Safety and Efficacy of Methanol Fraction of *Moringa oleifera* as Antihypertensive in L-NAME Induced Hypertensive Rabbits: Bedside to Bench, Implications for Bench Back to Bedside

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Abstract: *Context*: Hypertension, a global menace requires innovative research into the use of *Moringa oleifera* being promoted and traditionally used as alternative therapy.

Objective: To innovatively evaluate the mechanistic effect, safety and efficacy of the methanol fraction of *M. oleifera* (MMO) leaves on L-NAME induced hypertensive rabbits.

Methods: Rabbits were divided into six groups: Control, L-NAME alone, L-NAME with 100, 200 or 400 mg/kg of MMO and enalapril. Inclusion and exclusion criteria were similar baseline parameters and Day 3 systolic blood pressure (SBP) less than baseline SBP respectively. The primary outcome was a 10% reduction of SBP on Day 21. Enalapril group was excluded from analysis. Safety was assessed with liver and renal functions, hydrogen peroxide and nitric oxide concentrations to elucidate mechanistic effect.

Results: Moringa 100 mg/kg, 200 mg/kg and 400 mg/kg reduced SBP by 4.75, 18.00 and 15.25 mmHg ($F=22.123$, $p=0.000$). SBP control was achieved with MMO 200mg/kg, 14% reduction and 400mg/kg, 12% reduction. Nitric oxide concentration, 0.06, 0.094 and 0.114mmol ($F= 30.255$, $p= 0.000$) dose-dependently increased and was most predictive of SBP control ($r^2=0.802$, $p=0.000$). Nitric oxide production was inversely related to heart/body weight ratio which was dose-dependently reduced. MMO reduced hydrogen peroxide and ALT level but no significant effect on urea, HDL, and TG.

Conclusion: MMO reduced SBP and dose-dependently increased nitric oxide concentration in L-NAME induced hypertensive rabbits. The effect may be mediated via activation of nitric oxide pathway. MMO demonstrated a potent anti-oxidant activity and safety. Effect on ventricular hypertrophy needs further evaluation.

Keywords: *Moringa*, hypertension, nitric-oxide, anti-oxidant, intention-to-treat, per protocol.

INTRODUCTION

Hypertension, a global epidemic is of public health concern, and the prevalence is on the increase [1]. Hypertension or high blood pressure (BP) is a chronic medical condition in which arterial BP is elevated. Therapy includes the use of dietary modifications, antihypertensive medications, and exercise intervention. Herbal remedies and botanicals are employed by individuals for various medical conditions without appropriate disclosure to their physicians [2]. *Moringa oleifera* (MO) has been advocated as a remedy for hypertension [3]. It is argued that someone suffering from hypertension will benefit from MO because of its abundant calcium, magnesium, potassium, zinc and vitamins C and E [4]. MO has the potential for nutritional security [5] and as a pharmaceutical excipient [6].

Previous studies on MO have documented the antihypertensive effect and some proposed mechanism(s) of action [7,8,9]. However, the safety is yet to be established. This study aims to evaluate the safety and efficacy of methanolic fraction of *Moringa oliefera* (MMO) as an antihypertensive in L-NAME induced hypertensive rabbits and to elucidate the possible mechanism of action. Phytochemical analysis of methanolic leaf extract of MO has shown the presence of flavonoids, terpenoids, glycoside, tannins, and saponins [10].

L-NAME inhibits synthesis of nitric oxide, a potent vasorelaxant. Vascular endothelial cells produce nitric oxide (NO) which readily diffuses into the adjacent vascular smooth muscle (VSM) cells resulting in relaxation. This relaxation is achieved through the cGMP second messenger system that leads to activation of calcium pumps embedded in the plasma membrane and sarcoplasmic reticulum. The calcium pumps effectively lower the intracellular calcium concentration causing relaxation of VSM and dilation of the blood vessels [11]. Synthesis of NO is an enantiomer-specific reaction and is inhibited *in vitro* by

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Nω-monomethyl-L-arginine (L-NMMA), but not its D-enantiomer [12].

MATERIALS AND METHODS

Consumable Materials

Nω-nitro-L-arginine methyl ester (L-NAME) (Sigma Chemical Co. Estonia), AST, ALT, Triglycerides Randox kits, Methyl alcohol, Water, Ethanol, Kit for biochemical tests, Sterile gloves, Needles and syringes, Griess reagents, oxygenated Krebs-HEPES, NADPH oxidase, xanthine oxidase, Nitric oxide synthase and catalase

Non-Consumables

The tail-cuff computer-aided monitoring device (Automatic Blood Pressure Computer, Model LE 5007, LSI Letica Scientific Instruments, Barcelona Spain), ECG machine, Bucket Centrifuge, Pasteur pipette, Eppendorf tubes, Standing rack.

Methods

The leaves of MO were collected from the farm, in Ojoo Ibadan, Oyo State Nigeria and identified by Mr. Babalola in the Department of Botany, Faculty of Science, University of Ibadan. The leaf samples were air dried at room temperature and the dried samples were extracted with 100% methanol. 10kg of the dried leaves was used and soaked in 50 litres of 100% methanol and 198g was recovered as the extract. The extracts were collected at least three times and were filtered through Whatman number 1 filter paper and then concentrated on a rotary evaporator (Buchi, Flawil, Switzerland) at 45°C, dried and kept at 4°C till used for the assay. The sample and solvent mass ratio were 1: 2 during extraction. The extracts were diluted with sterile water to get the final concentration as per requirement.

Animals

Adult male rabbits weighing between 1.2kg and 1.95kg were obtained in a farmhouse at Ojoo, Oyo state. All animals were stabilized and maintained under laboratory conditions (12 h: 12 h light/ dark cycle, frequent air change) and had free access to tap water and food.

Study Design

The effect of methanol fraction of MO leaf was examined on blood pressure parameters; mean arterial, systolic and diastolic blood pressure (MABP, SBP, DBP) of rabbits previously treated with L-NAME.

Experimental protocols were carried out in line with the standard ethical guidelines for laboratory animal use and care. Blood pressure and heart rate measurements were determined by the non-invasive method. A stabilization period of 30 minutes was observed before any recording. Heart rate was determined by the use of the Computer-aided electrocardiogram (ECG) monitor.

The study was conducted in University of Ibadan Department of Pharmacology and Therapeutics and Veterinary Medicine. The animals were kept in the animal house while the experiments on the organs and sample extraction were done in the veterinary teaching hospital laboratory.

Rabbits were divided into six groups of four animals each.

Group A: Control rabbits (Distilled water)

Group B: L-NAME induced (40mg/kg) hypertensive rabbits

Group C: L-NAME induced (40mg/kg) hypertensive rabbits + Methanol fraction of *Moringa oleifera* (MMO)-treated (100mg/kg body weight)

Group D: L-NAME induced (40mg/kg) hypertensive rabbits + MMO-treated (200mg/kg body weight)

Group E: L-NAME induced (40mg/kg) hypertensive rabbits + MMO-treated (400mg/kg body weight) and

Group F: L-NAME induced (40mg/kg) hypertensive rabbits + Enalapril (40mg/kg body weight).

All drugs were administered daily by gastric intubation on non-anesthetized animals for 3 weeks. The intubation was done daily, on non-anesthetized animals. The dose of the extract was based on previous results [10, 13].

Hypertension was ascertained by blood pressure readings measured with tail cuff sphygmomanometer wrapped around the left forearm of the rabbits.

Baseline heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were done on day 0, 3, 7, 14, and at the end of the study on day 21. Safety was evaluated with liver function (aspartate transaminase, AST and

alanine transaminase, ALT) and renal function (urea) tests on the aforementioned days.

The hearts were collected, washed in saline and weighed. The samples were homogenized and centrifuged at 3, 000 rpm for 15 minutes. Hydrogen peroxide tissue content was evaluated. Nitric oxide tissue content was also evaluated using nitrite measurement at 550 nm [14]. Total cardiac nitrites concentration was determined according to the method of Granger and colleagues [14]. The heart samples were incubated with enzymatic cofactors and nitrate reductase for 30 min at room temperature to convert nitrite to nitrate. The reaction was evoked by adding Griess reagent to the solution. The colored substance obtained was determined by spectrophotometer at 540 nm, and its concentration reported as nmol/l.

Hydrogen Peroxide Production Evaluation

The heart sample homogenates were incubated in oxygenated Krebs-HEPES (composition in mmol L^{-1}: NaCl 118, KCl 4.5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, KH_2PO_4 1.2, Na-HEPES 25, $NaHCO_3$ 25 and glucose 5; pH 7.4), at 37°C. Sixty minutes later, H_2O_2 was measured in the incubation medium. These experiments were also performed in the presence of inhibitors of NADPH oxidase (diphenyleneiodonium, DPI, 500 µmol·L^{-1}; apocynin 500 µmol·L^{-1}), xanthine oxidase (oxypurinol, 500 µmol·L^{-1}), NOS (nitro-L-arginine methyl ester, L-NAME, 500 µmol·L^{-1}) and catalase (aminotriazole, 50 mmol·L^{-1}).

Serum Transaminases (AST, ALT)

Randox diagnostic kits were used and the manufacturer protocol followed. The respective parameters were determined by measuring the UV spectrophotometric absorbance.

Statistical Analysis

Data were analysed using IBM SPSS Statistics version 20 and GraphPad Prism 5. Data are expressed as a mean and standard error of the mean. One way

Table 1: Effect of Methanol Fraction of *Moringa oleifera* Leaf on Heart Rate (beats/min), Systolic (SBP) and Diastolic Blood Pressure (DBP) and Mean Arterial Pressure (MAP) (mmHg)

	Control	L-NAME	L-NAME + MMO 100mg/kg	L-NAME + MMO 200mg/kg	L-NAME + MMO 400mg/kg	F, P value
Heart rate						
Day 0	217.33±5.36	236.75±13.38	239.50±15.84	212.00±0.00	250.50±14.15	0.894, 0.500
Day 3	173.67±15.92	252.33±14.33	204.25±6.70	181.00±0.00	214.50±11.47	5.324, 0.015*
Day 7	181.33±8.09	237.25±12.36	191.00±1.29	-	210.50±8.85	7.723, 0.005**
Day 14	182.33±9.68	241.25±16.84	205.25±15.76	189.00±0.00	193.75±11.74	2.409, 0.112
Day 21	194.67±13.87	237.25±12.36	214.75±19.13	202.00±0.00	190.25±22.23	1.672, 0.226
SBP						
Day 0	128.33±2.90	113.50±2.40	110.75±5.44	121.00±0.00	119.75±4.99	2.243, 0.131
Day 3	125.33±2.03	137.50±4.66	117.00±1.68	130.00±0.00	127.00±5.70	3.326. 0.051
Day 7	111.67±1.20	130.50±3.10	120.50±7.00	121.00±0.00	118.25±2.18	2.340, 0.119
Day 14	112.00±3.60	129.25±2.50	121.50±6.13	119.00±0.00	111.75±1.60	3.478, 0.045*
Day 21	109.67±2.40	136.75±2.56	112.25±2.56	112.00±0.00	111.75±2.02	22.123,0.000***
DBP						
Day 0	88.67±6.06	71.00±2.58	68.75±5.33	91.00±0.00	74.50±4.50	3.188, 0.057
Day 3	78.00±2.08	91.25±1.89	87.75±2.39	89.00±0.00	83.00±1.78	5.609, 0.010*
Day 7	87.00±5.20	90.25±0.75	81.00±1.78	83.00±0.00	85.00±1.08	2.307, 0.123
Day 14	83.67±4.33	91.25±1.11	74.25±4.03	95.00±0.00	84.00±0.00	4.941, 0.016*
Day 21	85.33±0.88	91.50±1.56	79.75±1.70	80.00±0.00	83.50±2.60	5.743, 0.010*
MAP (mmHg)						
Day 0	103.67±2.33	86.00±1.78	86.00±5.64	103.00±0.00	90.75±3.82	3.596, 0.041*
Day 3	95.67±0.33	108.00±1.78	98.50±2.53	102.00±0.00	97.50±2.78	4.596, 0.020*
Day 7	94.67±4.06	104.75±1.93	95.25±3.45	95.00±0.00	95.75±1.55	2.383, 0.115
Day 14	92.67±3.93	103.75±1.32	89.25±4.80	103.00±0.00	93.25±2.14	3.269, 0.054
Day 21	93.33±1.20	106.75±1.25	90.50±1.32	91.00±0.00	92.50±1.85	20.801,0.000***

Values = Mean ± SEM *p≤0.05, **p≤0.01, ***p≤0.001.

ANOVA was used for comparison of multiple variables of continuous data, Newman-Keuls multiple comparison tests were carried as post hoc, the level of significance was taken at $p<0.05$.

RESULT

*Moringa*100mg/kg, 200mg/kg and 400mg/kg respectively reduced blood pressure (mmHg), systolic by 4%, (117.0 to 112.3); 14% (130.0 to 112.0) and 12%, (127 to 111.8), ($F=22.123$, $p<0.001$); diastolic by 9%, (87.8 to 79.8); 10%, (89.0 to 80.0) and about 1% increase, 83 to 83.5 ($F=5.743$, $p=0.010$) between day 3 and day 21; and mean arterial pressure by 8%, (98.5 to 90.5); 11%, (102.0 to 91.0) and 5%, (97.5 to 92.5) ($F=20.801$, $p<0.001$). Heart rate, beats/minute reduced by 6%, (252.3 to 237.3) in L-NAME only and L-NAME + MMO 400 mg/kg 11%, (214.5 to 190.3) but increased in distilled water control, 173.7 to 194.7, 12%; L-NAME + MMO 100 mg/kg 204.3 to 214.8, 5%;L-NAME + MMO 200 mg/kg 187.0 to 202.0, 8% ($F=1.672$, $p= 0.226$) Table **1**.

SBP control was best achieved with MMO 200mg/kg and positively correlated with nitrate level.

Effect of Methanol Extract of MMO Leaf on Nitric Oxide Production, Hydrogen Peroxide, and Heart Weight

The groups treated with MMO had a significant dose-dependent increase of 0.06, 0.094 and 0.144mmol in nitrite concentration in 100, 200 and 400mg/kg MMO groups respectively compared with L-NAME group ($F=30.255$, $p<0.001$) Table **2**. Hydrogen peroxide reduced by 20.4%, 26.2% and 8.6% in 100mg/kg, 200mg.kg and 400mg.kg MMO treated groups respectively compared to untreated hypertensive control group, Table **2**.

There was a dose-dependent reduction in the heart/body ratio of MMO treated groups. However, while the 400 mg/kg MMO caused a significant reduction, the 100 mg/kg caused a significant increase compared to normal and untreated hypertensive control ($p<0.05$). The group treated with 200 mg/kg MMO showed an insignificant reduction, Table **2**. Nitric oxide production was inversely related to heart/body ratio with a dose-dependent increase of nitric oxide in MMO treated rabbits. Body weight from baseline value increased on day 14 in 200mg/kg and 400mg/kg MMO treated groups, $p>0.05$, Table **3**.

Safety Profile of MMO on Renal and Liver Function

All the groups had an increase in urea concentration, though no significant difference between the experimental groups ($p\geq0.05$), 200 and 400 mg/kg MMO had more increase, Table **3**.

There was an increase in AST level for all experimental groups with 200 and 400mg/kg MMO treated group having the highest increase rate of 73 and 74.75µl, respectively, Table **3**.

Alanine transferase (ALT) was relatively reduced in the MMO treated groups, Table **3**.

SBP control was best achieved with MMO 200mg/kg and positively correlated with nitrate level (Table **4**). Nitrate was most predictive of SBP control ($r^2=0.802$, $p=0.000$) Table **5**. Other predictors of SBP control were baseline blood pressure as well as serum levels of albumin and triglyceride on day 7.

DISCUSSION

In this experiment, L-NAME induced hypertension model was used and the safety and efficacy of MMO were observed within 21 days. The rabbits showed a reduction in blood pressure and the antihypertensive effect of this extract was not dose-dependent. SBP control was achieved with MMO 200mg/kg, 14% reduction while 400mg/kg exhibited 12% reduction.

Table 2: Effect of Methanol Extract of *Moringa oleifera* (MMO) Leaf on Nitric Oxide Concentration, Hydrogen Peroxide Concentration (mmol) and Heart/Body Weight Ratio

GROUPS	Nitric oxide	Hydrogen Peroxide	heart/body weight ratio
Control(vehicle)	0.663±0.033	44.981±5.24	0.0020±0.0002
hypertensive rabbits (L-NAME only)	0.458±0.018***	58.94±16.97	0.0023±0.0005
hypertensive rabbits + 100mg/kg of MMO	0.518±0.011 ***	46.91±5.86	0.0028±0.0005 *
hypertensive rabbits + 200mg/kg of MMO	0.552±0.008***	43.471±1.65	0.0021±0.0004
hypertensive rabbits + 400mg/kg of MMO	0.602±0.039 ***	53.89±13.21	0.0019±0.0002*

Values = Mean ± SD, *p<0.05, ***p<0.001.

Table 3: Effect of Methanol Fraction of *Moringa oleifera* Leaf on Transaminases (ALT, AST) µl and Urea (mg/dl) and Body Weight of Rabbits

	Control	L-NAME	L-NAME + MMO 100mg/kg	L-NAME + MMO 200mg/kg	L-NAME + MMO 400mg/kg	F, P value
AST µl						
Day 0	12.33±0.33	14.50±1.76	12.25±1.60	12.00±0.00	12.50±1.04	0.484, 0.747
Day 3	44.67±16.19	32.25±9.32	30.25±6.71	29.00±0.00	38.25±3.01	0.402, 0.804
Day 7	55.50±17.50	50.50±9.92	31.75±4.09	75.00±0.00	61.50±4.33	2.861, 0.081
Day 14	60.33±10.14	55.75±10.07	36.75±4.99	82.00±0.00	76.25±3.79	4.468, 0.022*
Day 21	70.33±9.53	69.00±9.84	51.25±7.04	92.00±0.00	87.25±2.87	3.522, 0.044*
ALT µl						
Day 0	93.00±24.27	97.25±20.11	96.25±19.38	162.00±0.00	131.75±15.81	1.185, 0.370
Day 3	108.67±24.55	114.25±12.98	88.75±21.03	43.00±0.00	87.00±24.88	0.771, 0.566
Day 7	123.00±38.00	123.25±13.03	102.25±18.75	113.60±0.00	100.25±19.28	0.301, 0.871
Day 14	126.33±21.67	130.00±11.81	107.75±18.85	120.00±0.00	100.00±22.52	0.447, 0.772
Day 21	160.33±17.90	139.00±10.40	118.50±17.51	129.00±0.00	112.50±21.97	1.047, 0.427
Urea mg/dl						
Day 0	15.67±0.88	14.50±0.29	14.50±0.87	18.00±0.00	19.25±0.48	10.158,0.001**
Day 3	17.00±1.53	20.00±0.00	19.50±1.26	23.00±0.00	21.00±1.96	1.392, 0.299
Day 7	22.50±2.50	22.75±1.31	26.00±1.73	28.00±0.00	24.00±1.47	1.094, 0.411
Day 14	23.33±1.20	25.00±1.08	26.25±1.31	30.00±0.00	26.75±1.11	2.025, 0.160
Day 21	27.33±0.88	28.50±1.26	31.75±2.43	30.00±0.00	28.50±0.87	1.091, 0.408
Weight(kg)						
Day 0	1.50±0.10	1.45±0.07	1.74±0.09	1.60±0.00	1.56±0.16	0.965, 0.464
Day 3	1.52±0.07	1.43±0.08	1.71±0.10	1.65±0.00	1.53±0.11	1.353, 0.312
Day 7	1.57±0.07	1.46±0.10	1.66±0.09	1.55±0.00	1.53±0.10	0.631, 0.651
Day 14	1.60±0.13	1.50±0.10	1.66±0.12	1.70±0.00	1.54±0.09	0.444, 0.775
Day 21	1.65±0.15	1.51±0.08	1.61±0.14	1.70±0.00	1.59±0.07	0.273, 0.889

*P < 0.05, ***p≤ 0.001.

Table 4: Correlation Showing Relationship between Nitric Oxide Production and Heart Weight with other Variables in *Moringa oleifera* Methanol Fraction Treated L-NAME Induced Hypertensive Rabbits

		R	P value
Nitrate	SBP control	0.802	0.000
	SBP0	0.654	0.006
	DBP0	0.509	0.044
	MAP0	0.604	0.013
	HR3	-0.662	0.007
	DBP3	-0.782	0.000
	MAP3	-0.562	0.024
	Alb7	0.655	0.008
	Alb/Glob7	-0.525	0.044
	TG7	0.676	0.006
	HR7	-0.574	0.025
	SBP7	-0.593	0.015
	HR14	-0.612	0.012
	SBP14	-0.707	0.002
	HR21	-0.560	0.024
	SBP21	-0.681	0.004
	MAP21	-0.540	0.031
	LDL21	0.503	0.047
Heart weight	Heart/Bodyweight	0.752	0.001
	Urea0	-0.525	0.037
	TG7	-0.544	0.036
	AST7	-0.695	0.004
	AST14	-0.713	0.002
	AST21	-0.650	0.006
	Urea21	0.582	0.018

Table 5: Predictors of Systolic Blood Pressure Control in *Moringa oleifera* Methanol Fraction Treated L-NAME Induced Hypertensive Rabbits

	Standardized Coefficient	P-value
Nitrate	0.802	0.000
Heart weight	-0.270	0.205
Weight Day 0	0.317	0.120
SBP0	0.708	0.002
DBP0	0.575	0.020
MAP0	0.663	0.005
HR3	-0.631	0.005
DBP3	-0.498	0.050
HR14	0.569	0.022
SBP14	0.511	0.043
HR21	-0.531	0.034
Alb/Glob Day 0	0.564	0.023
TG3	0.504	0.046
Alb7	0.826	0.000
TG7	0.667	0.007
TG14	0.589	0.016
AST Day 14	0.509	0.044

This may suggest that MMO 200mg/kg is more potent. Drugs usually bind to receptors to elicit their response, as dose increases, the response may increase, but the increment may diminish with increasing dose due to receptor saturation. Prolonged exposure in terms of dose or duration may also result in internalization and downregulation of receptors [15].

However, there is a suggestion of a paradoxical effect of blood pressure lowering effect and presumably ventricular hypertrophy based on the increasing heart to body weight with reducing blood pressure. It has been argued that L-NAME model of hypertension may have an unexpected effect on ventricular hypertrophy. There was a significant reduction in heart rate of MMO treated groups when compared to the L-NAME treated group. Increase nitric oxide production for rabbits treated with MMO compared with L-NAME treated group suggests that MMO activates nitric oxide pathway as one of the mechanisms by which it exerts it's the anti-hypertensive effect. The leaves of MMO contain nitrile glycosides which are reported to have hypotensive and antioxidant activities [8, 9, 16]. Though muscarinic receptor activation was excluded in the previous study with fractions of ethanol extract of *Moringa oleifera* [8], the diuretic effect may be involved [4, 17, 18]. L-arginine esters are competitive muscarinic antagonists [17].

MMO has no significant effect on hydrogen peroxide concentration although it reduced its concentration when compared with L-NAME treated group. Plasma peroxide has been found to be higher in hypertensive patients compared normotensive subjects [19]. The reduction of hydrogen peroxide exhibited by MMO will be beneficial in a hypertensive patient and may be of therapeutic value. Oxygen free radicals, including hydrogen peroxide, may mediate oxidative stress in target organ tissues and contribute to cardiovascular complications in hypertension.

Also, reduction of hydrogen peroxide concentration, though not significant, indicates a potent antioxidant activity. This may explain some of its immune boosting related use. Vascular oxidative stress has been ameliorated with Moringa seeds in spontaneously hypertensive rats [20]. MMO reduced ALT level but no significant effect on urea, HDL, and TG.

Moringa had no significant effect on body weight compared to the control groups but reduced heart weight at a high concentration of 400mg/kg. Rabbits in the enalapril group died by day 21; the question is, is

enalapril poorly tolerated in rabbits or is *Moringa oleifera* more tolerable and safer than enalapril? Further studies are needed for this.

We innovatively adapted clinical research concept of "intention-to-treat" of randomized clinical trials to preclinical research. However, "per protocol" which focus on the subset of intention-to-treat which completed the study to day 21 was analysed [21]. This study has implications for dose recommendation of *Moringa oleifera* in humans as it suggests no beneficial effect of the 400 mg/kg over the 200mg /kg. Previous acute toxicity study showed no deaths in experimental animals administered 200mg/kg, 400mg/kg and 800mg/kg of methanolic leaf extract of commercially processed *Moringa oleifera* but increasing severity of lethargy and mortality were recorded with doses of 1600mg/kg and 3200mg/kg [10]. It is therefore suggested that the maximum dose in humans must not exceed 200mg/kg.

CONCLUSION

Methanol fraction of MMO leaf reduced the blood pressure, heart rate and cardiac hypertrophy in L-NAME treated rabbits. Our results showed that nitric oxide pathway activation might be one of the mechanisms of action for its antihypertensive effects.

Also, the reduction of hydrogen peroxide concentration indicated a potent antioxidant activity. It showed strong reducing power and free radical scavenging capacity. The methanol extract of the leaf was well tolerated by experimental animals and showed no toxicity. A reduced level of ALT and no effect on urea concentration may be suggestive of the safety.

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DECLARATION OF CONFLICTING INTERESTS

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