Websites:
http://www.nbmedicine.org
http://www.sciencepub.net/nurse

Emails:
editor@sciencepub.net
nbmeditor@gmail.com

Biomedicine and Nursing

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

A new combination of 4-hydroxyisoleucine and small dose amlodipine against DOCA-induced hypertension in rats: A comparative and mechanistic study

Mohamed Abdelmohsen Abdallah[1], Mohamed Farouk Ahmed[2] and Mohamed Elsayed Kelany[3*]

[1] Physiology Department, Faculty of Medicine, Menoufeya University, Egypt. Email: mwmdy@yahoo.com
[2] Clinical Pharmacology Department, Faculty of Medicine, Menoufeya University, Egypt. Email: m-pharma@windowslive.com
[3] Clinical Pharmacology Department, Faculty of Medicine, Zagazig University, Egypt. E-mail: drmkelany@hotmail.com

Abstract: Background: 4-Hydroxyisoleucine (4-OH-Ile) represents a new treatment of diabetes, dyslipidemia and subsequent complications. Amlodipine (AML) is an antihypertensive with many dose-related side effects. **Objective:** This study aimed to evaluate the effects of 4-OH-Ile and small dose amlodipine (s-AML) against DOCA-induced hypertension in rats, with comparison with high h-AML and the possible mechanisms. **Methods:** 48 adult male albino rats were divided into 6 groups (n = 8): **control group**: received vehicles: 0.3 mL olive oil/week subcutaneously and distilled water 1 ml/kg/day orally for 4 weeks (4w), **DOCA group**: received DOCA 50 mg/kg/week in vehicle subcutaneously and 1% NaCl drink for 4w, **4-OH-Ile/DOCA group**: received 4-OH-Ile 50 mg/kg/day orally and DOCA/saline as above, **s-AML/DOCA group**: received s-AML 2 mg/kg/day orally and DOCA/saline for 4w, **h-AML/DOCA group**: received h-AML 10 mg/kg/day orally and DOCA/saline, **s-AML/4-OH-Ile/DOCA group**: received s-AML, 4-OH-Ile and DOCA/saline for 4w. After 4-weeks, the parameters of blood pressures, lipids and oxidant/antioxidant status were estimated. **Results:** 4-OH-Ile/s-AML prevented DOCA-induced changes: it decreased SABP, DABP, serum CK-MB, LDH, cholesterol, triglycerides and LDL and cardiac tissue MDA, and increased cardiac TAC and serum HDL. These results had insignificant differences when compared to control and h-AML/DOCA rats**. Conclusion:** 4-OH-Ile/s-AML is an effective antihypertensive combination in rats, due to its ability to scavenge oxidants and improve cardiac function.
[Mohamed Abdelmohsen Abdallah, Mohamed Farouk Ahmed and Mohamed Elsayed Kelany. **A new combination of 4-hydroxyisoleucine and small dose amlodipine against DOCA-induced hypertension in rats: A comparative and mechanistic study**. *Biomedicine and Nursing* 2024;10(2):62-75]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 07. doi:10.7537/marsbnj100224.07.

Keywords: 4-hydroxyisoleucine, amlodipine, hypertension, antioxidant, rats.

1. Introduction

Essential hypertension is a major health problem which accounts for about 90% of cases of hypertension and is an important cause of cardiovascular morbidity and mortality around the world. It represents a complex interaction of genetic and environmental factors. However, the exact pathophysiologic mechanisms of developing essential hypertension are still unknown (**González et al., 2014; Siebenhofer et al., 2016**). **Sharma (2009)** stated that the increased vascular resistance in hypertensive patients is compensated by ventricular hypertrophy which in turn may elevate blood pressure upstream from the constricted arteriolar bed via elevation of cardiac output. Endothelial dysfunction, increased arteriole wall thickness, decreased lumen radius and large-vessel hypertrophy are considered as common vascular complications of hypertension (**Silvestre and Lévy, 2000**). Indeed, **Orlov et al. (1999)** mentioned that hypertension is considered as a vascular proliferative disorder. Hyperplasia is considered not only a result of elevated blood pressure, but also it can constitute a precipitating element for hypertension (**Lee et al., 2009**).

Many antihypertensive drugs, as a monotherapy or combination therapy, are used effectively for managing hypertension. Among the antihypertensive drugs, the calcium channel blockers (CCBs) are a very useful option for treating hypertension and minimizing its cardiac complications. They act through vasodilatation resulting in a reduction of myocardial oxygen demand (**Doroszko et al., 2016**). **Lu et al. (2016)** reported that amlodipine (AML) is a long-acting second-generation dihydropyridine calcium channel blocker which is very selective for vascular tissue and has showed clear therapeutic advantages in the control of arterial blood pressure in hypertensive patients and treatment of angina pectoris. Besides, previous clinical trials have suggested that AML reduces cardiovascular events in different patient populations (**Kishi and Sunagawa, 2012**). On the other hand, **Munoz et al. (2008)** showed

that AML as any chemical drugs, has many side effects specially with long duration usage; some of them are ccommon and dose-related e.g. peripheral edema, dizziness, palpitations and flushing. Others side effects are common but not dose-related e.g. fatigue, nausea, abdominal pain and somnolence. Others are rare e.g. blood disorders, impotence, depression, insomnia, tachycardia, gingival enlargement, hepatitis and jaundice.

Currently, herbal products are taking valuable acceptance from both public and medical eras due to introduction of new advances aiding in understanding their mechanisms of action by which they can positively influence health (**Nivethetha et al., 2009**). Again this issue recently becomes of a great need to avoid or minimize many side effects of the antihypertensive drugs by decreasing their doses (**Thippeswamy et al., 2009**). Fenugreek (*Trigonella foenum graecum*) is a plant traditionally used to treat many diseases especially diabetes. Fenugreek seeds extraction is returned to focus through its novel amino acid 4-hydroxyisoleucine (4-OH-Ile) which is not present in mammalian tissues (**Broca et al., 2004; Kalshetti et al. 2015**). Likewise, **Jetté et al. (2009)** have reported that 4-OH-Ile decreased elevated plasma triglyceride and total cholesterol levels and may represent an attractive new candidate for the treatment of dyslipidemia and all subsequent complications via its antioxidant effects.

Hence, we hypothesized that if 4-OH-Ile will be used in combination with small dose AML (s-AML), this new combination could give an antihypertensive effect comparable to that of the therapeutic high dose AML (h-AML) with avoidance of its side effects specially the dose-related ones. To address this hypothesis, **the present study** was aimed to evaluate the effects of the new combination of 4-OH-Ile and s-AML against hypertension induced by deoxycorticosterone acetate (DOCA) in rats, compare its effects with those of therapeutic h-AML and to explore the possible mechanisms involved.

2. Materials and Methods

Drugs and chemicals: DOCA (catalog number D7000, MW: 372.504 g/mol, CAS Number 56-47-3) was administered subcutaneously (SC) in olive oil. 4-OH-Ile (catalog number 50118, from fenugreek seed, ≥98.0% (TLC), MW: 147.17 g/mol, CAS Number 55399-93-4). AML was provided by Pfizer laboratories Div Pfizer Inc. Other chemicals were purchased from Sigma-Aldrich Chemical Co, USA. 4-OH-Ile and AML were administered orally in distilled water by gastric gavage (with an intragastric tube: a Portex 4FG cannula, Portex Ltd., Hythe, UK).

Animals: Forty-eight adult male Sprague-Dawley rats, weighing 200-250 grams, were obtained from the experimental animal center of the Faculty of Medicine, Zagazig University, Egypt. The experiment was carried out in the clinical pharmacology lab of the Faculty of Medicine, Menoufeya University, Egypt. The rats were housed in fully-ventilated cages, in a temperature-controlled environment at 22 ± 2ºC with a 12/12 hour light/dark cycle. All rats had free access to feed the standard laboratory chow (commercial standard pellets for rodents, purchased from local source in Egypt), and water for the control group rats and 1% saline for the other groups rats throughout the 4-weeks experimental period. All experiments were performed according to the guidelines for animal ethics and the Canadian Council on Animal Care (CCAC) guidelines.

The experimental design and animal groups: The rats were divided into 6 groups (n = 8 rats per group):

1. **Control group:** The rats received the vehicles: olive oil 0.3 ml/week SC and distilled water 1 ml/kg body weight/day orally for the 4-weeks experimental period. These rats had free access to water throughout the study. They did not receive DOCA or 1% NaCl.
2. **DOCA-treated group:** The rats were made hypertensive by DOCA 50 mg/kg/week SC in 0.3 ml olive oil for the 4-weeks experimental period. The rats had free access for drinking of 1% NaCl throughout the study. After 4 weeks, these rats showed increases in the SABP and DABP (**Magro et al., 1986; Haefliger et al., 1997**).
3. **4-OH-Ile/DOCA-treated group:** The rats received 4-OH-Ile 50 mg/kg/day orally **(Broca et al., 2004)** and DOCA and 1% NaCl as mentioned above for the 4-weeks period.
4. **s-AML/DOCA-treated group:** The rats received s-AML 2 mg/kg/day orally (**Kishi** and **Sunagawa, 2012; Hasegawa et al., 2016**) and DOCA and 1% NaCl as mentioned above for the 4-weeks period. The dose of amlodipine selected was based on a previous study in which it was observed that this dose caused a decrease in arterial blood pressure in rats (**Hasegawa et al., 2016**).
5. **h-AML/DOCA-treated group:** The rats received therapeutic h-AML 10 mg/kg/day orally (**Puzyrenko et al., 2013**) and DOCA and 1% NaCl as mentioned above for the 4 weeks.
6. **s-AML/4-OH-Ile/DOCA-treated group:** The rats received s-AML, 4-OH-Ile, DOCA and 1% NaCl as mentioned above for the 4-weeks experimental period.

The experimental procedures: At the end of the 4-weeks experimental period, we assessed the following parameters which could explain mechanistically the detectable beneficial effects of 4-OH-Ile and s-AML combination and therapeutic h-AML on the DOCA-induced hypertension in rats. Therefore, the rats had undergone the following procedures:

1. Measurement of the systolic and diastolic arterial blood pressures (SABP and DABP): These blood pressures were measured by a tail-cuff and a CODA monitor, a noninvasive blood pressure measurement system (Kent Scientific Apparatus, Torrington, CT, USA) which by its volume pressure sensor can measures both systolic blood pressure and diastolic blood pressure by 99 % accuracy as the direct invasive method **(Lv et al., 2013).** The conscious rats were pre-warmed for 10 min in a thermostatically-controlled restrainer and were tested once at the 1st and 28th days of treatments (the first and last days of the 4-weeks experimental period). Animals were trained to this procedure for 3 consecutive days (each cession consisting of 10 unrecorded measurements), before actual testing began, to familiarize the animal with rat tail cuff (**Makaritsis et at., 1998**). All rats were acclimated to the procedure for 15-30 minutes prior to measurement to minimize stress-induced variations in blood pressure. The mean of 3 separate pressure readings on 3 occasions was recorded. When the rat's SABP was maintained above 144 mmHg or DABP above 91 mmHg or both, the rats were considered hypertensive (**Magro et al., 1986; Haefliger et al., 1997; Mason et al., 2014**).

2. Collection of blood samples and separation of serum: After finishing the experiment, each rat was fasted overnight and held in a glass chamber to be anesthetized with diethyl ether. The blood sample was obtained, 24 hours after the last drugs administration, by heparinized microcapillary tubes from the retro-orbital plexus of rats after a 12-h overnight fast. Then, the blood sample (1-2 ml) was collected in a vacutainer plain tube without anticoagulant, and allowed to stand for 30 min at room temperature until blood clotted, and then centrifuged at 3000 *g* for 10 min for separation of serum. The serum was stored at −20°C till analyzed as described below **(Stone 1954; Ihedioha et al., 2013**).

3. Tissue sampling: After collection of the blood samples, all rats were sacrificed. Each heart was removed immediately, weighed and rapidly washed with ice-cold saline (0.9%) and then placed in ice-cold isotonic potassium chloride solution (1.15% KCl w/v) containing 0.1 mM EDTA. The left ventricle was then chopped in 4-5 volumes of 50 mM phosphate buffer (pH 7.4) and homogenized by a tissue homogenizer fitted with a Teflon pestle. The homogenate was then centrifuged at 3000 *g* for 10 min, the lipid layer was carefully removed and the resulting supernatant fraction was further centrifuged at 15,000 *g* for 60 min at 4°C. The supernatant was stored at −80°C until used (**Haidari et al., 2013**) for measuring the followings:

i. **The ventricle tissue total antioxidant capacity (t.TAC):** It was measured in 'nmol/mg protein' using the commercially available kits (Randox labs, Grumlin, UK). The assay principle was based on the ability of antioxidants to quench the absorbance of the radical cation that is formed by the reaction of a chromogen with the peroxide and H_2O_2 (**Miller et al., 1993**).

ii. **The ventricle tissue malondialdehyde (t.MDA):** It was measured, as the lipid peroxidation end-product, in 'nmol/mg protein' using MDA ELISA kit; ab118970, Abcam (**Yeh et al., 2013**). The assay principle was based on the MDA reaction with thiobarbituric acid (TBA), forming a MDA-TBA2 adduct that absorbs strongly at 532 nm.

4. Biochemical assays:

i. **Estimations of the serum lipids and lipoproteins:**

A. Total cholesterol (TC) was estimated using kits from Boehringer Mannheim's based on CHOD-PAP method.

B. Triglycerides (TGs) was assayed using kit from Human's which is based on enzymatic colorimetric method with lipid clearing factor.

C. High density lipoprotein-cholesterol (HDL-c) was determined using Menagent HDL-c reagent which allows the determination of HDL-c fraction after precipitation of LDL and very low density lipoprotein fractions with phosphotungstic acid and magnesium chloride (**Rao et al., 1986**). The estimation was carried out on a fully-automated analyzer (Hitachi, Japan).

D. Low density lipoprotein-cholesterol (LDL-c) level was calculated according to the following formula (**Friedewald et al., 1972**): LDL-c = TC – (HDL-c) – TGs/5

ii. **Estimations of the serum levels of lactate dehydrogenase (LDH) and creatine kinase isoenzyme MB (CK-MB):** The serum levels of these enzymes, used as cardiac injury markers, indicate the state of cardiac muscle and used to evaluate the effects of the blood pressure and drugs on the heart muscle. They were estimated using the commercial kits of Bechman by Bechman Coulter LX-2000 (Brea, CA, USA) by kinetic determination (**Bilginoğlu et al., 2014**).

Statistical analysis: The collected data were presented as means ± standard deviation of the mean (mean ± SD) for all groups. The results were statistically analyzed using Statistical Package for the Social Sciences (SPSS) software (version 16) (IBM Corp., Armonk, NY, USA). The one-way analysis of variance (one-way ANOVA) followed by the Post Hoc, Fisher's least significant difference (LSD) test were applied for

comparisons of means. In every case, significance difference was accepted at the probability $P < 0.05$.

3. Results

Table 1. Effects of DOCA, 4-OH-Ile, s-AML, h-AML and s-AML/4-OH-Ile treatments on the SABP and DABP and the ventricular tissue t.TAC and t.MDA levels and the serum CK-MB, LDH, TC, TGs, HDL-c and LDL-c levels in the different rats groups.

Group / Parameter	Control group	DOCA-treated group	4-OH-Ile /DOCA-treated group	s-AML /DOCA-treated group	h-AML /DOCA-treated group	s-AML/4-OH-Ile/DOCA-treated group
SABP (mm Hg)	108.63 ± 3.68	157.75 ± 3.79 #	155.63 ± 3.74 #	140.5 ± 2.46 # $	110.38 ± 4.67 $	111.13 ± 3.17 $
DABP (mm Hg)	76 ± 2.94	98.75 ± 2.12 #	97.88 ± 2.89 #	87.5 ± 2.46 # $	78.13 ± 2.01 $	78.63 ± 2.46 $
t.TAC (nmol/mg protein)	0.58 ± 0.034	0.18 ± 0.023 #	0.41 ± 0.023 # $	0.35 ± 0.028 # $	0.54 ± 0.057 $	0.6 ± 0.04 $ *
t.MDA (nmol/mg protein)	9.82 ± 0.18	21.87 ± 0.12 #	15.9 ± 0.22 # $	15.1 ± 0.31 # $	9.98 ± 0.4 $	10.05 ± 0.31 $
CK-MB (IU/L)	333.25 ± 15.28	620.5 ± 12.37 #	568.5 ± 19.8 # $	438.13 ± 9.76 # $	347.5 ± 16.4 $	347 ± 10.75 $
LDH (IU/L)	431.75 ± 10.4	769.88 ± 10.75 #	670.25 ± 11.89 # $	590.13 ± 17.83 # $	448.75 ± 9.1 # $	449.38 ± 9.34 # $
TC (mg/dl)	77.38 ± 2.66	128.5 ± 2.46 #	80.63 ± 2.66 $	121.63 ± 2.38 # $	120.25 ± 2.18 # $	79.25 ± 2.12 $ *
TGs (mg/dl)	68.25 ± 2.69	110 ± 3.2 #	71.63 ± 2.66 $	103.88 ± 2.04 # $	101.38 ± 2.77 # $	69. 5 ± 2.46 $ *
HDL-c (mg/dl)	60.25 ± 2.12	34.75 ± 2.12 #	58.38 ± 4.78 $	39.63 ± 1.7 # $	41.63 ± 2.01 # $	59.63 ± 1.84 $ *
LDL-c (mg/dl)	42.25 ± 4.16	91.25 ± 2.12 #	45.13 ± 2.01 $	83.2 ± 1.84 # $	81.11 ± 1.84 # $	42.38 ± 3.1 $ *

- Values are expressed as mean ± SD.
- #: Significant in comparison to the control group.
- $: Significant in comparison to the DOCA-treated group.
- *: Significant in comparison to the h-AML/DOCA-treated group.

1. Effects of 4-OH-Ile, s-AML, h-AML and s-AML/4-OH-Ile treatments on the SABP and DABP in DOCA-induced hypertensive rats (Table 1 and Fig. 1): At the 1st day of treatment, just before giving DOCA, no significant differences were found in the initial SABP and DABP between the different groups of rats. Thus, these data were excluded from tables in order to facilitate data comparison and interpretation.

At the end of experiment, 4-weeks DOCA treatment in the DOCA-treated rats induced significant ($P < 0.001$) increases in the SABP and DABP compared to the control rats. When compared to the DOCA-treated rats, 4-weeks 4-OH-Ile treatment to 4-OH-Ile/DOCA-treated

rats produced insignificant ($P > 0.05$) changes in the SABP and DABP. 4-weeks treatments with s-AML, h-AML or s-AML/4-OH-Ile to their respective/DOCA-treated rats produced significant ($P < 0.001$) decreases in the SABP and DABP compared to the DOCA-treated rats. From other aspect, when compared to the control group, 4-weeks treatments with h-AML or s-AML/4-OH-Ile to their respective/DOCA-treated groups produced insignificant ($P > 0.05$) changes in the SABP and DABP. Also compared to the h-AML/DOCA-treated rats, 4-weeks s-AML/4-OH-Ile treatment to its respective/DOCA-treated rats produced insignificant ($P > 0.05$) changes in the SABP and DABP. Thus, 4-OH-Ile potentiated the blood pressure-lowering effect of s-AML when compared to either s-AML or h-AML monotherapy.

Collectively, DOCA increased significantly the SABP and DABP in rats. The h-AML or s-AML/4-OH-Ile, administered concomitantly with DOCA in rats, prevented the DOCA-induced elevations in both blood pressures keeping them near their normal ranges. Thus, the combination s-AML/4-OH-Ile has equal efficacy to h-AML in decreasing the blood pressure and thereby, has fewer adverse effects than h-AML, and thereby, is favored and superior to h-AML as antihypertensive agent.

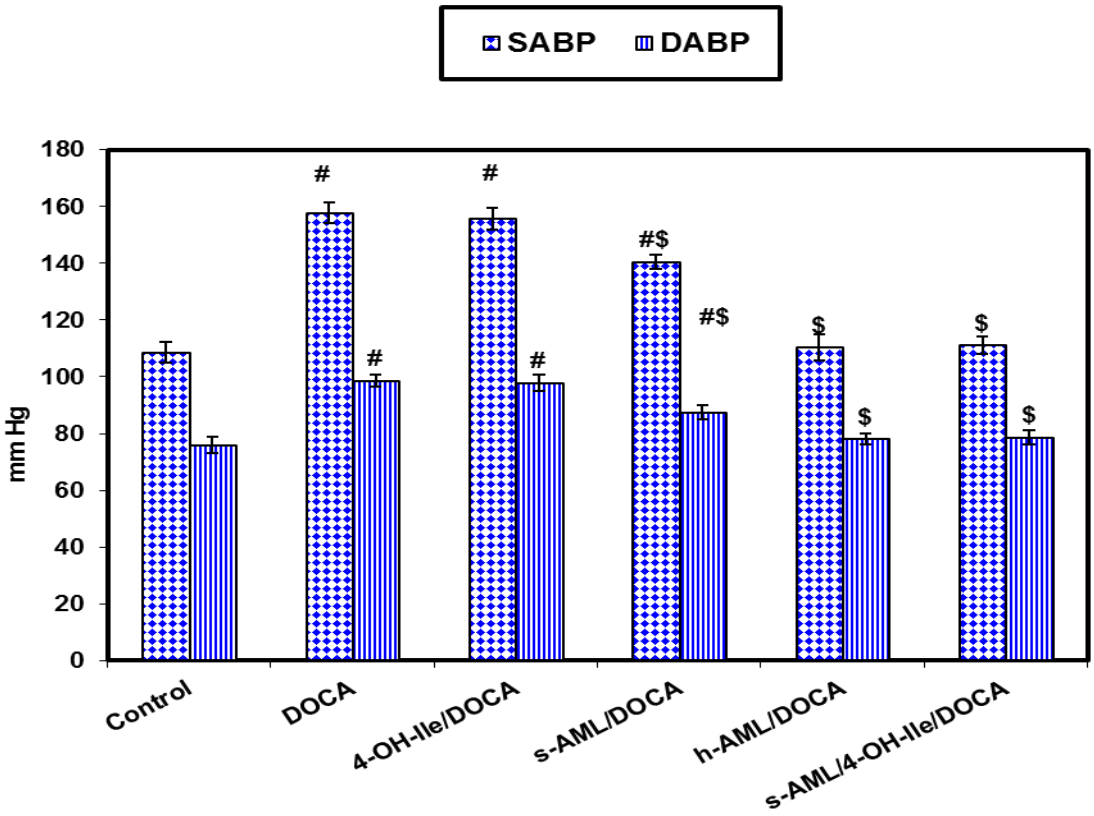

Figure. 1. Effects of DOCA, 4-OH-Ile/DOCA, s-AML/DOCA, h-AML/DOCA and s-AML/4-OH-Ile/DOCA treatments on the SABP and DABP in the different rats groups.
#: Significant in comparison to the control group.
$: Significant in comparison to the DOCA-treated group.

2. Effects of 4-OH-Ile, s-AML, h-AML and s-AML/4-OH-Ile treatments on the ventricle tissue TAC (t.TAC) in DOCA-induced hypertensive rats (Table 1 and Fig. 2): At the end of experiment, when compared to normal control, 4-weeks DOCA treatment induced a significant ($P < 0.001$) decrease in t.TAC in the DOCA-treated rats.

When compared to the DOCA-treated rats, 4-weeks treatments with 4-OH-Ile, s-AML, h-AML, and s-AML/4-OH-Ile, in their respective/DOCA-treated groups, produced significant ($P < 0.001$) increases in t.TAC.

From other aspect, 4-weeks treatments with h-AML and s-AML/4-OH-Ile in the respective/DOCA-treated rats produced insignificant ($P > 0.05$) changes in the t.TAC when compared to the control rats. Also, when compared to the h-AML/DOCA-treated rats, 4-weeks s-AML/4-OH-Ile treatment in the s-AML/4-OH-

Ile/DOCA-treated rats produced a significant ($P < 0.05$) increase in t.TAC.

Collectively, DOCA decreased significantly the t.TAC in rats. Both h-AML and s-AML/4-OH-Ile, administered concomitantly with DOCA, prevented the DOCA-induced reduction in t.TAC keeping it near its normal range in rats. Thus, the combination s-AML/4-OH-Ile has equal or even more efficacy than h-AML in increasing the t.TAC, and thereby, is superior to h-AML as anti-oxidant agent.

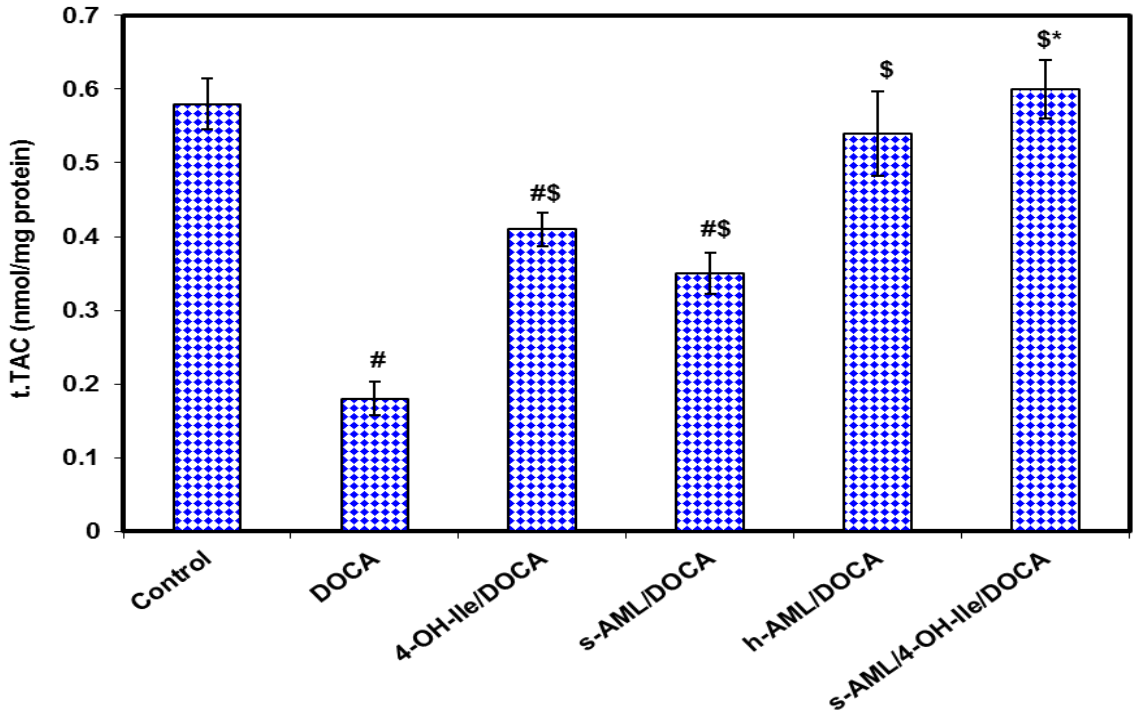

Figure 2. Effects of DOCA, 4-OH-Ile/DOCA, s-AML/DOCA, h-AML/DOCA and s-AML/4-OH-Ile/DOCA treatments on the t.TAC in the different rats groups.
#: Significant in comparison to the control group.
$: Significant in comparison to the DOCA-treated group.
*: Significant in comparison to the h-AML/DOCA-treated group.

3. Effects of 4-OH-Ile, s-AML, h-AML and s-AML/4-OH-Ile treatments on the ventricle tissue MDA (t.MDA) in DOCA-induced hypertensive rats (Table 1 and Fig. 3): At the end of experiment, 4-weeks DOCA treatment in the DOCA-treated rats induced a significant ($P < 0.001$) increase in t.MDA when compared to control group.

When compared to the DOCA-treated rats, 4-weeks treatments with 4-OH-Ile, s-AML, h-AML and s-AML/4-OH-Ile in their respective/DOCA-treated rats produced significant ($P < 0.001$) decreases in t.MDA. From other aspect, when compared to the control group, 4-weeks treatments with h-AML and s-AML/4-OH-Ile in their respective/DOCA-treated groups produced insignificant ($P > 0.05$) changes in t.MDA. Also, 4-weeks s-AML/4-OH-Ile treatment to its s-AML/4-OH-Ile/DOCA-treated rats produced an insignificant ($P > 0.05$) change in t.MDA when compared to the h-AML/DOCA-treated rats.

Collectively, DOCA increased significantly the t.MDA in rats. Both h-AML and s-AML/4-OH-Ile, administered concomitantly with DOCA, prevented the DOCA-induced increases in t.MDA keeping it near its normal range in rats. Thus, the combination s-AML/4-OH-Ile has equal efficacy to h-AML in decreasing the t.MDA, and thereby, is favored to h-AML as inhibitor of lipid peroxidation.

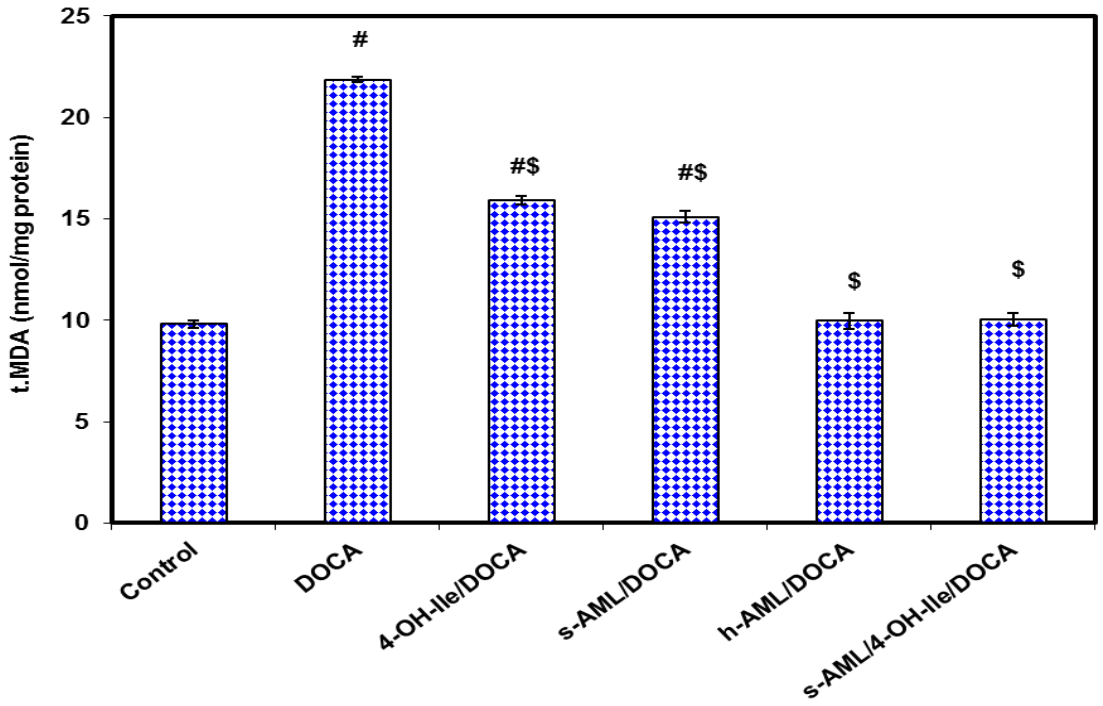

Figure 3. Effects of DOCA, 4-OH-Ile/DOCA, s-AML/DOCA, h-AML/DOCA and s-AML/4-OH-Ile/DOCA treatments on the t.MDA in in the different rats groups.
#: Significant in comparison to the control group.
$: Significant in comparison to the DOCA-treated group.

4. Effects of 4-OH-Ile, s-AML, h-AML and s-AML/4-OH-Ile treatments on the serum CK-MB and LDH in DOCA-induced hypertensive rats (Table 1 and Fig. 4): At the end of experiment, when compared to normal control, 4-weeks DOCA treatment to the DOCA-treated rats induced significant ($P < 0.001$) increases in the serum CK-MB and LDH.

4-weeks treatments with 4-OH-Ile, s-AML, h-AML and s-AML/4-OH-Ile to their respective/DOCA-treated rats produced significant ($P < 0.001$) decreases in the serum CK-MB and LDH compared to the DOCA-treated rats. While 4-weeks treatments with h-AML, and s-AML/4-OH-Ile to their respective/DOCA-treated groups produced insignificant ($P > 0.05$) changes in the serum CK-MB and a significant ($P < 0.05$) increase in the serum LDH compared to the control group. When compared to the h-AML/DOCA-treated rats, 4-weeks s-AML/4-OH-Ile treatment to its s-AML/4-OH-Ile/DOCA-treated rats produced insignificant ($P > 0.05$) changes in the serum LDH.

That is, DOCA increased significantly the serum CK-MB and LDH in rats. Both h-AML and s-AML/4-OH-Ile, administered concomitantly with DOCA, prevented the DOCA-induced elevations in the serum CK-MB and LDH keeping them near their normal values in rats. Thus, the combination s-AML/4-OH-Ile has equal efficacy to h-AML in decreasing the serum CK-MB and LDH, and thereby, is superior to h-AML as protective against cardiac injury.

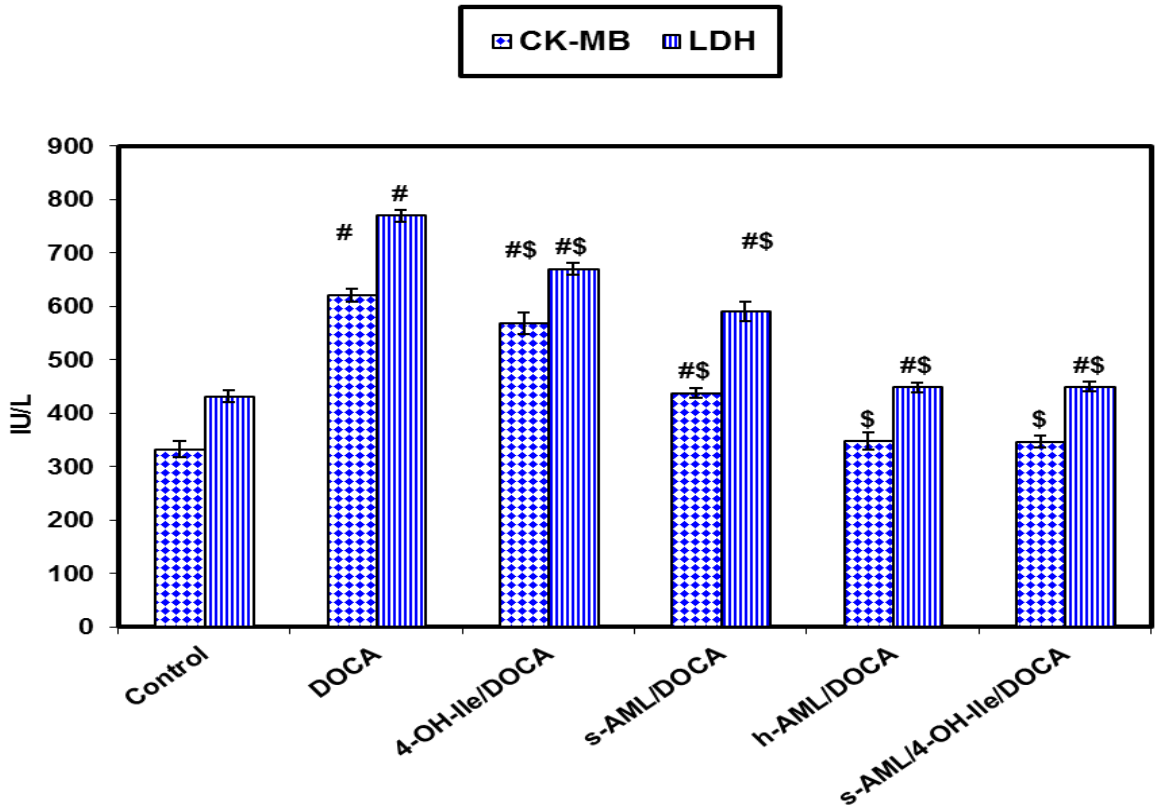

Figure 4. Effects of DOCA, 4-OH-Ile/DOCA, s-AML/DOCA, h-AML/DOCA and s-AML/4-OH-Ile/DOCA treatments on the serum CK-MB and LDH in in the different rats groups.
#: Significant in comparison to the control group.
$: Significant in comparison to the DOCA-treated group.

5. Effects of 4-OH-Ile, s-AML, h-AML and s-AML/4-OH-Ile treatments on the serum TC, TGs, HDL-c and LDL-c in DOCA-induced hypertensive rats (Table 1 and Fig. 5): At the end of experiment, when compared to normal control, 4-weeks DOCA treatment in the DOCA-treated rats induced significant ($P < 0.001$) increases in the serum total cholesterol, triglycerides and LDL-c, and a significant ($P < 0.001$) decrease in the serum HDL-c.

When compared to the DOCA-treated rats, 4-weeks treatments with 4-OH-Ile, s-AML, h-AML and s-AML/4-OH-Ile in the respective/DOCA-treated groups produced significant ($P < 0.001$) decreases in the serum total cholesterol, triglycerides and LDL-c, and a significant ($P < 0.001$) increase in the serum HDL-c. 4-weeks treatment with 4-OH-Ile and s-AML/4-OH-Ile, in their respective/DOCA-treated groups, produced insignificant ($P > 0.05$) changes in the serum total cholesterol, triglycerides and LDL-c when compared to the control rats. When compared to the h-AML/DOCA-treated rats, 4-weeks s-AML/4-OH-Ile treatment in the s-AML/4-OH-Ile/DOCA-treated rats produced a significant ($P < 0.001$) increase in the serum HDL-c.

That is, DOCA significantly increased the serum total cholesterol, triglycerides and LDL and decreased the serum HDL in rats. Both h-AML and more effectively s-AML/4-OH-Ile, when administered concomitantly with DOCA, prevented the DOCA-induced increases in the serum total cholesterol, triglycerides and LDL and a decrease in the serum HDL in rats, keeping them near their normal ranges in rats. Thus, the combination s-AML/4-OH-Ile has greater efficacy than h-AML, and thereby, is superior to h-AML as an antihyperlipidemic agent.

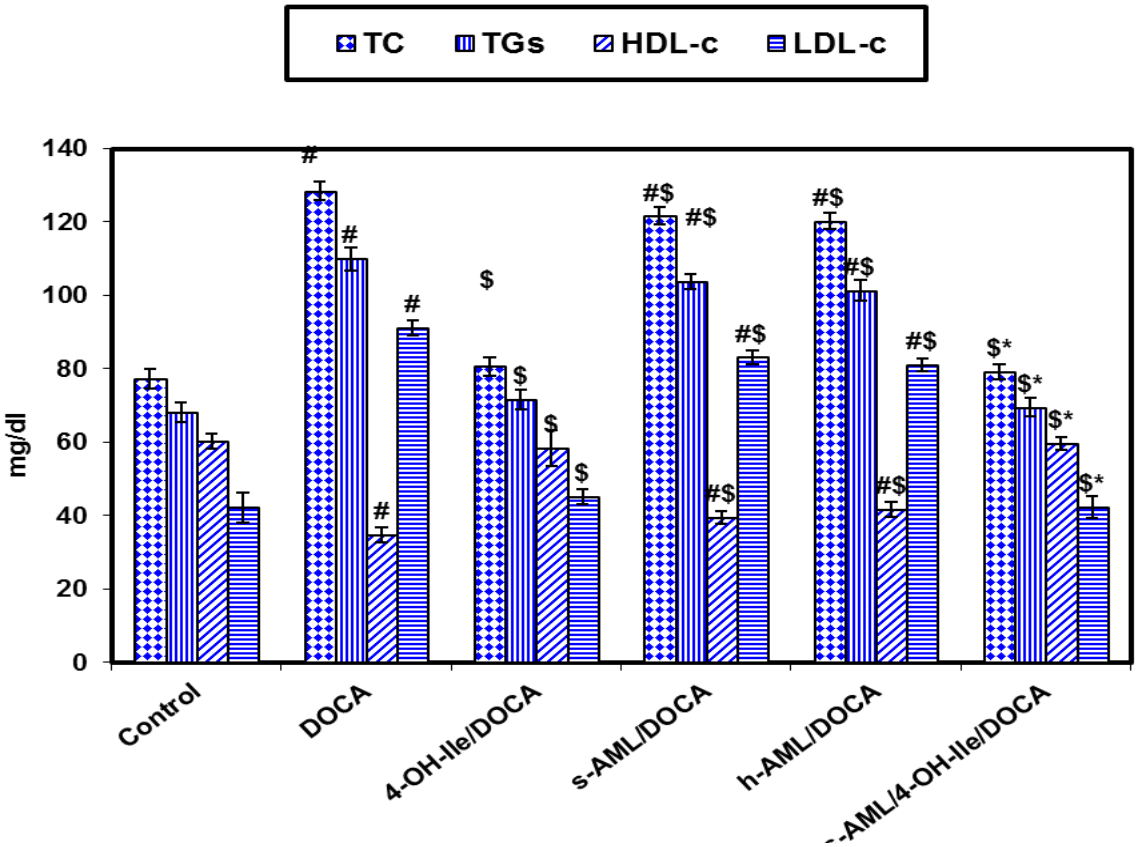

Figure 5. Effects of DOCA, 4-OH-Ile/DOCA, s-AML/DOCA, h-AML/DOCA and s-AML/4-OH-Ile/DOCA treatments on the serum TC, TGs, HDL-c and LDL-c in in the different rats groups.
#: Significant in comparison to the control group.
$: Significant in comparison to the DOCA-treated group.
*: Significant in comparison to the h-AML/DOCA-treated group.

4. Discussion

The present study evaluated the effects of combined 4-OH-Ile and s-AML on DOCA-induced hypertension in rats, and compared this effect with that of therapeutic h-AML and explored the possible mechanisms involved. In the present study, DOCA induced hypertension, cardiac injury (increased serum CK-MB and LDH), dyslipidemia (hypercholesterolemia, hypertriglyceridemia, increased LDL-c and decreased HDL-c), lipid peroxidation (increased MDA in heart) and oxidative stress (diminished total antioxidant capacity in heart) in rats. These present findings could be explained by and are in agreement with previous studies reported by **Iyer and Brown (2009**). In one of these studies, **Prahalathan et al. (2012)** found that DOCA-salt hypertensive rats showed increases in the concentrations of plasma total cholesterol, triglycerides, free fatty acids, phospholipids, plasma low-density and very low-density lipoproteins cholesterol, and decreased the plasma concentration of high-density lipoprotein cholesterol. It was reported that the DOCA-salt model is used as it rapidly induces cardiovascular remodeling similar to human chronic hypertension. Also, administration of DOCA and sodium chloride to rats provides a reliable animal model of cardiovascular oxidative and inflammatory stress because it enhances the action of many enzymes. These enzymes include peroxidases, lipoxygenases, myeloperoxidases, cytochrome P450 monooxygenase, xanthine oxidase, uncoupled nitric oxide synthase, cyclooxygenases, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, heme oxygenases and the enzymes of the mitochondrial electron transport chain producing reactive oxygen species e.g. superoxide (O_2^-) in the body cells (**Iyer and Brown, 2009**). In addition, **González et al. (2014)** mentioned that several

vasoconstrictor peptides (such as angiotensin II, endothelin-1 and urotensin II) stimulate the production of reactive oxygen species by activating many of those provided enzymes. O_2^- can react with nitric oxide (NO) to form peroxynitrite or converts to hydrogen peroxide to form hydroxyl radicals **(Iyer et al., 2010)**. Moreover, increased oxidative stress in the endothelium may contribute to the pathogenesis of hypertension and endothelial dysfunction via increased production of free radicals in the arterial wall. Furthermore, **Chistiakov et al. (2015)** showed that free radicals play an important role as mediators of cardiac and vascular smooth muscle damage and inflammation. Also, tonic release of NO from the endothelium exerts vasculo-protective and cardio-protective effects, while its reduction enhances apoptosis of endothelial cells. Besides, endothelial NO is mainly synthesized from the conversion of L-arginine to L-citrulline by endothelial nitric oxide synthase (eNOS). The uncoupled eNOS and other oxidases e.g. NADPH oxidase increased the production of O_2^- with associated loss of NO in animal models of hypertension and other models of cardiovascular risk (**Mason et al., 2014**). Moreover, **Pepine** and **Handberg (2001)** explained that peroxynitrite overproduction may contribute to the endothelial oxidative stress damage. Thereby, administration of antioxidants could reduce oxidative stress and high blood pressure levels and improve vascular function (**Bessa et al., 2009**). Indeed, chronic hypertension in humans and animal models is associated with cardiovascular diseases (CVDs) which are related to oxidative damage. Reactive oxygen species have a deleterious effect on cardiac functions **(Murugesan et al., 20120**. In addition, the elevated serum levels of the enzymes CK-MB and LDH (the diagnostic cardiac markers) are due to their leakage from the cytosol of damaged tissue to blood stream. This leakage occurred when cell membranes loss their integrity and become permeable or rupture due to any serious insult or damage to the heart muscle, for example in uncontrolled hypertension (**Chatterjea and Shinde, 2012**). At the same time, **Silvestre** and **Levy (2000)** mentioned that 3 abnormalities are present in the arterioles of hypertensive patients: i) high vasoconstrictor tone due to sympathetic hyperactivity, ii) hypertrophic remodeling promoting both the increase in vasoconstriction tone and reduction in the vascular lumen, and iii) rarefaction of the microvascular bed which contributes to the increase in the hemodynamic resistances. Hence, treatment of hypertension passed through 3 phases: i) correcting the structural abnormality, ii) limiting or correcting the hypertrophic remodelling of the arteries and arterioles of hypertensives, and recently iii) using vasodilator drugs or decreased arteriolar response to vasoconstrictor agents.

The current study also showed that h-AML prevented/corrected the DOCA-induced hypertension, cardiac injury enzymes, dyslipidemia and lipid peroxidation and oxidative stress in heart, thus keeping them near the normal ranges in rats. In consistence with the above findings regarding the therapeutic strategy of hypertension and our present results, **Mason et al. (2014)** documented that AML is a potent vasodilator and has many vascular benefits. It decreased nitrooxidative stress and increased NO release with concomitant reductions in peroxynitrite level. The vascular benefits of AML may be attributed to improved eNOS coupling efficiency associated with reduced oxidative stress through various enzymatic and nonenzymatic pathways. AML not only activates eNOS, but also enhances vasodilation through modulation of excitation-contraction mechanisms in smooth muscle cells while reducing oxidative stress. Correspondingly, AML could improve endothelial function in hypertensive patients and reduce cardiovascular events compared with other antihypertensive regimens in a manner correlated with enhanced vasodilation. In the same line, **Kishi and sunagawa (2012**) demonstrated that high dose of AML (10 mg /kg /day) caused sympathoinhibition via reduction of oxidative stress through the inhibition of NADPH oxidase and activation of manganese superoxide dismutase (Mn-SOD) in hypertensive rats; an effect which was not achieved by low dose of AML (3 mg /kg /day). **Zhang et al. (2016)** explained that AML reduced the angiotensin II level, thereby decreasing its arteriolar vasoconstrictor effect, thus lowering the elevated blood pressure. However, it had little effect on decreasing the thickness of arteriolar wall and opposing the cholesterolemic atheromatous deposition. Also, AML normalizes the ultrastructure of the myocardium and prevents the signs of over-contractility of myofibrils (**Puzyrenko et al., 2013**). Furthermore, the study of **Srinivasan et al. (1997)** revealed beneficial effects of AML treatment in diabetic and/or hypertensive rats producing reduction in cholesterol levels in SH rats. Also AML improved the clinical outcomes on lipid profiles in hypertensive patients **(Iyalomhe et al., 2012).** Notwithstanding this valuable antihypertensive effect of AML, **Munoz et al. (2008)** reported many dose-related side effects of AML, e.g. peripheral edema, dizziness, palpitations and flushing, in addition to other common side effects like fatigue, nausea, abdominal pain and somnolence. From this, we can deduce that there was a great need to develop a combination of s-AML with a new herbal product, like 4-OH-Ile, to minimize the h-AML side effect and to achieve the same antihypertensive effect.

Our current study also showed that combination of 4-OH-Ile and s-AML significantly corrected all the above DOCA-induced changes in the studied

parameters, keeping them near the normal ranges in rats which were similar to or may be better than those effects of h-AML. In agreement with the present results, 4-OH-Ile, an amino acid extracted from fenugreek seeds, produced beneficial effects on the plasma lipid profile (**Flammang et al., 2004**), glucose tolerance, inflammation, insulin action, liver function, cardiovascular health (**Fuller and Stephens, 2016**) and body weight (**Jetté et al., 2009**). Besides, 4-OH-Ile decreased the elevated plasma TGs and TC levels and may represent a new candidate for the treatment of dyslipidemia and all subsequent complications, e.g. thickening of arteriolar walls and atherosclerosis in hypertensive patients via its antioxidant effects (**Jetté et al, 2009**).

Furthermore, **Dutta et al. (2014)** stated that 4-OH-Ile possesses its antioxidant effects via its capacity to scavenge O_2^-, hydroxyl, hydrogen peroxide and NADPH radicals and prevents the increase in lipid peroxidation and protein carbonyl levels. In cultured rat muscle cells, 4-OH-Ile could inhibit the palmitate-induced production of reactive oxygen species **(Fuller and Stephens, 2015)**. Moreover, **Dutta et al. (2014)** revealed that the 4-hydroxyisoleucine (28%) and trigonelline enriched fraction extract (TF4H (28%)), isolated from the seed of *Trigonella foenum graecum*, possesses good antioxidant characteristics which is evident from its capacity to scavenge hydroxyl radical ($^-$OH), superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and DPPH radical in chemically-defined *in vitro* system using Cu2+ and ascorbic acid. This fraction prevented the increases in cardiac mitochondrial lipid peroxidation and protein carbonyl levels, and prevented the decrease in reduced glutathione level in Cu-ascorbate induced oxidative stress in goat heart mitochondria. Also, this fraction caused inhibition of the increase in manganese superoxide dismutase (Mn-SOD) activity, and caused induction of glutathione reductase (the reduced glutathione synthesizing enzyme) and Krebs cycle enzyme activity, which makes its antioxidant phenomenon. Also, they reported that fenugreek seeds also lower serum TGs, TC and LDL-c (**Narender et al., 2006**). The 4-hydroxyisoleucine has been reported to be responsible for the antidiabetic activity of fenugreek which is possibly due to their antioxidant activities. In India, fenugreek is commonly used as a condiment and used medicinally as an anti-dyslipidemic agent (**Narender et al., 2006; Dutta et al., 2014**). In the same line, **Zafar** and **Gao (2016)** added that 4-OH-Ile improved the renal dysfunction and dyslipidemia parameters, and increased the antioxidants and hormones levels altered in different metabolic disorders.

Conclusion: The current study confirmed that the new combination of 4-OH-Ile and s-AML produced comparable beneficial effects, to those of therapeutic h-AML, in the treatment of hypertension and in protection against the deleterious effects on biochemical parameters estimated, all induced by DOCA in rats, thus, restoring the levels of the serum lipids and cardiac enzymes and the cardiac tissue oxidative stress parameters; TAC and MDA to normal values. This antihypertensive effect of combined 4-OH-Ile and s-AML might be due to their combined strong antioxidant activities opposing the oxidative damages accompanying DOCA-induced hypertension in rats, and would owe also to their anti-dyslipidemic effects which improve blood vessel elasticity and thereby improve the vasodilator effect of s-AML. These results suggest that the combination therapy with 4-OH-Ile and s-AML has a potential to be a novel treatment for hypertension with protection against cardiac dysfunction and oxidative stress. Thus, to potentiate these results, it is recommended to perform more studies to clarify some possible mechanisms of interaction between 4-OH-Ile and AML, like its effect on the pharmacokinetics of AML e.g. its plasma concentrations. Also, for more reliable evidences supporting a clinical trial on 4-OH-Ile, it is preferably recommended to use the most widely used preclinical spontaneously-hypertensive rats (SHR) model. These evidences will be accredited for the use of this combination in clinical trials on humans to obtain concrete evidence of its beneficial impact before it can be approved as a clinically-effective new antihypertensive combination.

Acknowledgements: The authors are thankful to the technician staff of the physiology and clinical pharmacology departments in the Faculties of Medicine, King Abdul-Aziz and Jazan universities, KSA, for their valuable help in this study.

Disclosure Statement: No Conflicts Of Interest Exist Among Author.

*** Corresponding author:** Mohamed Abdelmohsen Abdallah, MBBS, PhD, Physiology Department, Faculty of Medicine, Menoufeya University, Egypt.
Phone: 00201099127688 – 00966596504807.
Email: mwmdy@yahoo.com

References

[1]. **Bessa, S.S., Ali, E.M. and Hamdy, S.M. 2009.** The role of glutathione S- transferase M1 and T1 gene polymorphisms and oxidative stress-related parameters in Egyptian patients with essential hypertension. Eur. J. Intern. Med. 20(6): 625-630. doi: 10.1016/j.ejim.2009.06.003.

[2]. Bilginoğlu, A., Aydın, D., Özsoy, S. and Aygün, H. 2014. Protective effect of melatonin on adriamycin-induced cardiotoxicity in rats. Türk. Kardiyol.

Dern. Arş. – Arch. Turk. Soc. Cardiol. 42(3):265-273 doi: 10.5543/tkda.2014.36089.

[3]. Broca, C., Breil, V., Cruciani-Guglielmacci, C., Manteghetti, M., Rouault, C., Derouet, M., et al. 2004. Insulinotropic agent ID-1101 (4-hydroxyisoleucine) activates insulin signaling in rat. Am. J. Physiol. Endocrinol. Metab. 287(3):E463-E471. doi:10.1152/ajpendo.00163.2003.

[4]. Chatterjea, M.N. and Shinde, R. 2012. Serum enzymes in heart diseases. In: Textbook of Medical Biochemistry, 8th ed., edited by Chatterjea M.N. and Shinde R., Jaypee Brothers Medical Publishers (P) Ltd, New Delhi. pp. 639.

[5]. Chistiakov, D.A., Orekhov, A.N. and Bobryshev, Y.V. 2015. Endothelial Barrier and Its Abnormalities in Cardiovascular Disease. Front. Physiol. 6:365. doi: 10.3389/fphys.2015.00365.

[6]. Doroszko, A., Janus, A., Szahidewicz-Krupska, E., Mazur, G. and Derkacz, A. 2016. Resistant Hypertension. Adv. Clin. Exp. Med. 25(1):173-83. doi: 10.17219/acem/58998.

[7]. Dutta, M., Ghosh, A.K., Mohan, V., Shukla, P., Rangari, V., Chattopadhyay, A., et al. 2014. Antioxidant mechanism(s) of protective effects of Fenugreek 4-hydroxyisoleucine and trigonelline enriched fraction (TF4H (28%)) Sugaheal against copper-ascorbate induced injury to goat cardiac mitochondria in vitro. Journal of Pharmacy Research, 8(6): 798-811. ISSN: 0974-6943.

[8]. Flammang, A.M., Cifone, M.A., Erexson, G.L., and Stankowski, L.F.Jr. 2004. Genotoxicity testing of a fenugreek extract. Food Chem. Toxicol. 42(11): 1769-1775. PMID: 15350674.

[9]. Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18(6): 499-502. PMID: 4337382.

[10]. Fuller, S. and Stephens, J.M. 2015. Diosgenin, 4-hydroxyisoleucine, and fiber from fenugreek: mechanisms of actions and potential effects on metabolic syndrome. Adv Nutr. 6 (2):189-97. doi: 10.3945/an.114.007807.

[11]. Haefliger, J.-A., Castillo, E., Waeber, G., Bergonzelli, G.E., ois Aubert, J.-F., Sutter, E., et al. 1997. Hypertension increases connexin43 in a tissue-specific manner, Circulation, 95:1007-1014. doi: 10.1161/01.CIR.95.4.1007.

[12]. Haidari, F., Omidian, K., Rafiei, H., Zarei, M. and Mohamad Shahi, M. 2013. Green tea (Camellia sinensis) supplementation to diabetic rats improves serum and hepatic oxidative stress markers. Iran J. Pharm. Res. 12(1): 109-114. PMID: 24250578.

[13]. Hasegawa, Y., Nakagawa, T., Uekawa, K., Ma, M., Lin, B., Kusaka, H., et al. 2016. Therapy with the Combination of Amlodipine and Irbesartan Has Persistent Preventative Effects on Stroke Onset Associated with BDNF Preservation on Cerebral Vessels in Hypertensive Rats. Transl. Stroke Res. 7(1):79-87. DOI 10.1007/s12975-014-0383-5.

[14]. Ihedioha, J.I., Noel-Uneke, O.A. and Ihedioha, T.E. 2013. Reference values for the serum lipid profile of albino rats (*Rattus norvegicus*) of varied ages and sexes. Comp. Clin. Pathol. 22: 93–99 DOI 10.1007/s00580-011-1372-7

[15]. Iyalomhe, G.B.S., Omogbai, E.K.I., Isah, A.O., Iyalomhe, O.O.B., Dada, F.L. and Iyalomhe, S.I. 2012. A Comparison of the Effects of Amlodipine and Hydrochlorothiazide Monotherapy on Lipid Metabolism in Hypertensive Nigerians with Type 2 Diabetes Mellitus. J Diabetes Metab 3(10): 229. doi:10.4172/2155-6156.1000229.

[16]. Iyer, A. and Brown, L. 2009. Is mycophenolate more than just an immunosuppressant? - An overview. Indian J Biochem Biophys. 46(1):25–30. PMID: 19374250

[17]. Iyer, A., Chan, V. and Brown, L. 2010. The DOCA-Salt Hypertensive Rat as a Model of Cardiovascular Oxidative and Inflammatory Stress. Curr Cardiol Rev. 6(4): 291–297. doi: 10.2174/157340310793566109.

[18]. Jetté, L., Harvey, L., Eugeni, K., and Levens, N. 2009. 4-Hydroxyisoleucine: a plant-derived treatment for metabolic syndrome. Curr. Opin. Investig. Drugs (London), 10(4):353-358. PMID: 19337956.

[19]. Kalshetti, P.B., Alluri, R., Mohan, V., and Thakurdesai, P.A. 2015. Effects of 4-hydroxyisoleucine from Fenugreek Seeds on Depression-like Behavior in Socially Isolated Olfactory Bulbectomized Rats. Pharmacogn. Mag. 11(Suppl. 3): S388-S396. doi: 10.4103/0973-1296.168980.

[20]. Kishi, T. and Sunagawa, K. 2012. Combination therapy of atorvastatin and amlodipine inhibits sympathetic nervous system activation and improves cognitive function in hypertensive rats. Circ. J. 76(8):1934-1941. doi:10.1253/circj.CJ-12-0276

[21]. Lee, Y.H., Kim, T.Y. and Hong, Y.M. 2009. Metalloproteinase-3 Genotype as a Predictor of Cardiovascular Risk in Hypertensive Adolescents. Korean Circ. J. 39(8): 328-34. doi: 10.4070/kcj.2009.39.8.328.

[22]. Lu, J., Liu, F., Liu, D., Du, H., Hao, J., Yang, X. and Cui, W. 2016. Amlodipine and atorvastatin improved hypertensive cardiac hypertrophy through regulation of receptor activator of nuclear

factor kappa B ligand/receptor activator of nuclear factor kappa B/osteoprotegerin system in spontaneous hypertension rats. Exp. Biol. Med. (Maywood). pii: 1535370216630180. [Epub ahead of print] PMID: 26908571.

[23]. Lv GY1, Zhang YP, Gao JL, Yu JJ, Lei J, Zhang ZR, Li B, Zhan RJ, Chen SH 2013. Combined antihypertensive effect of luteolin and buddleoside enriched extracts in spontaneously hypertensive rats J Ethnopharmacol. 2013 Nov 25;150(2):507-13. doi: 10.1016/j.jep.2013.08.058. Epub 2013 Sep 28.

[24]. Magro, A., Osswald, W., Reis, D., and Vanhoutte, P. 1986. Central and peripheral mechanisms of cardiovascular regulation. Series A: Life sciences, Vol. 109, 1st edition, Plenum Press, New York.

[25]. Makaritsis, K.P., Gavras, H., Du, Y., Chobanian, A.V. and Brecher, P. 1998. α_1- adrenergic plus angiotensin receptor blockade reduces atherosclerosis in apolipoprotein E-deficient mice. Hypertension, 32(6): 1044-1048. doi: 10.1161/01.HYP.32.6.1044

[26]. Mason, R.P., Jacob, R.F., Corbalan, J.J., Kaliszan, R. and Malinski, T. 2014. Amlodipine Increased Endothelial Nitric Oxide and Decreased Nitroxidative Stress Disproportionately to Blood Pressure Changes. Am. J. Hypertens. 27(3): 482-488 doi:10.1093/ajh/hpt202.

[27]. Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V. and Milner, A. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci. 84(4): 407-412. PMID: 8482045.

[28]. Munoz, R., Schmitt, C.G., Roth, S.J., and da Cruz, E. (Eds.). 2008. Handbook of Pediatric Cardiovascular Drugs. Springer-Veralog London. DOI: 10.1007/978-1-84628-953-8. Print ISBN: 978-1-84628-952-1 Online ISBN: 978-1-84628-953-8.

[29]. Murugesan, M., Ragunath, M., Nadanasabapathy, S., Revathi, R. and Manju, V. 2012. Protective role of fenugreek on isoprotenelol induced myocardial infarction in rats. International Research Journal of Pharmacy (IRJP), 3(2): 211-216. ISSN 2230-8407.

[30]. Narender, T., Puri, A., Shweta, Khaliq, T., Saxena, R., Bhatia, G. and Chandra, R. 2006. 4-hydroxyisoleucine an unusual amino acid as antidyslipidemic and antihyperglycemic agent, Bioorg Med. Chem. Lett. 16(2): 293-296. DOI: 10.1016/j.bmcl.2005.10.003

[31]. Nivethetha, M., Jayasri, J., and Brindha, P. 2009. Effects of Muntingia calabura L. on isoproterenol-induced myocardial infarction. Singapore Med. J. 50(3):300-302. PMID: 19352575.

[32]. Orlov, S.N., Thorin-Trescases, N., Kotelevtsev, S.V., Tremblay, J. and Hamet, P. 1999. Inversion of the intracellular Na^+/K^+ ratio blocks apoptosis in vascular smooth muscle at a site upstream of caspase-3. J. Biol. Chem. 274(23): 16545-16552. doi: 10.1074/jbc.274.23.16545.

[33]. Pepine, C.J. and Handberg, E.M. 2001. The vascular biology of hypertension and atherosclerosis and intervention with calcium antagonists and angiotensin-converting enzyme inhibitors. Clin Cardiol. 24(11): V1-5. PMID: 11712769

[34]. Prahalathan, P., Saravanakumar, M. and Raja, B. 2012. The flavonoid morin restores blood pressure and lipid metabolism in DOCA-salt hypertensive rats. Redox Rep. 17(4): 167-75. doi: 10.1179/1351000212Y.0000000015. Epub 2012 Jul 9.

[35]. Puzyrenko, A.M., Chekman, I.S., Kuftyreva, T.P., and Horchakova, N.O. 2013. The effect of amlodipine, bisoprolol on the myocardial ultrastructure of the hypertensive rats. Fiziol. Zh. 59(3):39-49. PMID: 23957163.

[36]. Rao, K.N., Kottapally, S., Eskander, E.D., Shinozuka, H., Dessi, S. and Pani, P. 1986. Acinar cell carcinoma of rat pancreas: Regulation of cholesterol esterification. Br. J. Cancer, 54(2): 305-310. PMID:3741765 PMCID:PMC2001518.

[37]. Sharma, J.N. 2009. An Overview on Relation between the Bradykinin System and Hypertension and Diabetes. Am. J. Biomed. Sci. 1(3): 242-249. DOI: 10.5099/aj090300242.

[38]. Siebenhofer, A., Jeitler, K., Horvath, K., Berghold, A., Posch, N., Meschik, J., et al. 2016. Long-term effects of weight-reducing drugs in people with hypertension. Cochrane Database Syst. Rev. 3:CD007654. DOI: 10.1002/ 14651858.CD007654.pub4.

[39]. Silvestre, J.S. and Lévy, B.I. 2000. Hypertension: microvascular complications. Arch. Mal. Coeur Vaiss. 93(11 Suppl):1387-92. PMID:11190285.

[40]. Srinivasan, P.S., Hakim, Z.S., Santani, D.D and Goyal, R.K. 1997. Effects of chronic treatment with amlodipine in streptozotocin-diabetic and spontaneously hypertensive rats. Pharmacol Res. 35(5): 423-8. DOI: 10.1006/phrs.1997.0154

[41]. Stone, S.H. 1954. Method of obtaining venous blood from the orbital sinus of a rat or mouse. Science, 119(3081):100. DOI: 10.1126/science.119.3081.100

[42]. Thippeswamy, B.S., Thakker, S.P., Tubachi, S., Kalyani, G.A., Netra, M.K., Patil, U., et al. 2009. Cardioprotective effect of Cucumis trigonus Roxb

on isoproterenol-induced myocardial infarction in rats. Am. J. Pharmacol. Toxicol. 4(2): 29-37. ISSN 1557-4962.URLhttp://www.scipub.org/fulltext/AJPT/AJPT4229-37.pd...

[43]. Yeh, J.H., Yang, Y.C., Wang, J.C. and Wang, J.J. 2013. Curcumin attenuates renal ischemia and reperfusion injury-induced restrictive respiratory insufficiency. Transplant. Proc. 45(10): 3542-5. doi: 10.1016/j.transproceed.2013.09.004.PMID:24314955.

[44]. Zafar, M.I. and Gao, F. 2016. 4-Hydroxyisoleucine: A Potential New Treatment for Type 2 Diabetes Mellitus. BioDrugs. 2016 May 5. [Epub ahead of print]. DOI: 10.1007/s40259-016-0177-2

[45]. Zhang, L., He, D., and Lin, J. 2016. Prehypertensive treatment with losartan, however not amlodipine, leads to long-term effects on blood pressure and reduces the risk of stroke in spontaneously hypertensive stroke-prone rats. Mol Med Rep. 13(2):1304-10. doi: 10.3892/mmr.2015.4641.

.

5/26/2024