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Original Article

Evaluation of Hypotensive and Vasodilatory Effects of *Murraya koenigii* Leaves Extract in L-NAME Induced Hypertensive Rats in Relation with Muscarinic Receptors

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ABSTRACT

Background: High Blood Pressure (BP) is a dreadful condition worldwide. Available scientific data confirms the antihypertensive potential of traditional plants. Present study was aimed to assess the blood pressure lowering effect of hexane (MURK-HEX), ethyl acetate (MURK-EA) and methanol (MURK-MeOH) extracts of *Murraya koenigii* leaves in L-NAME induced hypertensive rats with possible mechanism of action. **Materials and Methods:** Air dried leaves of "*Murraya koenigii*" were extracted with different organic solvents namely methanol (MURK-MeOH), Hexane (MURK-HEX) and ethyl acetate (MURK-EA). Wistar rats of either sex were used in the study. Hypertension was developed by L-NAME and then data was collected after jugular vein cannulation using Oscillograph and powerlab data acquisition system. For possible mode of action muscarinic pathway was chosen. Data was analysed using SPSS-20 by applying independent sample *t*-test. **Results and Discussion:** The invasive method revealed that "MURK- HEX", "MURK-EA" and "MURK- MeOH" extracts exhibited significant dose dependent (18.8-50.1) % fall in MABP at the log doses (1, 3, 5, 10 and 30 mg/kg) in normotensive rats. Except, "MURK-HEX", the "MURK-EA" and "MURK-MeOH" altered the BP lowering effect, when these extracts followed by "Atropine" (Atr 10^{-4}M) via intravenous route. Results demonstrate that MURK- EA and MURK-MeOH showed the involvement of muscarinic receptors while hypotensive effect of MURK-HEX may mediate via another pathway. In L-NAME induced hypertensive rats, MURK-HEX, MURK- EA and MURK-MeOH exhibited an immediate (18.37-51.93% falls) in MABP but it did not persist for longer time. **Conclusion:** *Murraya koenigii* caused a significant decrease in MABP by the stimulation of muscarinic receptor in activation of EDRF which release nitric oxide to produce vasorelaxation.

Keywords: *Murraya koenigii*, L-NAME, Muscarinic receptor, Hypotensive activity.

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INTRODUCTION

Cardiac diseases are one of the principal reasons of mortality all over the world and are continuously increasing day by day.[1] Amongst them hypertension has a complex incidence as all kind of heart diseases. The major factors contributing in occurrence of High BP are smoking; metabolic syndrome marked by (obesity and diabetes mellitus) and altered endothelial function.

"Nitric oxide" is a very potent cardio-protective agent which is an integral component of "Endothelium Dependant Relaxing Factor (EDRF)" whose release is facilitated by various vasorelaxants. These vasorelaxants (like acetylcholine, serotonin and bradykinin etc) mainly produce "eNOS (enothelium Nitric Oxide Synthase)" by Ca^{2+} pump mechanism.[2]

To confirm that a drug is improving blood pressure using Nitric oxide pathway a nitric oxide deficient model is required. To produce Nitric oxide deficient experimental model "L-NAME (NG- nitro-L- arginine methyl- ester)" is most appropriate[3] as it blocks nitric oxide release and cause hypertension. Plant "polyphenolic compounds" tend to improve such hypertension by reverting endothelial abnormality and thereby increasing NO release.[4]

Commonly used antihypertensive agents like diuretic etc are treatment of choice but are associated with adverse reactions.[5] The Intention of the current study is to find agents with fewer harmful effects.

"Murraya koenigii" (Rutaceae) is cultivated in "Pakistan", "Sri Lanka" and "East India" to "China" and "Hainan".[6] Locally, it is name as Karipatta and curry tree. Phytochemical analysis shows

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mahanine, mahanimbine, rheiin, girimimbine and mangiferin along with essential oils as main component of the plant. It is used traditionally in many diseases. In the sub-continent it is widely used in common household cooking. It is supposed to purify blood, relief stomach pain and flatus[7] relieve diarrhoea and vomiting.[8] Leaf oil is used to treat skin problems[9] Leaves and roots are said to relief pain, inflammation and rash.[10] The powder of leaves has antioxidant[11] and anti-amnesic[12] effects. It water based extract has been revealed anti-nociceptive[13] anti-diabetic[14] and anti-microbial[15] activities.

Hepato-protective effect is shown by carbazole alkaloids and tannins isolated from hydro-alcoholic extract.[16] Murraya leaves exhibit anti-cancer effect with their methanolic extract[17] and improved obesity and serum lipid levels by ethanolic, ethyl acetate and dichloromethane extracts.[18] The alkaloid fraction of the leaves has shown anti-inflammatory, anti-ulcerogenic[19] and neuroprotective[20] activities.

Aim of the current study is to explore hypotensive and anti-hypertensive activity and to determine mode of action of hexane, ethylacetate and methanolic extracts of *Murrayya koenigii* leaves in normotensive and L-NAME induced hypertensive rats.

MATERIALS AND METHODS

Plant

Leaves were collected in March 2013 from Azizabad No. 2. The plant was authenticated by Prof. Dr. Anjum Perveen at "Centre for plant conservation", from Botany Department at University of Karachi with receipt sample number "(GH No. 86543-4 KUH)".

Extraction of Plant *Murraya koenigii*

Murraya koenigii leaves in the form of fresh air-dried and uncrushed form were taken for sequential extraction. The procedure was carried out with different organic solvents e.g., "hexane", "dichloromethane", "ethyl acetate", "methanol" and "acetone" at room temperature in increasing polarity order. The process of each extraction was executed thrice.

A rotary evaporator was used for evaporation of extracts separately[21] to obtain their respective dry extracts like "hexane, MURK- HEX" (25.798 g, 1.289%), "dichloromethane, MURK-DC" (60.487, 3.024%), "ethyl acetate, MURK-EA" (51.98, 2.59%), "methanol, MURK-MeOH" (116.43 g, 5.82%) and "acetone, MURK-AC" (5.68 g, 0.284%) residues.

For the blood pressure regulation evaluation "MURK- HEX", "MURK- EA" and "MURK- MeOH" were used as other extracts were not soluble in available vehicles. Extracts were kept in refrigerator at 4°C until further used.

Drugs

"NG-nitro-L-arginine methyl ester hydrochloride (L-Name)" was purchased from ("Sigma Aldrich"). "Acetylcholine" from "E. Merck", "Atropine sulphate" from "C. H. Beohringer Sohn Ingelheim Rhein," Germany. "Urethane and heparin" were obtained from "Unichem" and "Leo Pharma" respectively.

Animals

"Normotensive Wistar rats" (200-240 g) of either sex were used in the study. Animals were "procured" from animal house of "Dr HMI Institute of Pharmacology and Herbal Sciences" and placed under standard condition. Rats were nourished with standard diet with free access to water *ad libitum*. Handling of laboratory animals and the procedure of cannulation were approved by "Hamdard University Ethical Review Board, HU-ERB" (Ref No. AEC-17-01). The protocols were followed according to international standard guidelines for animal use.

Blood pressure lowering effect

1.2-1.5 g/kg intraperitoneal "Urethane" was used to anesthetize the rats and procedure was performed on animals in supine position. Plastic canula was used for tracheal cannulation which cleans to facilitate respiration throughout the whole procedure. Juglar vein and carotid artery were cannulated using polyethylene canula by making small incisions with the help of scissor, probe and forceps. A saline flush followed all the drug administration's via jugular vein (0.2 mL) and the "carotid artery" was heparinized. Cannulated carotid artery with PE-50 polyethylene tubing was used to measure "blood pressure". The "blood pressure transducer" (model ML 4856) was connected with "N12128 bridge amplifier" and "power lab data acquisition system".

To maintain the body temperature of animal the overhead lamp was used. After 15-20 min of stabilization period, the extracts of *Murraya koenigii* leaves "MURK-HEX, MURK EA and MURK MeOH" were given in log doses of 1 mg/kg, 3 mg/kg, 5 mg/kg and their 10 folds higher doses i.e., "10 mg/kg, 30 mg/kg and 50 mg/kg respectively". Blood pressure was permitted to return to normal basal value between each drug administration. Alterations in mean arterial blood pressure were determined as percentage difference from normal basal values, which were obtained immediately before the drug administration. "Mean Arterial Blood Pressure (MABP)" was calculated by adding 1/3rd of pulse pressure to "diastolic blood pressure".[22] Acetylcholine (10^{-6} M) was used as a positive control which produced 43.47±0.18% fall (mean±S.E.M, n=3) in MABP. The "blood pressure" was recorded on oscillograph at a chart speed of 10 mm/sec.[5]

Mode of action of blood pressure lowering effect

By Muscarinic pathway

Urethane (1.2-1.5g/kg, i.p.) was administered to anesthetize Wistar rats (200-250 g). Rats then cannulated to determine arterial blood pressure as described previously. After 15-20 min of stabilization period, Atropine (Atr 10^{-4} M), the cholinergic antagonist was administered, Acetylcholine (Ach 10-6 M), then

cholinergic agonist was given for confirmation of muscarinic receptor blockade, thereafter from *Murraya koenigii* leaves extracts of "hexane (MURK-HEX)", "ethyl acetate (MURK-EA)" and "methanol (MURK-MeOH)" at the dose of 30 mg/kg was administered and the subsequent variations in mean arterial blood pressure were then noted.[22]

Acute anti-hypertensive activity

The procedure was performed on normotensive rats by measuring mean arterial blood pressure. The invasive method of hypotension activity is same as described earlier. Intravenous administration of "L-NAME", a "nitric oxide synthase" inhibitor (20 mg/kg) induced hypertension.[23] When "L-NAME" induced maximum rise mean "arterial blood pressure (MABP)", the "hexane (MURK-HEX)", "ethyl acetate (MURK-EA)" and "methanol (MURK-MeOH)" extracts of leaves of "*Murraya koenigii*" were then given at 30 mg/kg dose through jugular vein. The variation in mean arterial blood pressure was monitored through BP transducer.

Statistical analysis

The results were analysed via the Software of "SPSS-20". Data was considered significant when p was found <0.05. The results are displayed as "mean±Standard Error Mean (SEM)" by dependent sample t-test.

RESULTS

Blood pressure lowering effect of "MURK-HEX", "MURK-EA" and "MURK-MeOH"

Results of extracts "MURK-HEX", "MURK-EA" and "MURK-MeOH" at the doses of 1 mg/kg, 3 mg/kg, 5 mg/kg and 10 mg/kg caused a dose dependent decrease in systolic and diastolic blood pressure in Table 1. The doses 10 and 30 mg/kg in MURK- HEX and doses 30 and 50 mg/kg in MURK-EA and MURK-MeOH displayed significant and comparable hypotensive effect. However, in all three extracts, the dose 30 mg/kg was exhibited the optimum % drop in blood pressure.

Mode of action of blood pressure lowering effect

By muscarinic pathway

Rats pretreated with Atropine (Atr, 10-4M) abolished the hypotensive effect of "MURK- MeOH" and "MURK- EA" at the dose of 30mg/kg, whereas when Atropine was given prior to "MURK- HEX", the blood pressure lowering effect of "MURK-HEX" did not change as shown in Figure 1.

Antihypertensive effect of "MURK- HEX", "MURK- EA" and "MURK- MeOH"

The results demonstrated that "MURK-HEX", "MURK-EA" and "MURK-MeOH" from *Murraya koenigii* leaves ameliorates the blood pressure related with nitric oxide deficient L-NAME induced hypertensive rats. The L-NAME (20 mg/kg) via intravenous administration, induced hypertension which produced significant rise in MABP of 165.4±9.04 from 111.1±7.58 mmHg± that persist for about half an hour.

Following the L-NAME injection, MURK-HEX and MURK-MeOH exhibited correspondingly decrease (18.3% and 31.1%) in elevated MABP by 162.7 mmHg±3.8 to 132.7 mmHg±11.1 and 145.8 mmHg±3.2 to 101.2 mmHg±5.31 respectively. MURK-EA produced the maximum fall (51.9%) at 0.15 min in MABP from 152.4 mmHg±4.3 to 73.1 mmHg±3.8 just after 0.15 min of drug administration. Apart from MURK-HEX, which showing little antihypertensive effect, MURK-EA and

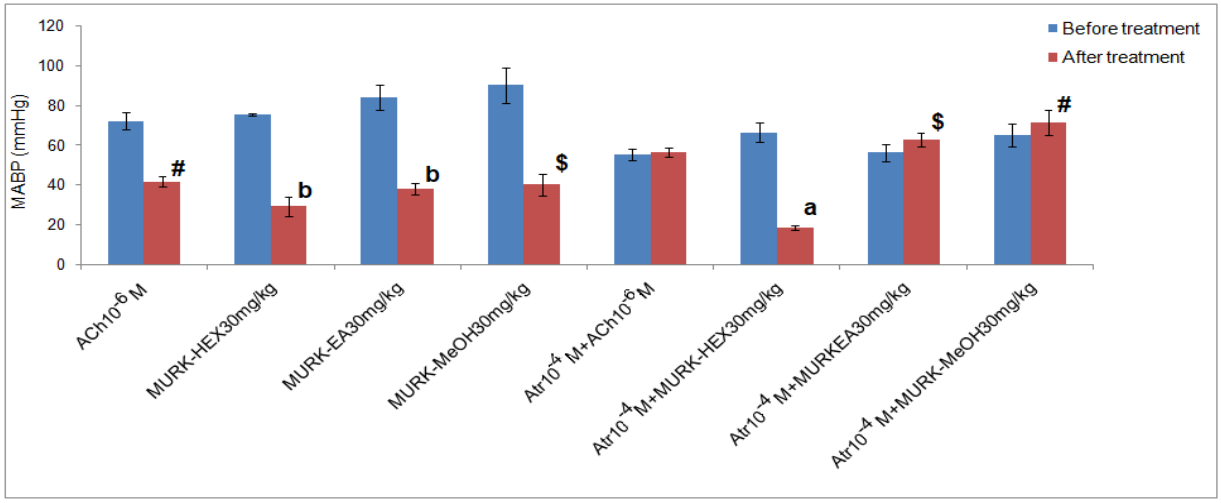

Figure 1: Effect of methanol, ethyl acetate and hexane extract of *Murraya koenigii* leaves on Muscarinic receptors.

Results are expressed as mean±S.E.M. ap<0.0005, bp<0.005, cp<0.01, \$$p$<0.025, #$p$<0.05 Anti-hypertensive effect of "MURK- HEX", "MURK- EA" and "MURK- MeOH"

MURK-MeOH returned to their initial values in 15 min as shown in Figure 2.

DISCUSSION

Blood pressure is a serious health condition which affects various organs of the body and deteriorates their function. Therefore, the use of medicinal plants gains popularity due to safe therapeutic agent, low toxicity and curative remedy for numerous diseases. Several studies showed that *Murraya koenigii* leaves possess cardioprotective potential[24,25] in various experimental models. Data obtained in this study (Table 1) indicates that leaves of *Murraya koenigii* of hexane, "MURK-HEX", ethyl acetate, "MURK-EA" and methanol, "MURK-MeOH" extracts at the doses of 1 mg/kg, 3 mg/kg, 5 mg/kg and 10 mg/kg displayed the dose dependent decrease in systolic and diastolic blood pressure. The doses 10 and 30 mg/kg in MURK- HEX and doses 30 and 50 mg/kg in MURK-EA and MURK-MeOH showed significant and comparable hypotensive effect. However, MURK-HEX when compared with "MURK-EA" and "MURK-MeOH" exhibited maximum % decrease (61.09±3.21) at the dose of 30 mg/kg in MABP which persists for more than 2 min (Table 1).

In addition to this, the duration of action in lowering blood pressure was increased in rats treated with MUR-HEX at the doses of 10, 30 and 50 mg/kg, which persisted for about 2.5-3 min. However, the longevity in lowering blood pressure at the dose of 10 mg/kg produced a more pronounced effect in comparison to 30 and 50 mg/kg doses. At the dose of 30 mg/kg, the maximum % reduction in MABP was found in all three extracts of "MURK-HEX", "MURK-EA" and "MURK-MeOH."

The cholinergic pathway plays a vital role in regulating the vascular tone, by stimulating the production of endothelial nitric oxide.[26] Therefore, there is a possibility of compromising vascular relaxation when muscarinic receptors are blocked by given Atropine 10-4 M prior to extracts.

Intravenous injection of MURK-MeOH and MURK-EA when pretreated with Atropine 10-4 M abolished hypotensive effect of both extracts (Figure 1). However, MURK- HEX failed to alter its hypotensive effect when the extract, followed by Atropine. Results demonstrate that the mode of hypotensive effect of "MURK-EA" and "MURK- MeOH" might comprise the muscarinic receptors, whereas "MURK-HEX" is not depending on cholinergic path.

Liu *et al.*[27] is reported that L-NAME-induced hypertensive rats has been widely used in research purposes to antagonize the effect of Nitric Oxide Synthase (NOS).[27]

The results demonstrated that "MURK-HEX", "MURK-EA" and "MURK-MeOH" from *Murraya koenigii* leaves ameliorates the blood pressure related with nitric oxide deficient L-NAME induced hypertensive rats. The L-NAME (20 mg/kg) via intravenous administration, induced hypertension which produced significant rise in MABP of 165.4±9.04 from 111.1±7.58 mmHg± that persist for about half an hour.

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Vasodilation is the primary response of the muscarinic pathway caused by the contribution of endothelial muscarinic receptor subtypes.[28] MURK-EA and MURK-MeOH exhibited the vasodilating and relaxant effect when atropine blocked the muscarinic receptors. This emphasized that both MURK-EA and MURK-MeOH possess the same mechanism as acetylcholine to produce endothelium-dependent relaxation leading hypotensive action. The involvement of the muscarinic pathway also supports the results of L-NAME-induced acute anti-hypertension. However, these findings determined that both "MURK-EA" and "MURK-MeOH" may ameliorate the nitric oxide release from the endothelium but might not re-establish the vasorelaxation for a longer duration. Nitric Oxide Synthase (NOS) is responsible for releasing Nitric Oxide (NO) using L-arginine substrate found in endothelium. Nitric oxide is the major endogenous vasodilator. Its production activates the guanyl cyclase by increasing cGMP, which leads to the stimulation PKG. This PKG potentiates the NO-cGMP pathway and produces relaxation by inhibiting Ca^{+2} influxes.[29]

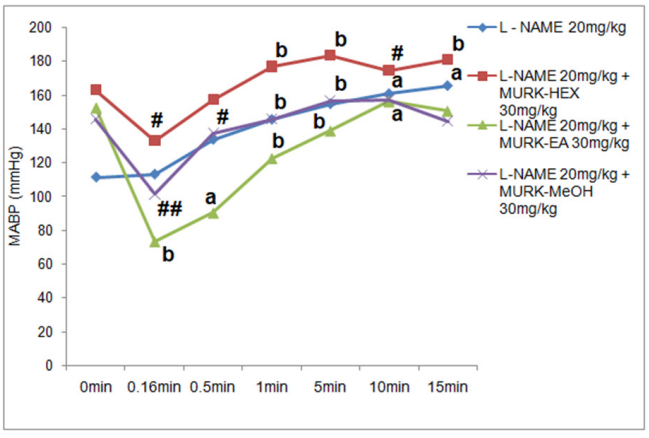

Figure 2: Effect of the hexane, ethyl acetate and methanol extracts from Murraya koenigii leaves on the mean arterial blood pressure in L-NAME induced hypertensive rats. Each point represents the mean±SEM (n=3); $^{a}p<0.0005$, $^{b}p<0.005$, $^{\#}p<0.025$ and $^{\#\#}p<0.01$ significantly different when compared to the initial value (0 min).

Table 1: Effect of different doses of Methanol, Ethyl acetate and Hexane extract of *Murraya koenigii* leaves in Mean Arterial Blood Pressure.

Extract	Dose	% fall in MABP±SEM	Duration of action (sec)±SEM
MURK- HEX	1	22.4±0.9[a]	77.9±20.8
	3	34.9±4.2[b]	58.0±40.8
	5	40.8±1.4[a]	56.2±25.0
	10	50.1±1.4[a]	190.0±29.4
	30	61.1±3.3[b]	155.3±35.0
	50	51.6±1.4[a]	149.7±23.9
MURK- EA	1	18.9±3.8[#]	20.5±4.5
	3	20.5±2.5[$]	56.3±0.9
	5	41.6±3.1[b]	69.3±1.5
	10	48.4±1.5[b]	85.3±34.7
	30	54.9±5.2[b]	28.0±2.5
	50	51.8±3.7[a]	47.8±4.0
MURK- MeOH	1	18.9±1.4[b]	12.7±1.9
	3	29.3±2.7[c]	51.7±11.7
	5	33.9±5.5[#]	34.7±2.85
	10	49.8±1.4[a]	71.0±42.0
	30	55.0±3.7[$]	57.3±9.5
	50	54.3±1.8[a]	61.3±18.7

Results are expressed as mean±S.E.M. [a]$p<0.0005$, [b]$p<0.005$, [c]$p<0.01$, [$]$p<0.025$, [#]$p<0.05$.

CONCLUSION

Extracts of "hexane" ("MURK-HEX"), "ethyl acetate" ("MURK-EA") and "methanol" ("MURK-MeOH") isolated from *Murraya koenigii* leaves appear to exhibit favourable effect for the treatment and deterrence of hypertension. The mechanism of L-Name-induced hypertension comprises the inhibition of NO production with a subsequent decline of vasorelaxant activity. However, the L-Name-induced acute antihypertensive effect of *Murraya koenigii* leaves may maintain their endothelium- derived vasodilation to some extent. Thus, additional work is required to improve vascular endothelial cells relaxation for prolonged intervals of time by increasing the potency of these extracts and to determine the chronic L-Name induced anti-hypertensive study. We could not isolate the marker compounds liable for these activities. However, this study will have a significant impression on further research on this distinct natural herb.

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ETHICAL APPROVAL

The study was approved by the Hamdard University Ethical Review Board (HU-ERB) vide letter number AEC-17-01.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Farzana Sadaf: Conceptualization, data curation, write-up, Initial drafting; Sumbul Shamim: Methodology, Resources, Supervision, Final approval of current version; Humera Ishaq: Data curation, Formal analysis, final drafting; Ata-ur-Rehman: Review, Methodology; Ishrat Imran: Review, Methodology; Faheema Siddiqui: Data curation, Review drafting.

ABBREVIATIONS

L-NAME-N(G): Nitro-L-arginine methyl ester; **NO:** Nitric Oxide; **eNOS:** Endothelium Nitric oxide synthase; **MABP:** Mean arterial blood pressure; **BP:** Blood Pressure; **MURK-HEX:** *Murrayya koenigii* Hexane extract; **MURK-MeOH:** *Murrayya koenigii* methanolic extract; **MURK-EA:** *Murrayya koenigii* ethyl acetate extract; **EDRF:** Endothelium dependent relaxing factor; **HU-ERB:** Hamdard university-Ethical review board.

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