

## Drug-Herb Interactions: Selected Antihypertensive Drugs with *Moringa oleifera* Leaves Extract

(Interaksi Ubat-Herba: Ubat Antihipertensi Terpilih dengan Ekstrak Daun *Moringa oleifera*)

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### ABSTRACT

*Moringa oleifera* is a local plant which is commonly used in cooking and as a health supplement. It has been reported to possess blood pressure (BP) lowering effect and little is known about its possible interactions with cardiovascular drugs. This study looked into the possible drug-herb interactions between *M. oleifera* leaves extract and selected antihypertensive drugs. Ten groups of spontaneously hypertensive rats (SHRs) and one group of normotensive rats (NTs) were administered either *M. oleifera* extract alone, drugs alone or drugs in combination with *M. oleifera* extract for 14 days and BP of the rats were measured. Angiotensin converting enzyme (ACE) activity was also determined *in vitro* and *ex vivo*. Treated groups were found to produce significant BP reduction on day 15 when compared with the negative control but there was no significance difference when compared with positive controls (drugs alone). *M. oleifera* extract administered alone significantly reduced BP of SHRs on day 15 and this is comparable with the BP reduction observed when antihypertensive drugs were administered alone. However, no additive effect was observed when drugs were used in combination with *M. oleifera* extract. Similar results were seen in the *in vitro* and *ex vivo* ACE inhibitory activity of *M. oleifera* extract and enalapril. It can be concluded that there is a possibility of drug-herb interaction between *M. oleifera* extract and the selected antihypertensive drugs.

Keywords: ACE activity; antihypertensive drugs; blood pressure; drug-herb interactions; *Moringa oleifera*

### ABSTRAK

*Moringa oleifera* ialah tumbuhan tempatan yang biasanya digunakan dalam masakan dan sebagai makanan tambahan kesihatan. Ia dilaporkan mempunyai kesan penurunan tekanan darah (TD), tetapi amat sedikit yang diketahui jika ia mungkin berinteraksi dengan ubat kardiovaskular. Kajian ini melihat jika terdapat interaksi antara ekstrak daun *M. oleifera* dengan ubat hipertensi terpilih. Sepuluh kumpulan tikus hipertensi secara spontan (SHR) dan satu kumpulan tikus normotensif (NT) diberi sama ada ekstrak *M. oleifera* sahaja, ubat sahaja atau gabungan ubat dan ekstrak *M. oleifera* selama 14 hari dan TD tikus tersebut diukur. Aktiviti enzim pengubah-angiotensin (ACE) juga ditentukan secara *in vitro* dan *ex vivo*. Kumpulan yang dirawat didapati menurunkan TD secara ketara pada hari ke-15 jika dibandingkan dengan kawalan negatif tetapi tidak menunjukkan perbezaan yang bererti jika dibandingkan dengan kawalan positif (ubat sahaja). Ekstrak *M. oleifera* sahaja dapat mengurangkan TD SHR secara bererti pada hari ke-15 dan ini setanding dengan penurunan TD yang diperolehi daripada ubat hipertensi sahaja. Walau bagaimanapun, tiada kesan tambahan yang diperolehi apabila ubat diberi bersama ekstrak *M. oleifera*. Hasil yang serupa dilihat dalam aktiviti perencatan ACE secara *in vitro* dan *ex vivo* oleh ekstrak *M. oleifera* dan enalapril. Dengan ini dapat disimpulkan bahawa berkemungkinan terdapat interaksi ubat-herba antara ekstrak *M. oleifera* dengan ubat antihipertensi terpilih. Mekanisme terperinci mengenai interaksi yang berlaku perlu dikaji dengan lebih lanjut untuk memastikan penggunaan ekstrak *M. oleifera* bersama ubat antihipertensi adalah selamat.

Kata kunci: Aktiviti ACE; interaksi ubat-herba; *Moringa oleifera*; tekanan darah; ubat antihipertensi

## INTRODUCTION

Hypertension is estimated to affect 9.4 million people annually worldwide - approaching the number of all infectious diseases combined (Angell et al. 2015). This increasing trend is predicted to persist, with 29.2% of global population (1.5 billion people) predicted to suffer from hypertension by 2025 (Forouzanfar et al. 2017; Kearney et al. 2005). Elevated blood pressure is the strongest modifiable risk factor for cardiovascular diseases and related disabilities (O'Brien 2017). Hypertension is treated with four groups of drugs namely ACE inhibitors, calcium channel blockers,  $\beta$ -blockers and angiotensin II receptor blockers (ARBs). Apart from conventional drugs, herbal medicines have now gained public interest in controlling hypertension (Al Disi et al. 2016).

A handful of Malaysian plants namely, soursop (*Annona muricata*), papaya (*Carica papaya* L.), mempelas (Dilleniaceae) and merunggai (*Moringa oleifera*) have been used traditionally to lower blood pressure (BP) (Coria-Télez et al. 2018; Lima et al. 2014; Stohs & Hartman 2015; Vij et al. 2015). *M. oleifera* is widely found in the tropical and subtropical regions and is rich in simple sugars (glucosinolates and isothiocyanates), alkaloids (moringine and moringinine), flavanoids (kaempferol, rhamnetin, isoquercitrin and kaempferitrin), amino acids (methionine, cysteine, tryptophan, and lysine) and fatty acids (oleic oils and tocopherols) (Vongsak et al. 2013). The presence of diverse bioactive compounds in this highly valued plant may contribute to the pharmacological activities reported; antihypertensive, antispasmodic, hepatoprotective, antitumour, and antimicrobial (Stohs & Hartman 2015).

It must be noted that with the universal increased use of complementary and alternative medicines (CAM), especially those marketed as herbal supplements, more incidences of drug herb interactions are now documented including drugs used to treat cardiovascular diseases (Agbabiaka et al. 2018; Aworte et al. 2018; Izzo et al. 2016; Peng et al. 2004). Antiplatelet agents and anticoagulants were found to interact with *Ginkgo biloba*, *Panax ginseng*, *Piper methysticum*, *Serenoa repens*, and *Camellia sinensis* causing cardiovascular collapse, cardiotoxicity, bradycardia, and inflammatory reactions (Posadzki et al. 2013). *Allium sativum* (garlic) was shown to increase the availability of propranolol (Asdaq & Inamdar 2009). Moreover, studies have shown that metabolism of nifedipine was inhibited by *Ginkgo biloba* (Baxter 2010) but induced by *Hypericum perforatum* (St

John's wort) (Wang et al. 2007). Research focuses mainly on common herbs used in Chinese traditional medicines (*Ginkgo biloba*, *Panax ginseng*, and *Camellia sinensis*) and Western herbs (St John's wort, *Echinacea* and valerian) and little is known about drug interactions with Malaysian herbs particularly those which are used with cardiac agents. Herbal medicines are a mixture of more than one active ingredients and therefore the likelihood of herb-drug interactions occurrence is high (Aworte et al. 2018). Phytochemicals in herbal medicines may be substrates for enzymes or transporters that act on drugs, potentially inhibiting metabolism or transport of drugs (Li et al. 2014). On the other hand, bioactive constituents of herbal medicines might interact with receptors inducing production of enzymes or transporters (Koe et al. 2014). These can alter the absorption, distribution, metabolism, and elimination of drugs which might result in toxicity or reduced efficacy of drugs when taken together with herbal medicines (Izzo et al. 2016). *M. oleifera* extract which was reported to lower BP might produce an additive effect if used in combination with antihypertensive drugs thus poses the risk of hypotension (Stohs & Hartman 2015; Ray et al. 2003).

In this study, the possible drug-herb interactions between *M. oleifera* aqueous leaves extract and antihypertensive drugs namely enalapril (ACE inhibitor), amlodipine (calcium channel blocker), atenolol ( $\beta$ -blocker) and losartan (ARB) were investigated using NTs and SHRs.

## MATERIALS AND METHODS

*M. oleifera* leaves were obtained from a farm in Kuala Terengganu, Terengganu in October 2018. The plant was identified and authenticated by Emeritus Professor Dato' Dr. Abdul Latiff Mohamad of the Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM) and a voucher specimen (HF-135) was deposited in the UKM Herbarium. The leaves were cut into small pieces, dried, ground, and stored at room temperature (29 °C) until further use. The ground *M. oleifera* leaves were extracted with water by boiling the leaves (0.1 kg) in distilled water (1.2 L) for 6 h. The extract was filtered, kept at -80 °C for 3 days prior to a freeze drying process and the powdered extract obtained was kept at -20 °C until further use (Rathi et al. 2006).

Reference standards used in this study were purchased from Wuhan ChemFaces Biochemical Co. Ltd., Wuhan, China ( $\beta$ -sitosterol, 98% purity by high performance liquid chromatography (HPLC);

campesterol, 98% purity by HPLC) and Extrasynthese, Lyon, France (kaempferol-3-*O*-rutinosides, 98% purity by HPLC; stigmasterol, 90% purity by HPLC; chlorogenic acid, 99% purity by HPLC). Tragacanth powder and ACE from rabbit lungs  $\geq 2.0$  unit/mg were purchased from Sigma-Aldrich (United States). Enalapril maleate 5 mg, amlodipine 5 mg, atenolol 50 mg and losartan 50 mg tablets were purchased from the Standard Pharmacy Sdn. Bhd. (Malaysia). Enalaprilat was purchased from Santa Cruz Biotechnology (United States). Hippuryl-L-Histidyl-L-Leucine (HHL) 100 mg, boric acid and sodium tetraborate were purchased from Nacalai Tesque Inc. (Japan). All other reagents were of analytical grade. Distilled water, prepared from deionised water was used throughout the study.

#### HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS

Chemical markers were dissolved in HPLC grade methanol to produce standard solutions of 1.0 mg/mL for  $\beta$ -Sitosterol, kaempferol-3-*O*-rutinosides and stigmasterol and 0.5 mg/mL for chlorogenic acid and campesterol. Octacosanoic acid was prepared at a concentration of 0.5 mg/mL in methanol and chloroform (7:3). *M. oleifera* extract was accurately weighed (1.0 g), dissolved in 10 mL distilled water and mixed thoroughly with vortex, followed by 15 min of sonication and 5 min centrifugation at 4000 rpm. The supernatant was collected and filtered with 0.45  $\mu$ m membrane filter prior to injection into the high performance liquid chromatography (HPLC) column. HPLC (Waters 1515 Isocratic HPLC pump) with dual wavelength absorbance detector (Waters 2487) was utilised to analyse general profiles and quantify phytochemical compounds in the leaf extract. The 5  $\mu$ m C18 column (XBridge Reverse Phase) was set at room temperature (29 °C) with a flow rate of 1.0 mL/min. HPLC grade methanol and water was mixed in a ratio of 70:30 v/v and used as a mobile phase. The mobile phase was filtered and degassed for 15 min prior to use. Samples (20  $\mu$ L) were injected with running time of 10 min and peaks were detected at 205 nm. Accuracy, precision and specificity of the method were determined for method validation.

#### EXPERIMENTAL DESIGN

Sixty male SHR and six male Wistar Kyoto rats (WKR) weighing between 180-300 g were purchased from the Animal Unit, Monash University (Subang Jaya,

Malaysia). The research was conducted in accordance with the internationally accepted principles for laboratory and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). Experimental protocols were approved by the UKM Animal Ethics Committee (FF/2018/MALINA JASAMAI/14-MAY/925-AUG.-2018-JAN.-2019). The rats were housed two per cage and given free access to food and water in a temperature-controlled room ( $26 \pm 1$  °C) with 12-h/12-h light/dark cycle (0700-1900 h lights on). Rats were divided into eleven groups with six rats in each group (normotensive (NT), SHR treated with tragacanth (SHR), SHR treated with *M. oleifera* (MO), SHR treated with enalapril maleate (E), SHR treated with *M. oleifera* and enalapril maleate (MOE), SHR treated with amlodipine (A), SHR treated with *M. oleifera* and amlodipine (MOA), SHR treated with atenolol (T), SHR treated with *M. oleifera* and atenolol (MOT), SHR treated with losartan (L) and SHR treated with *M. oleifera* and losartan (MOL)).

#### BLOOD PRESSURE MEASUREMENT

Doses of drugs (1.92 mg/kg for enalapril, 5.7 mg/kg for losartan, 4.8 mg/kg for atenolol and 0.5 mg/kg for amlodipine) were calculated based on dose conversions from humans to animals (CDER 2005). The dose of the extract used (1 g/kg) was determined in a preliminary study carried out prior to the actual study. Extract, drugs and controls were administered to the rats by oral gavage once daily for 14 days. Systolic (SBP) and diastolic BP (DBP) were measured using CODA high throughput tail-cuff system (CODA-HT2) on day 1 (prior to the administration of extract, drugs and controls) and day 15 (post treatments) (Daugherty et al. 2009). Temperature of the tail was maintained between 32 and 35 °C throughout the experiment using a warming cover. After the blood pressure measurement on day 15, NT, SHR, MO, E, and MOE groups of rats were sacrificed and their lungs were used for the *ex vivo* experiment.

#### IN VITRO DETERMINATION OF ACE INHIBITORY ACTIVITY

*In vitro* ACE inhibitory was carried out as described by Nakamura et al. (1995). A volume of 80  $\mu$ L containing different concentrations of enalaprilat (1, 3, 5, 10, 20, 40, 60, 80, and 100  $\mu$ g/mL) and *M. oleifera* extract (5, 10, 20, 40, 60, 80, and 100  $\mu$ g/mL) was added to 200  $\mu$ L of HHL (5 mmol/L) and preincubated at 37 °C for 3 min. Enalaprilat is an active metabolite of enalapril and it was

used in this study as the metabolising enzyme which metabolises enalapril prodrug is not available in an *in vitro* set up. Test samples and HHL were prepared in 100 mmol/L borate buffer (pH of 8.3) containing 300 mmol/L NaCl. The reaction was initiated by adding 20  $\mu$ L of ACE (0.1 U/mL) prepared in the same buffer and the mixture was incubated for 30 min. The enzymatic reaction was quenched by an addition of 250  $\mu$ L of hydrochloric acid (HCl) (0.05 mol/L). Hippuric acid (HA) from the reaction was extracted with ethyl acetate (1.7 mL) and evaporated. The residue was dissolved in distilled water (1 mL) and the absorbance at 228 nm was determined using an ultra violet (UV)-visible spectrophotometer. ACE inhibitory activity was calculated as follow:

$$\text{ACE inhibition (\%)} = \left( \frac{B-A}{B-C} \right) \times 100 \quad (1)$$

where A is the absorbance of HA generated in the presence of test samples; B is the absorbance of HA generated without test samples; and C is the absorbance of HA generated without ACE.

#### ISOLATION AND PURIFICATION OF ACE FROM RAT LUNGS

The rats were sacrificed under anesthesia and dissected. Their lungs were isolated, washed, chopped (1 g) and homogenised in potassium phosphate buffer (50 mL, 0.1M) at pH 8.3 for 5 min. The homogenate was

centrifuged (2000 rpm) for 15 min at 4 °C and the supernatant formed was subjected to dialysis. A 10 cm dialysis tube was filled with water, tightened and squeezed to ensure there was no leaking. The water was replaced with the supernatant (5 mL) and the dialysis tube was immersed in a beaker containing potassium phosphate buffer (250 mL, pH 8.3). The beaker was left for 24 h at 4 °C with a change of buffer after the first 5 h. After the dialysis, sample inside the tube was removed and was subjected to the enzymatic assay as described earlier (Abdulazeez et al. 2016).

#### STATISTICAL ANALYSIS

Data are presented as mean  $\pm$  SEM and values obtained were statistically analysed using GraphPad Prism 7.0 program. The statistical comparisons were made using paired sample t-test, one-way ANOVA and Bonferroni post hoc analyses. Significance was set at  $p \leq 0.05$ . The enzymatic assay was carried out in triplicate.

#### RESULTS AND DISCUSSION

##### HPLC ANALYSIS

The presence of different phytochemical compounds in *M. oleifera* extract which correspond to the respective standards used (chlorogenic acid,  $\beta$ -sitosterol, sigmasterol, campesterol and kaempferol-3-*O*-rutosides) is shown in Table 1. The presence of the respective chemical markers was confirmed by spiking the extract with the markers.

TABLE 1. Phytochemical compounds identified from *M. oleifera* plant part extracts by HPLC

Leaves extract/Standards	Retention time (min)	RSD (%)	Area (mAU)	Concentration (mg/mL)
Leaves extract	5.33 $\pm$ 0.120	0.013	83956154	1.026
Chlorogenic acid	5.226 $\pm$ 0.145	0.014	40916458	1.000
$\beta$ -Sitosterol	2.824 $\pm$ 0.010	0.025	2270104	1.000
Sigmasterol	4.278 $\pm$ 0.003	0.017	1899873	1.000
Campesterol	4.276 $\pm$ 0.003	0.017	1955984	0.500
Kaempferol-3- <i>O</i> -rutosides	3.150 $\pm$ 0.007	0.022	2230220	1.000

*M. oleifera* extract is rich with various phytochemical compounds and the presence of these chemical markers were confirmed by the standards used; chlorogenic acid,

$\beta$ -sitosterol, sigmasterol, campesterol and kaempferol-3-*O*-rutosides. Similar compounds have also been reported previously in various *M. oleifera* plant parts (Anwar et al. 2007; Vongsak et al. 2013).

EFFECT OF *M. OLEIFERA* EXTRACT ON BP WHEN USED  
IN COMBINATION WITH ANTIHYPERTENSIVE DRUGS

All treatment groups showed significantly lower SBP and DBP than the negative control and significant SBP and DBP reductions ( $p \leq 0.001$ ) after 14 days of treatment when compared with day 1 (Figure 2). In addition, after 14 days MO was able to decrease BP comparable to the treatment with of all antihypertensive drugs tested (Figure 1). It was hypothesised that a combination of *M.*

*oleifera* extract with antihypertensive agents will show an additive effect if there was no interaction between antihypertensive drugs and extract in the BP lowering activity. However, when antihypertensive drugs were used in combination with *M. oleifera* extract at the concentrations tested in this study, it was found that there was no additive effect of BP lowering activity so it can be concluded that there might be an interaction between antihypertensive drugs and extract.

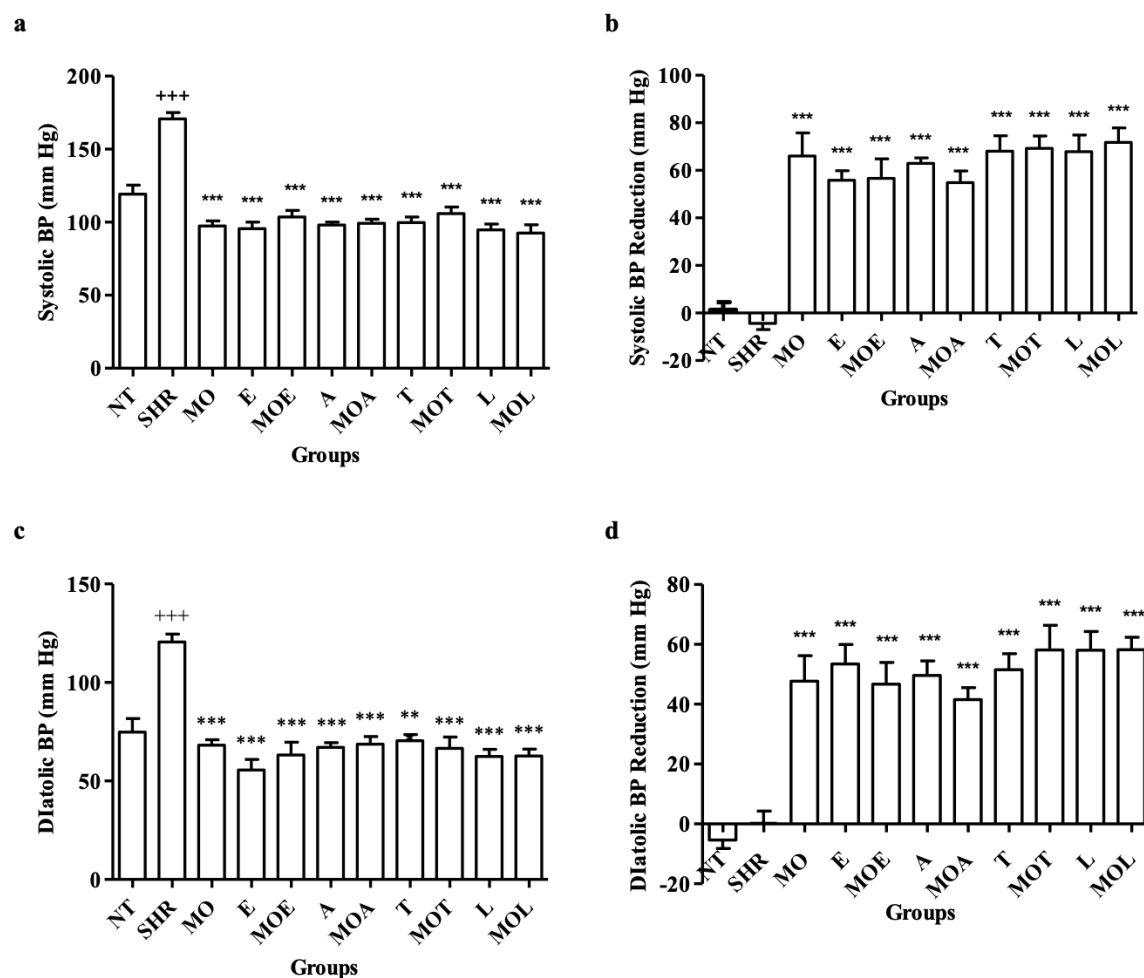


FIGURE 1. Systolic BP (a), systolic BP reduction (b), diastolic BP (c) and diastolic BP reduction (d) in SHR after 14 days of treatment with *M. oleifera* extract

NT: normotensive rats (negative control), SHR: spontaneously hypertensive rats, MO: SHR given *M. oleifera* extract, E: SHR given enalapril, MOE: SHR given enalapril and *M. oleifera* extract, A: SHR given amlodipine, MOA: SHR given amlodipine and *M. oleifera* extract, T: SHR given atenolol, MOT: SHR given atenolol and *M. oleifera* extract, L: SHR given losartan, MOL: SHR given loartan and *M. oleifera* extract, <sup>\*</sup> $p \leq 0.05$ ; <sup>\*\*</sup> $p \leq 0.01$ , <sup>\*\*\*</sup> $p \leq 0.001$  compared with NT, <sup>\*</sup> $p \leq 0.05$ ; <sup>\*\*</sup> $p \leq 0.01$ , <sup>\*\*\*</sup> $p \leq 0.001$  compared with SHR (negative control), n=6



*M. oleifera* extract showed a comparable BP lowering effect with the antihypertensive drugs studied (Figure 1). This is in agreement with several other studies on BP lowering effect of *M. oleifera* extract. Nitrile, mustard oil glycosides, carbamate glycosides, niacinin A, niacinin B, niazimicin, and niacinin A+B and alkaloids play an important role in the *M. oleifera*-mediated BP lowering activity (Anwar et al. 2007; Dangi et al. 2008; Gilani et al. 1994). Furthermore, significant reduction of BP has also been observed with a long term intake of *M. oleifera* aqueous leaf extract compared to a single dose administration (Tahkur et al. 2016).

*M. oleifera* extract was proposed to reduce BP through diuretic effect, calcium channel blocking and potassium ions inhibition (Faizi et al. 1995; Gilani et al. 1994; Tahkur et al. 2016). However, these mechanisms are not conclusive and their site of actions remain unclear. The reduced BP observed in SHR might be through the inhibition of ACE (enalapril), blockage of calcium channels (amlodipine), blockage of  $\beta$ -receptors (atenolol) and blockage of angiotensin receptors (losartan). A greater drop of BP was expected when combination treatments (*M. oleifera* extract and an antihypertensive drug) were given to the SHR should there be no drug-herb interactions. A comparable drop in BP observed when treated with *M. oleifera* extract singularly or in combination with antihypertensive drugs suggest that there were drug-herb interactions between *M. oleifera* extract and antihypertensive drugs.

#### IN VITRO AND EX VIVO ACE INHIBITORY ACTIVITY OF *M. OLEIFERA* EXTRACT WHEN USED IN COMBINATION WITH ENALAPRIL

Although ACE inhibitors are the second most prescribed antihypertensive drugs in Malaysia after calcium channel blockers (Teh et al. 2020), this group was chosen to be studied further in *in vitro* and *ex vivo* studies due to the availability of the assay in the laboratory. The inhibitory effect of ACE in this study was determined quantitatively by measuring the amount of HA produced as ACE will convert HHL to HA. ACE used in this study was either obtained commercially or extracted from rat lungs. Commercially available ACE was used to determine the minimum and maximum inhibitory concentrations of enalapril and *M. oleifera* extract. Minimum inhibitory concentrations of enalapril and *M. oleifera* extract were 1 and 5  $\mu\text{g/mL}$  with inhibitory activities of  $29.54 \pm 1.62$  ( $p < 0.001$ ) and  $40.62 \pm 1.06\%$  ( $p < 0.001$ ), respectively (Figure 2). Maximum inhibitory concentration for both enalapril and *M. oleifera* extract was 60  $\mu\text{g/mL}$  with inhibitory activities of  $64.62 \pm 0.97$  ( $p < 0.001$ ) and  $52.17 \pm 0.96\%$  ( $p < 0.001$ ), respectively (Figure 2). The maximum and minimum concentrations obtained were used to investigate a possible interaction between enalapril and *M. oleifera* extract when used in combination (Figure 3). At 60  $\mu\text{g/mL}$  both enalapril and *M. oleifera* extract inhibited ACE at  $60.57 \pm 2.27$  ( $p < 0.001$ ) and  $53.60 \pm 2.60\%$  ( $p < 0.001$ ), respectively. On the other hand, when both were used in combination,

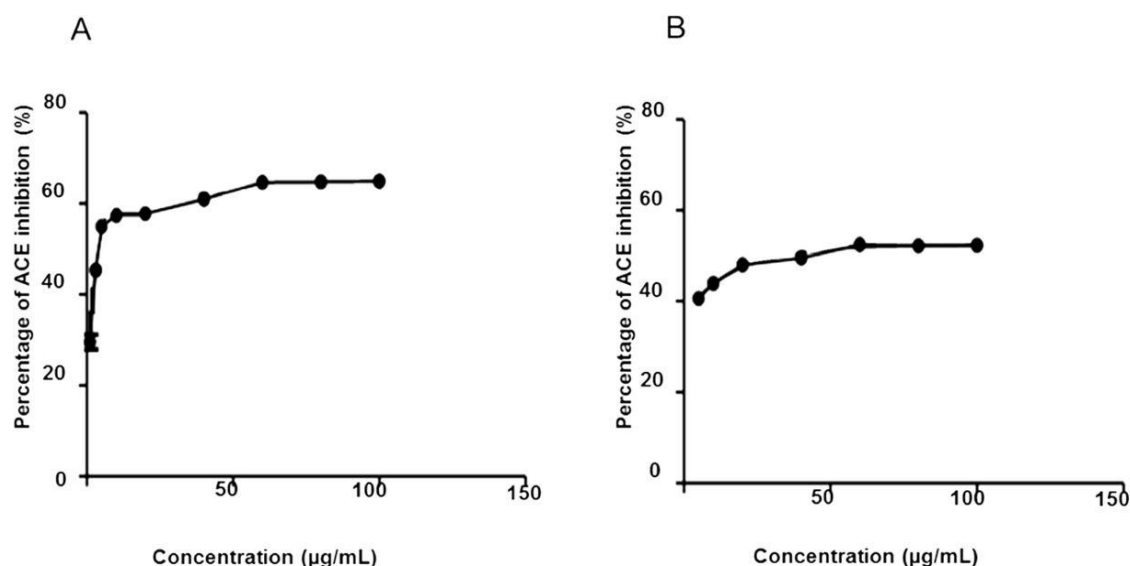


FIGURE 2. *in vitro* ACE inhibitory activity of enalapril (A) and *M. oleifera* extract (B) Borate buffer was used as a negative control, n=3

the inhibitory activity observed was  $47.57 \pm 2.80\%$  ( $p < 0.001$ ). Similar result was seen at minimum concentrations for both (Figure 4). At 1 and 5  $\mu\text{g/mL}$ , both enalapril and *M. oleifera* extract inhibited ACE at  $31.86 \pm 0.81$  ( $p < 0.001$ ) and  $35.95 \pm 0.92\%$  ( $p < 0.001$ ), respectively. However, when used in combination, the inhibitory activity of  $37.53 \pm 0.80\%$  ( $p < 0.001$ ) was

obtained. The ACE inhibitory activity of enalapril and *M. oleifera* extract used in combination at maximum and minimum concentrations is significantly different when compared with the inhibitory activity of enalapril alone. The  $\text{IC}_{50}$  values for enalapril and *M. oleifera* extract were also determined in this study (Figure 5). They were  $3.91 \pm 0.22$  and  $36.70 \pm 6.14$   $\mu\text{g/mL}$ , respectively.

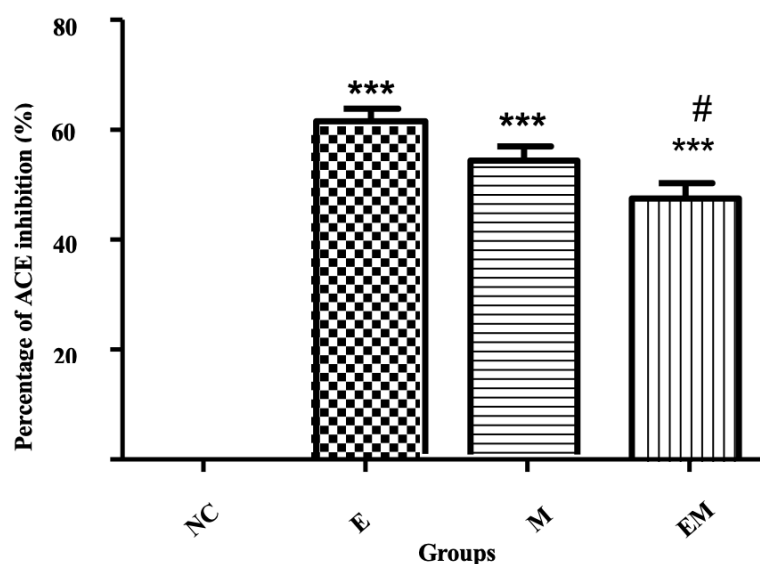


FIGURE 3. *In vitro* ACE inhibitory activity of enalapril (E) and *M. oleifera* extract (M) and the combination of enalapril and *M. oleifera* extract (EM) at 60  $\mu\text{g/mL}$  (maximum inhibitory concentration)

\*\*\*  $p < 0.001$  compared with negative control (NC), #  $p < 0.05$  compared with E, borate buffer was used as a negative control,  $n=3$

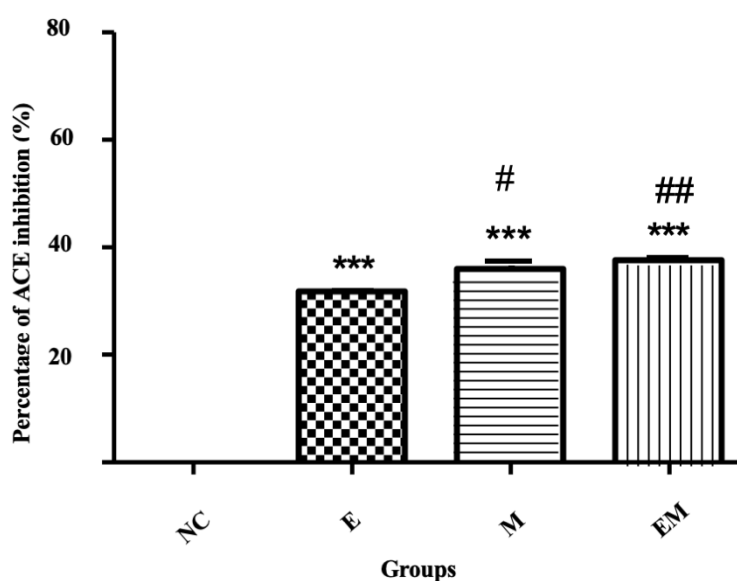


FIGURE 4. *In vitro* ACE inhibitory activity of enalapril (E) at 1  $\mu\text{g/mL}$  and *M. oleifera* extract (M) at 5  $\mu\text{g/mL}$  and the combination of enalapril and *M. oleifera* extract (EM) at 1  $\mu\text{g/mL}$  and 5  $\mu\text{g/mL}$  respectively (minimum inhibitory concentration)

\*\*\*  $p < 0.001$  compared with negative control (NC), #  $p < 0.05$  compared with E, borate buffer was used as a negative control,  $n=3$

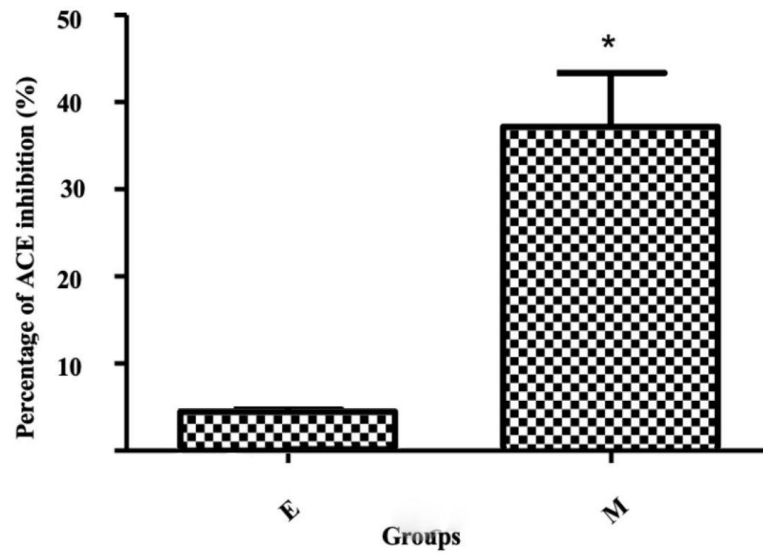


FIGURE 5. IC<sub>50</sub> values of enalapril (E) (3.91±0.22 µg/mL) and *M. oleifera* extract (M) (36.70±6.14 µg/mL)

\*  $p \leq 0.05$  compared with E (positive control), borate buffer was used as a negative control, n=3

The experiment above was repeated using ACE isolated from rat lungs. At the concentrations administered to the rats, both enalapril and *M. oleifera*

extract inhibited ACE at 32.56±6.37 ( $p < 0.001$ ) and 29.58±6.21% ( $p < 0.01$ ), respectively. An inhibitory activity of 20.82±4.50% ( $p < 0.05$ ) was obtained when they were used in combination (Figure 6).

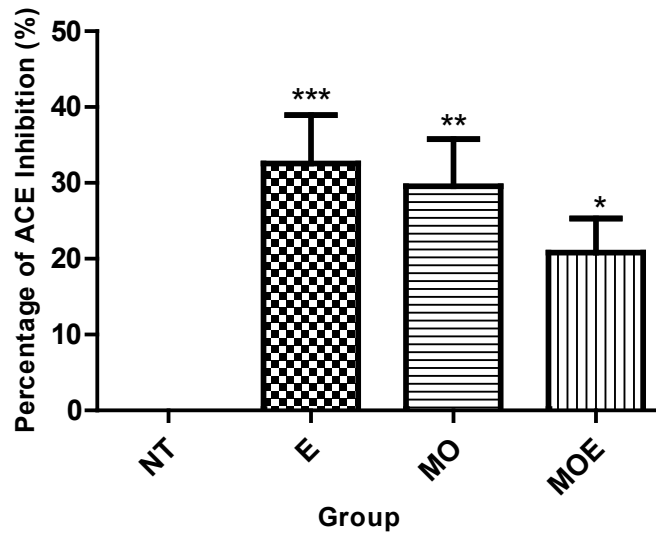


FIGURE 6. *Ex vivo* ACE inhibitory activity of enalapril (E), *M. oleifera* extract (MO) and the combination of enalapril and *M. oleifera* extract (MOE)

NT: normotensive rat lungs (negative control), E: SHR given enalapril lungs, MO: SHR given *M. oleifera* extract lungs, MOE: SHR given enalapril and *M. oleifera* extract lungs, \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$  compared with NT, n=6, E and MO are not significantly different from MOE



In the *in vitro* study (ACE was obtained commercially), both enalapril and *M. oleifera* extract showed maximum ACE inhibitory activity at 60 µg/mL. Phenolic compounds, flavanoids such as rutin, isoquercitrin, kaempferol, quercitrin and quercetin, alkaloids and glycosides might be responsible for the ACE inhibitory activity observed in the *M. oleifera* extract (Obboh et al. 2015; Saputri et al. 2015). Quercetin glycosides also was shown to have optimal binding affinity with ACE (Muhammad et al. 2015). The ACE inhibitory activity of enalapril, *M. oleifera* extract and the combination of enalapril and *M. oleifera* extract was significantly different ( $p < 0.001$ ) when compared with negative control. Both *M. oleifera* extract ( $p < 0.05$ ) and the combination of enalapril and *M. oleifera* extract ( $p < 0.01$ ) also showed significant differences when compared with enalapril. This observation might be due to the interaction between enalapril and *M. oleifera* extract when used in combination thus causing a lesser amount of both substances to competitively inhibit ACE. Similar finding was reported by Obboh et al. (2018) where lisinopril did not give an additive effect when used in combination with *M. oleifera* extract.

Overall, the same profile of ACE inhibitory activity was achieved with maximum and minimum concentrations of enalapril and *M. oleifera* extract used in this study. At both concentrations, enalapril and *M. oleifera* extract used in combination did not give an additive effect. Further studies can look at the possibilities of using *M. oleifera* extract as an alternative to enalapril as an ACE inhibitor since there was no significant difference between them in inhibiting ACE activity at both maximum and minimum concentrations.

It can be concluded from the  $IC_{50}$  values obtained that lower concentration of enalapril ( $3.91 \pm 0.22$  µg/mL) is required to achieve ACE inhibitory activity compared to *M. oleifera* extract ( $36.70 \pm 6.14$  µg/mL). Enalapril might have higher capacity to displace HHL and inhibit ACE than *M. oleifera* extract or have higher binding affinity towards ACE (Dzau et al. 2002).

In the *ex vivo* study (ACE was obtained from the treated rat lungs), the results obtained are comparable with the *in vitro* study where the ACE inhibitory activity of E ( $p < 0.001$ ), MO ( $p < 0.01$ ) and MOE ( $p < 0.05$ ) were significantly different with negative control. Enalapril and *M. oleifera* extract used in combination (MOE) also did not show a synergistic effect as seen in the *in vitro* study.

Physicochemical reactions such as hydrolysis, oxidation, neutralisation, precipitation or complexation

between drugs and phytochemicals are possible (Rodríguez-Fragoso et al. 2011). Insoluble complexes formed at the sites of absorption can greatly affect the absorption of drugs (D'arcy et al. 2012). The effect of physicochemical drug-herb interactions has only been studied in a handful of plants even though herbal medicines are commonly consumed with medicines. The chelation of metformin and glibenclamide by psyllium and alginates were reported in both *in vitro* and *in vivo* studies and *Capsicum annum* was found to affect aspirin absorption in rats due to complexation (Colalto et al. 2010; Tarirai et al. 2010). *M. oleifera* on the other hand has been shown to reduce the absorption rate of amodiaquine due to its high fibre content (Olawoye et al. 2017). Drug-herb interactions can also happen in a pharmacokinetic manner where the drug metabolising enzymes and transporters are inhibited or induced by herbal medicines taken concomitantly with drugs and *vice versa* (Brantley et al. 2014; D'arcy et al. 2012). This could easily lead to changes of plasma concentration of either the drug or the herbal active constituents leading to the decreased effects of either the former, the latter or both (Mamindla et al. 2016). Moreover, pharmacodynamic drug-herb interactions are also possible where the herbs administered concomitantly with drugs will alter the pharmacological effects of the drugs. This is demonstrated by the increased values of prothrombin time (PT) and international normalized ratio (INR) of warfarin when given together either with pomegranate peel or guava leaf extracts (Alnaqeeb et al. 2019).

Numerous classes of phytochemicals have been reported in *M. oleifera* and these phytoconstituents could be responsible for the pharmacokinetic drug-herb interactions mentioned earlier. Enalapril and most ACEIs are substrates of intestinal human peptide transporters 1 (PepT1) for systemic absorption (Li et al. 2017). Enalapril is also a substrate for human organic anion transporting polypeptides 1B1 (OATP1B1) which responsible for enalapril transportation into liver where it is metabolised into active metabolites (Verbeeck et al. 2017). Flavanoids were found to interact with transporter proteins and hepatic cytochrome enzymes and *M. oleifera* with high flavonoid contents might interact with enalapril in this manner (Rodríguez-Fragoso et al. 2011). There are many possibilities where pharmacokinetic interactions can occur during concomitant use of *M. oleifera* extract and antihypertensive drugs. Further studies are required to identify detailed mechanisms on how *M. oleifera* extract interacts with antihypertensive drugs.

## CONCLUSION

*M. oleifera* extract administered alone significantly reduced SBP and DBP of SHR after 14 days and this is comparable with antihypertensive drugs administered alone. Similar result was observed with the ACE inhibitory activity of *M. oleifera* extract when compared with enalapril. However, no additive effect of reduction in BP was observed when *M. oleifera* extract was given concomitantly with antihypertensive drugs. There is a possible interaction between *M. oleifera* extract and enalapril as shown in the *in vitro* and *ex vivo* studies on the ACE inhibitory activity. Furthermore, the use of *M. oleifera* extract with enalapril may have no additional therapeutic value. However, *M. oleifera* extract can be considered as an alternative to ACE inhibitors. *M. oleifera* extract might interact with antihypertensive drugs in a pharmacokinetic or/and pharmacodynamic manners and detailed mechanism of interactions are worth to be explored further.

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