungs were removed and weighed. These tissues were subsequently homogenized in cold saline using glass homogenizer. The homogenate was centrifuged. Hundred microliters of tissue homogenate supernatant was mixed with a mixture containing 0.1 M Tris–HCl buffer (pH 7.4), study samples and pro-oxidants (250 µM Fe^{2+}). The volume was made up with 300 µl of distilled water before incubation at 37 °C for 2 h. The color reaction was developed by adding 8.1% sodium dodecyl sulphate (SDS) to the reaction mixture containing the homogenate, followed by the addition of acetic acid/HCl (pH 3.4) and 0.8% thiobarbituric acid. This mixture was incubated at 100 °C for 1 h. The absorbance of thiobarbituric acid reactive species (malondialdehyde) produced was measured at 532 nm (Ohkawa et al. 1979; Belle et al. 2004).

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability

The free radical scavenging ability of the moringa extracts and lisinopril against DPPH free radical was evaluated as

described by Gyamfi et al. (1999). Briefly, appropriate dilutions of the extracts (1 ml) were mixed with 1 ml 0.4 mM DPPH radicals dissolved in methanol. The mixture was left in the dark for 30 min, and the absorbance was taken at 516 nm. The experiment was controlled by using 2 ml DPPH solution without the test samples. The DPPH free radical scavenging ability was subsequently calculated as percentage of the control.

Determination of 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonate (ABTS) radical scavenging ability

The ABTS* scavenging ability of lisinopril and moringa leaf extract were determined according to the method described by Re et al. (1999). The ABTS* was generated by reacting an (7 mmol/l) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mmol/l, final concentration) in the dark for 16 h and adjusting the Absorbance at 734 nm to 0.700 with ethanol. 0.2 ml of appropriate dilution of lisinopril and moringa leaf extract was added to 2.0 ml ABTS* solution and the absorbance were measured at 734 nm after 15 min. The trolox equivalent antioxidant capacity was subsequently calculated.

Determination of ferric reducing antioxidant property

The reducing property of the lisinopril and moringa leaf extract were determined by assessing the ability of the samples to reduce $FeCl_3$ solution as described by Oyaizu (1986). 2.5 ml aliquot was mixed with 2.5 ml 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. and then 2.5 ml 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property was subsequently calculated.

Fe^{2+} chelating assay

The Fe^{2+} chelating ability of lisinopril and moringa leaf extract were determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel et al. (2005). Freshly prepared 500 µM $FeSO_4$ (150 µl) was added to a reaction mixture containing 168 µl 0.1 M Tris–HCl (pH 7.4), 218 µl saline and the samples (0–100 µl). The reaction mixture was incubated for 5 min, before the addition 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer.

Data analysis

The results of replicates will be pooled and expressed as mean standard error and the least significance difference and one—way analysis of variance (ANOVA) were determined (Zar 1984).

Results

The results of the phenolic distribution of the moringa leaf extracts are presented in Table 1. The total phenol content is 14.3 mg/100 g and the total flavonoid content is 4.6 mg/100 g. The effect of moringa leaf extracts, lisinopril and combinations on ACE activity in rat heart homogenates is presented in Fig. 1. Moringa extract and a combination of 75% moringa with 25% lisinopril (1L + 3M) had the highest ACE inhibitory abilities, while lisinopril (71.6%) and a 50% moringa and 50% lisinopril (L + M) (73.4%) had the least inhibitory abilities. Similarly, 100% lisinopril (62.31%) had the least ACE inhibition in rat lung homogenates, while 100% moringa (74.6%) had the highest (Fig. 2). Furthermore, incubation of rat heart homogenate in presence of Fe^{2+} caused a significant increase ($P < 0.05$) in the MDA content (Fig. 3); however, the samples inhibited MDA production, with moringa leaf extract having the highest inhibitory ability. Combinations of moringa leaf extract and lisinopril reduced MDA production to 55.4, 57.5 and 47.5% for 1L + 3M, L + M and 3L + 1M respectively. Similarly, incubation of rat lungs homogenate in presence of Fe^{2+} caused a significant increase ($P < 0.05$) in the MDA content (Fig. 4). 100% moringa extract and a combination of 75% lisinopril and 25% moringa had the highest inhibition of MDA production.

Figure 5 presents the DPPH* scavenging ability of the study samples. 100% moringa leaf extract had the highest scavenging ability (78.14%), while 100% lisinopril had the least scavenging ability (52.25%). The scavenging abilities of the combinations ranged from 66.11% (L + M) to 76.32% (3L + 1M). 100% moringa also had the highest ABTS* scavenging ability (Fig. 6), while lisinopril had the least. Figure 7 shows the ferric reducing antioxidant properties of the samples. 3L + 1M had the least reducing ability, while 1L + 3M had the highest reducing ability. The Fe2 + chelating ability of the study samples (Fig. 8) revealed that 100%

Table 1 The total phenolic and flavonoid content of Moringa leaf extract

Total phenol (gallic acid equivalent) (mg/100 g)	14.3 ± 2.1
Total flavonoid (quercetin equivalent)(mg/100 g)	4.6 ± 0.6

Values represent means ± standard deviation of triplicate readings

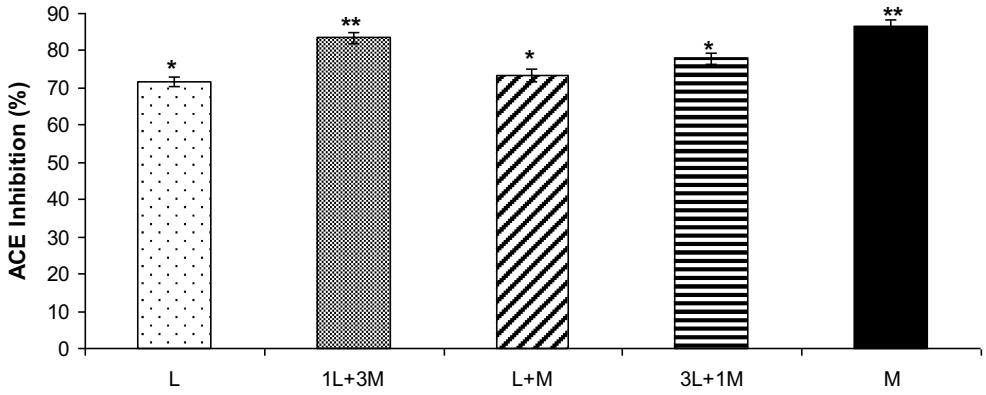

Fig. 1 The effect of moringa leaf extracts, lisinopril and combinations on ACE activity in rat heart homogenates. L = 100% lisinopril (1 mg/ml); M = 100% moringa extract (1 mg/ml); 1L + 3M = 25% lisinopril + 75% moringa extract; L + M = 50% lisinopril + 50% moringa extract; 3L + 1M = 75% lisinopril + 25% moringa extract. Values represent mean ± standard deviation of triplicate readings. Bars with same annotation (*, **, ***) are not significantly different

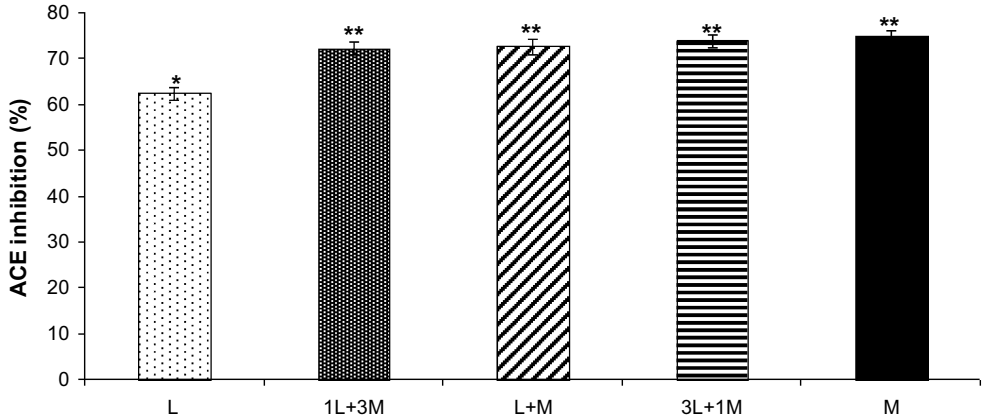

Fig. 2 The effect of moringa leaf extracts, lisinopril and combinations on ACE activity in rat lung homogenates. L = 100% lisinopril (1 mg/ml); M = 100% moringa extract (1 mg/ml); 1L + 3M = 25% lisinopril + 75% moringa extract; L + M = 50% lisinopril + 50% moringa extract; 3L + 1M = 75% lisinopril + 25% moringa extract. Values represent mean ± standard deviation of triplicate readings. Bars with same annotation (*, **, ***) are not significantly different

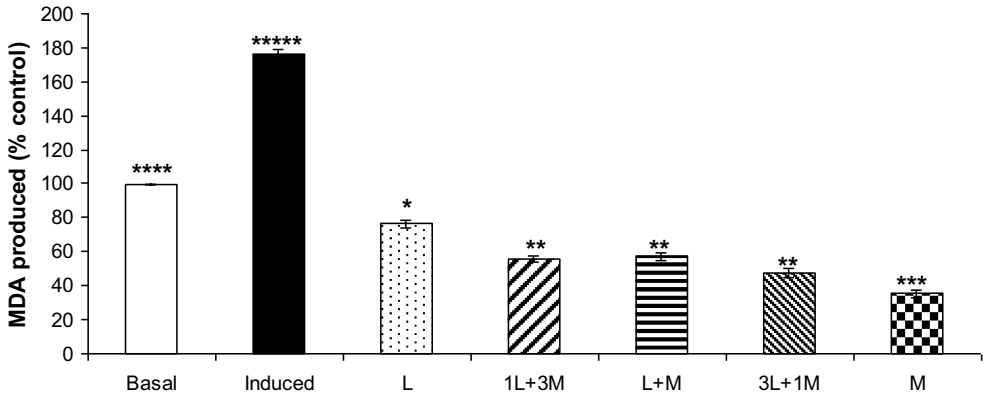

Fig. 3 The effect of moringa leaf extracts, lisinopril and combinations on Fe^{2+}-induced lipid peroxidation in rat heart homogenates. L = 100% lisinopril (1 mg/ml); M = 100% moringa extract (1 mg/ml); 1L + 3M = 25% lisinopril + 75% moringa extract; L + M = 50% lisinopril + 50% moringa extract; 3L + 1M = 75% lisinopril + 25% moringa extract. Values represent mean ± standard deviation of triplicate readings. Bars with same annotation (*, **, ***) are not significantly different

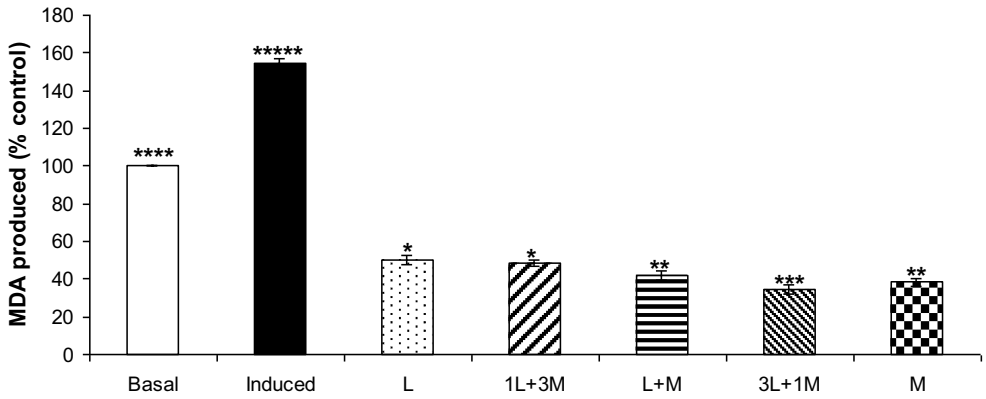

Fig. 4 The effect of moringa leaf extracts, lisinopril and combinations on Fe^{2+}-induced lipid peroxidation in rat lung homogenates. L = 100% lisinopril (1 mg/ml); M = 100% moringa extract (1 mg/ml); 1L + 3M = 25% lisinopril + 75% moringa extract; L + M = 50% lisinopril + 50% moringa extract; 3L + 1M = 75% lisinopril + 25% moringa extract. Values represent mean ± standard deviation of triplicate readings. Bars with same annotation (*, **, ***) are not significantly different

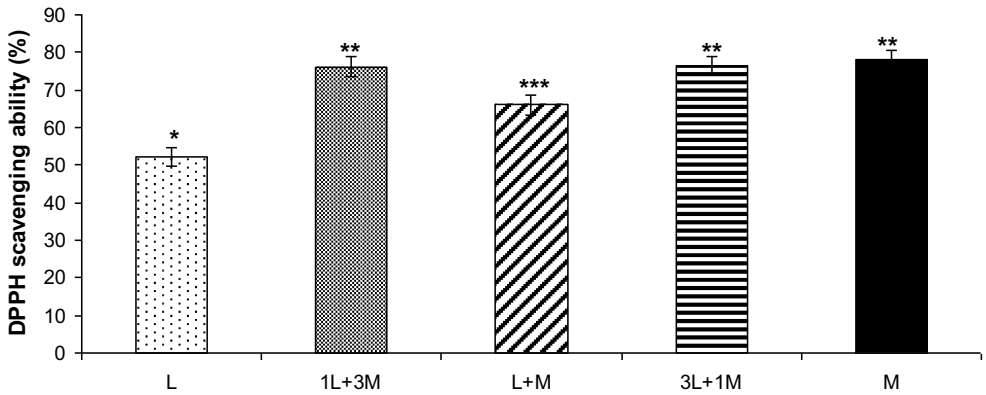

Fig. 5 DPPH* scavenging abilities of moringa leaf extracts, lisinopril and combinations. L = 100% lisinopril (1 mg/ml); M = 100% moringa extract (1 mg/ml); 1L + 3M = 25% lisinopril + 75% moringa extract; L + M = 50% lisinopril + 50% moringa extract; 3L + 1M = 75% lisinopril + 25% moringa extract. Values represent mean ± standard deviation of triplicate readings. Bars with same annotation (*, **, ***) are not significantly different

moringa (63.54%) had the highest chelating ability, 100% lisinopril had the least chelating ability (40.87%), while the chelating abilities of the combinations ranged from 46.36 to 59.86%.

Discussion

Several studies have reported the ACE inhibitory properties of natural products (Oboh and Ademosun 2011). More specifically, the ACE inhibitory property of Moringa leaf extracts had been previously reported. The ACE inhibitory properties of Moringa leaf extracts as shown in this study could be linked to the flavonoid contents as previous studies have reported the ACE inhibitory properties of flavonoids (Guerrero et al. 2012). We had previously reported that flavonoids such as rutin, quercitrin, isoquercitrin, quercetin and kaempferol were present in moringa leaves (Oboh et al. 2015). These flavonoids inhibit ACE activity competitively based on their possession of a double bond between C2 and C3 at the C-ring, presence of acetone group at the C4 carbon of the C-ring and the presence of a catechol group in the B-ring (Guerrero et al. 2012). This study revealed that moringa leaf extract had significantly higher ACE inhibition in the heart and lung homogenates than lisinopril. However, combinations of the extract and lisinopril did not show any synergistic effect. More specifically, in the rat lung homogenates, there was no significant difference in the inhibitory abilities of the combinations. We hypothesized that the phenolic compounds present in the moringa leaf extract and the lisinopril compete for the enzyme active site. This is due to the fact that two competitive inhibitors do not act synergistically on the same target enzyme (Breitinger 2012). It is noteworthy that the various combinations showed higher

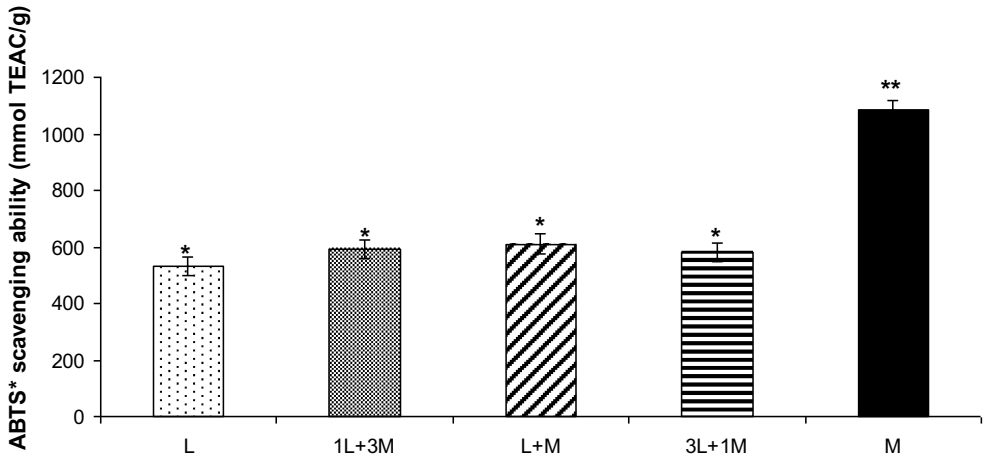

Fig. 6 ABTS* scavenging abilities of moringa leaf extracts, lisinopril and combinations. L = 100% lisinopril (1 mg/ml); M = 100% moringa extract (1 mg/ml); 1L + 3M = 25% lisinopril + 75% moringa extract; L + M = 50% lisinopril + 50% moringa extract; 3L + 1M = 75% lisinopril + 25% moringa extract. Values represent mean ± standard deviation of triplicate readings. Bars with same annotation (*, **, ***) are not significantly different

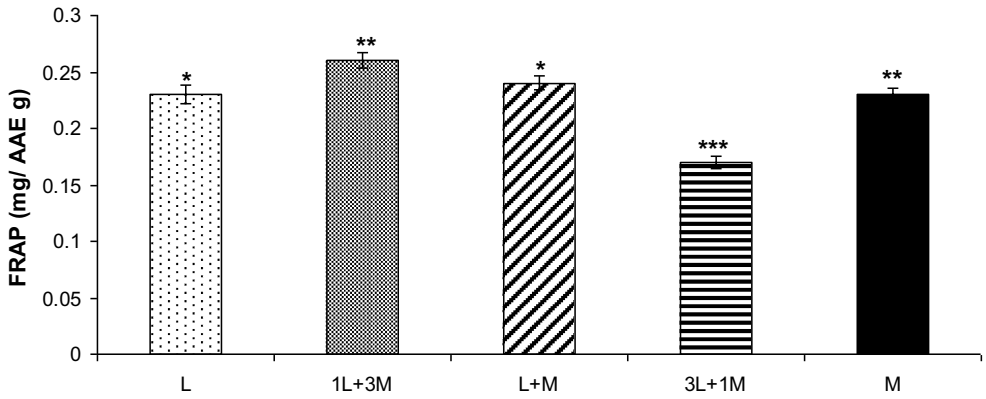

Fig. 7 Ferric reducing antioxidant properties of moringa leaf extracts, lisinopril and combinations. L = 100% lisinopril (1 mg/ml); M = 100% moringa extract (1 mg/ml); 1L + 3M = 25% lisinopril + 75% moringa extract; L + M = 50% lisinopril + 50% moringa extract; 3L + 1M = 75% lisinopril + 25% moringa extract. Values represent mean ± standard deviation of triplicate readings. Bars with same annotation (*, **, ***) are not significantly different

inhibitory effect than lisinopril, suggesting that moringa leaf could improve the ACE inhibitory effects of lisinopril.

Lisinopril is a non-SH containing ACE inhibitor and this has contributed to its ability to inhibit lipid peroxidation (Chopra et al. 1992). Kirbas et al. (2013) observed that lisinopril reduced MDA production and caused an increase in the activities of enzymes such as superoxide dismutase, catalase and glutathione peroxidase in rats with L-NAME induced hypertension. Thus, suggesting the antioxidant potential of lisinopril. However, this study revealed that moringa leaf extracts could improve the ability of lisinopril to prevent lipid peroxidation in rat heart and lung tissue homogenates. Moringa leaves have been shown from previous studies to prevent oxidative damage in tissues (Oboh and Ademosun 2011) and the antioxidant properties of the extracts could be attributed to the presence of phenolic compounds with hydroxyl groups at the 3 and 5 positions or a catechol moiety in the B-ring (Bourne and Rice-Evans 1998). The increase in MDA content when heart and lung homogenates were incubated in the presence of Fe^{2+} as shown in this study is through the decomposition of H_2O_2 to produce OH*. Therefore, the ability of the lisinopril and moringa leaves to chelate Fe^{2+} would prevent it from participating in the initiation of lipid peroxidation (Oboh and Rocha 2007; Dastmalchi et al. 2007).

Furthermore, the most commonly used method for the determination of reducing property is the ferric reducing antioxidant activity. Our results showed that the combination of lisinopril and moringa showed antagonistic effect as the ratio of lisinopril to moringa increased. An important

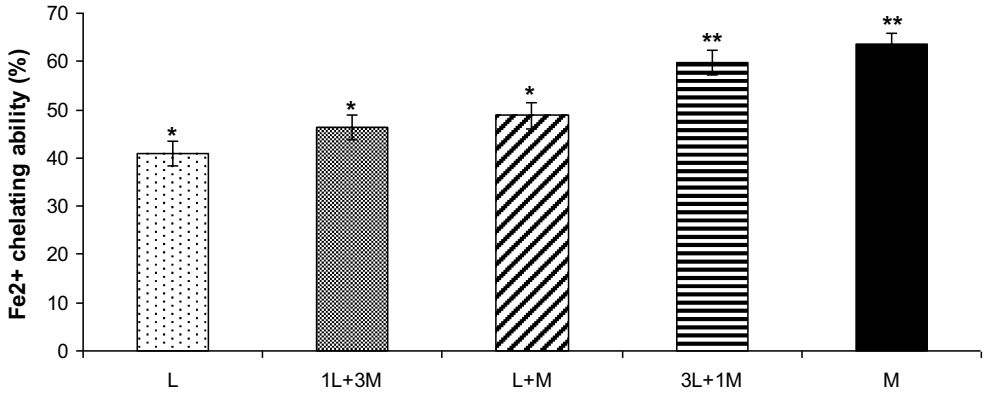

Fig. 8 Fe^{2+} chelating abilities of moringa leaf extracts, lisinopril and combinations. L = 100% lisinopril (1 mg/ml); M = 100% moringa extract (1 mg/ml); 1L + 3M = 25% lisinopril + 75% moringa extract; L + M = 50% lisinopril + 50% moringa extract; 3L + 1M = 75% lisinopril + 25% moringa extract. Values represent mean ± standard deviation of triplicate readings. Bars with same annotation (*, **, ***) are not significantly different

antioxidant mode of action is the scavenging of various reactive species such as free radicals (Dastmalchi et al. 2007). The higher DPPH* scavenging ability of moringa could be due to the presence of phenolic compounds possessing 3′,4′-*o*-dihydroxyl group in the B-ring, a 3-hydroxyl group and a 2,3-double bond combined with a 4-keto group in the C-ring. The radical scavenging properties of lisinopril, moringa leaf extract and their combinations were further studied using a moderately stable nitrogen-centred ABTS* due to the drawbacks of the DPPH* scavenging assay such as colour interference and sample solubility (Re et al. 1999; Dastmalchi et al. 2007). The ABTS* scavenging ability of the samples further revealed that moringa extract enhanced the scavenging ability of lisinopril. Some studies have shown that the radical scavenging ability of ACE inhibitors is influenced by the presence of SH in their chemical structures (Evangelista and Manzini 2005). However, some other studies showed that the presence of SH is not relevant to the radical scavenging abilities of ACE inhibitors (Mira et al. 1993). However, one obvious deduction from this study is that the radical scavenging properties of lisinopril were enhanced by combination with moringa extracts.

Conclusion

This study revealed that moringa leaf extracts could improve the antioxidant and ACE inhibitory properties of lisinopril. However, further in vivo experiments and clinical trials are recommended.

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Compliance with ethical standards

Ethical statement The handling and use of the animals were in accordance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals. The handling and use of the experimental animals was as approved by the Animal Ethics Committee of the School of Sciences, Federal University of Technology, Akure, Nigeria, with protocol reference SOS/14/02a.

Conflict of interest The authors declare no conflict of interests.

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