

Evaluation of Anti-Hypertensive Activity of *Mitragyna Parvifolia* (Roxb.) Korth. Root Extract and its Vasorelaxation Potential on Calcium Chloride Induced Contractions in Isolated Rat Aorta

Rimi Ghosh¹, *Anita Murali² and Gowri R.³

*Contact Author E-mail: anitamurali.pg.ph@msruas.ac.in

Contributors

¹ Post Graduate, ² Professor, ³ Assistant Professor, Department of Pharmacology, Dept. of Pharmacognosy, Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bangalore – 560054 India

Abstract

The present study was conducted to evaluate the anti-hypertensive activity and vasorelaxant potential of alcohol extract of *Mitragyna parvifolia* root. Hypertension was induced by uninephrectomy followed by the administration of 1%w/v sodium chloride solution with drinking water and s.c injection of desoxy corticosterone acetate (20 mg/kg). The alcohol extract of *M. parvifolia* root was administered at doses 200 and 400 mg/kg. Systolic blood pressure and heart rate were measured along with serum levels of TC and TG. Vasorelaxation property of the extract was evaluated on isolated rat thoracic aorta against calcium chloride induced contractions. Test extract at dose 400 mg/kg was found to be effective in controlling blood pressure. The extract of *M. parvifolia* root at concentrations 1 and 10 mg/ml was found to be effective in countering CaCl₂ induced contractions on the isolated tissue. The study thus concludes the anti-hypertensive and vasorelaxation potentials of alcohol extract of *M. parvifolia* root.

Keywords: *Mitragyna Parvifolia*, Anti-Hypertensive Activity, DOCA-Salt, Vasorelaxation, Rat Aorta

1. INTRODUCTION

Hypertension and other cardiovascular disease are leading cause of morbidity and mortality now days.¹ Many drugs are already in use as anti-hypertensives, either alone or in combination. Research is still on to find out safe, suitable and cost effective drugs for treatment of hypertension. Herbal medicines have been found to be quite effective in lowering the increased blood pressure and correcting other cardiovascular problems. *Mitragyna parvifolia* (Roxb.) Korth. is a substitute for Kadamba,² for which the accepted botanical source is *Neolamarckia cadamba* (Roxb.) Boiss, The root of Kadamba is traditionally used for urinary calculi and studies have been reported on its diuretic property also.³ *M. parvifolia* is frequently found in moist and warm type of deciduous and evergreen forests, in the sub- Himalayan tract from Nepal eastwards, on the lower hills of

Darjeeling terrain in West Bengal, Karnataka, Kerala (west costs), in the Western Ghats and the Andamans.⁴ Traditionally *M. parvifolia* is widely used for treating various disorders such as bark and root for fever, pain, poisoning, gynecological problems and edema; also as diuretic, aphrodisiac and for alleviating kapha and pitta. Fruit juice is a lactagogue;² leaf is used as liver protective, anti-hypertensive, anti-microbial, anxiolytic, anti-nociceptive and anti-oxidant.⁴ *Mitragyna* genus is reported to contain anti- malarial, and analgesic indole alkaloids.⁵ Phytoconstituents reported on *M. parvifolia* are tannins and flavonoids in root, bark, alkaloids rhynchophylline and isorhynchophylline isolated from root bark,⁶ alkaloid mitraphylline and flavonoids in stem.⁷ Alkaloids angustine, tetrahydro-alstonine, aquammigine, pteropodine, isopteropodine, speciophylline, uncraine F, rotudifoline, iso-



rotudifoline, isomitrophylline, hirsutine, hydrocorynantheine, khirsultine, dihydrocorynantheine, demethoxyisohortiamine in the leaf.^{4,7} Mitraphylline is not present in new leaves and become the predominant alkaloid during July to November.⁵ *M. parvifolia* has been validated for activities like anthelmintic,⁸ anxiolytic⁹ and anti-microbial activity¹⁰ on the stem bark; anti-convulsant¹¹, anti-inflammatory, antinociceptive,^{12,13} analgesic, anti-pyretic and anti-arthritis activity¹⁴ on leaf; anti-diabetic activity on the root.¹⁵ Sharda¹⁶ has reported that the methanol extract of leaf possess good antioxidant activity due to its high phenolic content.

Leaf of *M. parvifolia* is traditionally used for hypertension and Kadamba possesses diuretic property. Based on these reports, the present study was designed to provide pharmacological evaluation of the effectiveness of *M. parvifolia* root (a substitute for Kadamba) in the treatment of hypertension.

2. METHODOLOGY

2.1 Collection of Plant Material and Preparation of Alcohol Extract

M. parvifolia was collected from the forest of Sivagangai, Tamil Nadu, India, during January 2013, in flowering condition along with the root. The plant was identified and authenticated by Dr. S. N. Yoganarasimhan, Plant taxonomist following various floras.^{17,18} Herbarium specimen along with voucher specimen was deposited in the crude drug museum of Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bangalore, Karnataka, India. Shade dried roots of *M. parvifolia* were powdered and extracted with 95% v/v ethanol in a Soxhlet apparatus by continuous hot percolation. The alcohol extract was filtered and concentrated to dryness under reduced pressure (yield 8% w/w). A suspension of the extract was prepared in 2% w/v gum acacia for the pharmacological studies.

2.2 Preliminary Phytochemical Studies

Preliminary phytochemical studies have been carried out as per Kokate (1999).¹⁹

2.3 HPTLC Studies

Chromatographic studies were carried out following Harborne (1973),²⁰ Wagner and Bladt, (1996).²¹ HPTLC is a technique used for separation and identification of components. HPTLC studies were carried out using Camag HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by Win CATS- 4 software.

2.4 Animals

Wistar albino rats bred and reared in the animal house facility of our institution and weighing between 200-250 g were used in the study. The experiments were performed with prior approval from the Institutional Animal Ethics Committee (IAEC) (IAEC certificate no. Ref No. MSRCP/M-42/2014).

2.5 Acute Toxicity Studies

Acute toxicity test was conducted as per the limit test specification of OECD guidelines (423).²²

2.6 *In vivo* Anti-Hypertensive Activity

Wistar albino rats of either sex were used for this study. They were divided into 5 groups contained 6 animals each. Group I served as the normal control and received 2% w/v acacia. Group II- V were served with desoxy corticosterone acetate (DOCA-salt) (20 mg/kg) and 1% of NaCl solution instead of drinking water for induction of hypertension. Group II served as the hypertension (positive) control. Group III received standard antihypertensive drug captopril (1 mg/kg) from 7th to the 21st day. Groups IV and V received the test extract at doses 200 and 400 mg/kg respectively from 7th to the 21st day, as curative regimen. All treatments were administered by oral route once daily.²³



Animals were anaesthetized by ketamine 40 mg/kg (Neon Laboratory Pvt Ltd). The left kidney was removed through a flank incision. The renal blood vessels and ureter was ligated and the incision was sutured. After 2-3 days of recovery period, groups 2 to 5 were administered 20 mg/kg DOCA- salt (Desoxycorticosterone acetate, Sigma Life Science) in olive oil (s.c.) for 21 days on every 4th day. Drinking water was replaced with 1% w/v NaCl solution for 21 days. Blood pressure was found to rise progressively after 1 week. Treatment was started from the 8th day onwards.

Systolic blood pressure and heart rate were measured at 7th, 14th and 21st day. On day 22 the animals were subjected to estimate biochemical parameters such as triglyceride and total cholesterol. Systolic blood pressure and heart rate were measured by tail cuff plethysmography method using NIBP controller instrument (Pulse transducer and Cuff for rats, Model LE5160R, Panlab, NIBP).²⁴ Following this, blood was collected from the retro orbital plexus under light ether anaesthesia. Blood samples were allowed to clot at room temperature and centrifuged at 3000 rpm for 10 min (Centrifuge, REMI Motors Ltd, Mumbai). The resulting upper serum layer was collected and analyzed for the biochemical parameters such as total cholesterol and triglyceride. Serum triglyceride was estimated by GPO-PAP Trinder's method²⁵ and serum total cholesterol was estimated by CHOD/PAP's method.²⁶

2.7 Effect on Vascular Contraction

Adult albino female rat of the Wistar strain weighing between 200-250 g was killed by excess thiopentone anaesthesia. A midline incision was made through the sternum to open the thoracic cavity and excise the aorta. The thoracic aorta was quickly removed and cleaned of all adhering tissue. The aorta was mounted in the organ bath under a resting tension of 500 mg. The organ bath contained 20

ml of Krebs solution (NaCl-130; KCl-5.6; CaCl₂-2.6; NaHCO₃-11.9; NaH₂PO₄- 0.91; MgCl₂-0.24; Glucose-11 nmol/L, pH 7.4). The organ bath was maintained at 37°C and bubbled with air. The strip was allowed to stabilize for 15-30 min, before being challenged with doses of the calcium chloride (100 µg/ml), extract (1 mg/ml, 10 mg/ml) and standard drug, verapamil (100 µg/ml).^{27,28} Log Dose versus % response graph was plotted to check the shift in DRCs as a measure of the vasorelaxation property.

2.8 Statistical Analysis

The results were expressed as Mean ± SEM. Results were analyzed by using one way ANOVA followed by Tukey-Kramer multiple test.

3. RESULTS

3.1 Preliminary Phytochemical Studies

The preliminary phytochemical analysis of ethanol extract of *M. parvifolia* root indicated the presence of phytoconstituents carbohydrates, glycosides, flavonoids and mucilage.

3.2 HPTLC Studies

HPTLC fingerprint was developed in mobile phase ethyl acetate: formic acid: glacial acetic acid: water (100: 11: 11: 26). When the chromatogram was scanned at 254 nm, 13 peaks were revealed, at 366 nm 5 peaks were revealed and at 425 nm 7 peaks were revealed (Figure 1).

3.3 Acute Toxicity Study

The ethanol extract of *M. parvifolia* was found to be safe and no mortality was observed up to a dose of 2000 mg/kg p.o. The dose for anti-hypertensive activity was taken as 400 and 200 mg/kg, i.e 1/5th and 1/10th of the maximum dose tested during acute toxicity studies.



3.4 Anti-Hypertensive Activity

A significant ($P<0.001$) rise in systolic blood pressure and heart rate were noticed on day 14 to 16 following uninephrectomy and DOCA-salt administration. Administration of *M. parvifolia* root extract of 400 mg/kg for 21 days to the hypertensive rats revealed (Table 1,2) a significant ($P<0.001$) decrease in systolic blood pressure and heart rate. The onset of action was observed on day 14 post dosing. Uninephrectomy and DOCA- salt administration in rats produced significant ($P<0.001$) elevation in total cholesterol and triglyceride levels (Table 3). The extract treatment significantly altered the elevated TG levels when estimated on the 21st day (200 mg/kg $P<0.01$, 400 mg/kg $P<0.001$).

Table 1. Effect of alcohol extract of *M. parvifolia* root on systolic blood pressure (mm-Hg)

Group	Dose	7 th Day	14 th Day	21 st Day
Normal Control	2% acacia	122.82±1.139	121.82 ± 0.79	122.15 ±0.74
Disease Control	DOCA - salt	140 ^a ±1.390	151.33 ^a ± 1.60	159.66 ±0.76 ^a
Captopril	1 mg/kg	140.5±1.088 ^{ns}	133.66 ±1.30 [*] **	124.66 ±1.1 ^{***}
Alcohol Extract	200 mg/kg	140.83±0.74 ^{ns}	149.66 ±0.6 ^{ns}	148.33 ±1.72 [*] **
Alcohol extract	400 mg/kg	140±0.730 ^{ns}	152±0.73 ^{ns}	148.66 ±1.11 [*] **

Values expressed as Mean ± SEM, n = 6; One Way Anova P value is <0.0001; Tukey-Kramer multiple comparison test *** $P<0.001$, ^{ns} $P>0.5$ in comparison with the disease control, ^a $P<0.001$ in comparison with normal control

3.5 Effect on Vascular Contraction

Vasorelaxant potential was measured against calcium chloride induced contractions on isolated rat aorta. Results were interpreted graphically (Figure 2). Calcium chloride at different concentrations showed dose dependent contractions (A).

Table 2. Effect of alcohol extract of *M. parvifolia* root on heart rate (beats/ min)

Group	Dose	7 th Day	14 th Day	21 st Day
Normal Control	2% gum acacia	424.16±1.90	423.50±0.67	424±1.19
Disease Control	DOCA -Salt	434±1.54 ^a	447.66±2.15 ^a	458±0.84 ^a
Captopril	1 mg/kg	431±0.80 ⁿ _s	436.66±0.80 [*]	427±0.79 ^{***}
Alcohol Extract	200 mg/kg	434±1.50 ⁿ _s	443±1.09 [*]	453±1.22 [*]
Alcohol Extract	400 mg/kg	435±1.09 ⁿ _s	445±0.9 [*]	450±0.73 ^{**}

Values expressed as Mean ± SEM, n = 6; One Way Anova P value is <0.0001; Tukey-Kramer multiple comparison test *** $P<0.001$, ** $P<0.01$, * $P<0.5$, ^{ns} $P>0.5$ in comparison with the disease control, ^a $P<0.001$ in comparison with normal control.

The vasorelaxation potential of the test extract was compared with the effect of verapamil on isolated aorta (D). Calcium chloride with verapamil showed dose dependent relaxation. Extract at doses 1 and 10 mg/ml showed more dose dependent vasorelaxant potential compared to 1 mg/ml dose (C, D).

A. Calcium chloride alone; B. CaCl₂ +1 mg/ml alcohol extract; C. CaCl₂ +10 mg/ml alcohol extract; D. CaCl₂+Verapamil



4. CONCLUSION

Elevated serum urea and creatinine is an indication of impaired kidney function.¹⁴ No significant fluctuation in serum urea level is noticed in control as well as treated groups. Though there is a significant decrease in serum creatinine, the value has been found to be within normal range.

Table 3. Effect of alcohol extract of *M. parvifolia* root on total cholesterol and triglycerides

Group	Dose	TC (mg/dl)	TG (mg/dl)
Normal control	2% gum acacia	129.31±1.37	123.24±0.87
Disease Control	DOCA-salt	144.24±1.32 ^a	139.78±1.68 ^a
Captopril	1 mg/kg	125.35±1.60 ^{***}	124.96±1.08 ^{***}
Alcohol Extract	200 mg/kg	140.31±1.20 ^{ns}	130.97±1.91 ^{**}
Alcohol Extract	400 mg/kg	141.97±0.35 ^{ns}	127.49±1.24 ^{***}

Values expressed as Mean ± SEM, n = 6; One Way Anova P value is < 0.0001; Tukey-Kramer multiple comparison test ***P<0.001, **P<0.01, ^{ns}P >0.5 in comparison with the disease control, ^aP<0.001 in comparison with normal control.

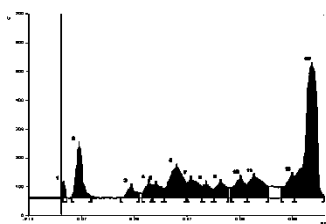


Fig. 1 HPTLC fingerprint of alcohol extract of *M. parvifolia* root at 254 nm

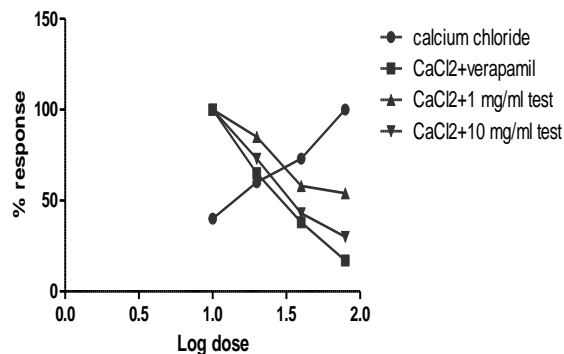


Fig. 2 Effect of alcohol extract of *M. parvifolia* root on isolated rat aorta

Thus, Absence of any marked deviation in serum protein, urea, creatinine and glucose level is suggestive of absence of any toxic impact on these metabolic parameters.

Effective control of hypertension is essential to reduce the risk of cardiovascular complications, such as heart attack and stroke. Alcohol extract of *M. parvifolia* root at 400 mg/kg exhibited significant ($P<0.001$) antihypertensive effects by lowering the systolic blood pressure and heart rate, following continued administration for 14 days, compared to the untreated hypertension group. Peak effects were observed on the 21st day and the results were independent of dose. Herbal products usually contain many active ingredients which may alone or in combination is responsible for the observed antihypertensive effect.

On isolated rat aorta, the extract was found to elicit a concentration dependent relaxation effect against calcium chloride induced contraction. Hence it can be assumed that the high concentration of the extract (10 mg/ml) is capable of antagonizing calcium channels, while a low concentration 1 mg/ml is capable of exhibiting mild antagonizing action against calcium induced contraction on rat aorta, in

comparison with the widely known vasorelaxant, verapamil. The phytoconstituents responsible for the antihypertensive effect is not yet identified. However preliminary phytochemical screening revealed the presence of flavonoids, mucilage and glycosides in the alcohol extract of *M. parvifolia* root.

To confirm the role of phytoconstituents, isolation of phytoconstituents(s) and their pharmacological evaluation is required. This study validates the anti- hypertensive effect of *M. parvifolia* root. However no conclusions could be drawn from this study regarding the role of phytoconstituents(s) and mechanism(s) of the anti- hypertensive effect, for which further investigations are required.

Conflict of Interest Statement

The authors declare no conflict of interest.

5. Acknowledgements

The authors are thankful to the authorities of M. S. Ramaiah University of Applied sciences, Bangalore for supporting this work.

References

1. Burt P, Whelton EJ. Prevalence on hypertension in the US adult population. *Polish J Pharmacol* 1995; 54(7): 305-13.
2. Yoganarasimhan SN. Medicinal Plants of India- Tamil Nadu. Bangalore: Cyber Media; 2000.
3. Elizabeth W. Major herbs of Ayurveda Bangalore: 1 st ed. Churchill Livingstone; 2000.
4. Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in Ayurveda Delhi: Central Council for Research in Ayurveda and Siddha; 2000.
5. Anonymous. The Ayurvedic Pharmacopoeia of India New Delhi: Ministry of health and family welfare, Govt of India: 1st ed. 1986.
6. Subhramanian A, Mohan VR, Kalidaas A. Pharmacognostic Studies on the Trunk Bark of *Mitragyna parvifolia* (ROXB.) KORTH. *Phcog. Mag* 2009; 2: 116-20.
7. Rastogi RP, Mehrotra BN. Compendium of Indian medicinal plants New Delhi: CDRI, National Institute of Science Communication 1999.
8. Badgujar VB, Sanjay SJ. *In vitro* investigation of anthelmintic activity of *M. parvifolia* (Roxb.) Korth (Rubiaceae). *Vet. World* 2012; 3(7): 326-38.
9. Vishal B, Sanjay S.J. Investigation of anxiolytic effects of *Mitragyna parvifolia* stem-bark extracts on animal models. *Sch. Res. Lib* 2009, (4)188-94.
10. Pundir RK, Bishnoi S. Antimicrobial activity of *Mitragyna parvifolia* barks and *Butea monosperma* leaves extracts against human pathogenic microbial strain. *Int J Drug Dev Res* 2011; 3(4): 141-47.
11. Dhirender K, Sukhbir LK, Pawan K, Ankit S, Divya A. Anticonvulsant Activity of *Mitragyna parvifolia* Leaves Extract. *Pharmacologyonline* 2009; (3): 101-06.
12. Vikas G, Pawan K, Parveen B, Ranjit S. Anti-inflammatory and Anti-nociceptive Activity of *Mitragyna parvifolia*. *Asian J Med Sci* 2009; 1(3): 97-9.
13. Kaushik D, Kaushik P, Saneja A, Sharma C, Saneja KR. A study of analgesic potential of *Mitragyna parvifolia*. *Int J Pharm Sci Drug Res* 2009; 1(1): 6-8.



14. Jain AP, Tote MV, Mittal A, Mahire NB, Undale VR, Bhosale AV. Antiarthritic and antipyretic activity of *Mitragyna parvifolia* leaves. Pharmacologyonline 2009; (2)739-49.
15. Shukla A, Srinivasan BP. 16,17-Dihydro-17b-hydroxy isomitrephylline alkaloid as an inhibitor of DPP-IV, and its effect on incretin hormone and β -cell proliferation in diabetic rat. Eur J Pharm Sci 2012; 47(2): 509-12. Sharda RA. Comparative Study of Anti-oxidant potential of successive chloroform methanol extracts of leaves of *Mitragyna parvifolia*. J Nat Rem 2012; 2: 151-56.
16. Murthy, KKR, Yoganarasimhan SN. Flora of Croog (Karnataka, India) Bangalore: Vimsat Publisher; 1990.
17. Gamble JS. Flora of the Presidency of Madras Dehradun: Bishen Singh Mahendra Pal Singh, 2005; 2.
18. Kokate, CK. Practical Pharmacognosy New Delhi: 4th ed. Vallabh Prakashan; 2008:107-11.
19. Harbone JB. Phytochemical methods London: 3rd ed. Chapman's Hall; 1998.
20. Wagner H, Bladt S. Plant drug Analysis Berlin: 2nd ed. Springer Verlag; 1996:1-32.
21. OECD (2001) Guidance document on acute oral toxicity testing Series on testing and assessment No. 24, Organisation for Economic Cooperation and Development, OECD, Environment of Health and Safety Publications. Available from: Paris (<http://www.oecd.org/ehs>) Understanding oral toxicity
22. Vogel GH. Drug Discovery and evaluation pharmacological assay New York. 3rd ed. Springer Publications; 2008.
23. Gupta M, Mazumder UK, Das S. Effect of leaf extract from *Clerodendron colebrookianum* on blood pressure in rats. Indian J Exp Bio 1994; 32: 216-17.
24. Bucolo G, David M. Estimation of serum triglyceride'. Clin Chem 1973; 19(24): 476-82.
25. Jacobe NJ, Vandenmark PJ. Estimation of total cholesterol. Arch Biochem. Biophys 1960; 88: 250.
26. Hiren MM, Palak KP, Sen DJ. Synthesis and *in vitro* Screening of dihydropyrimidine for antihypertensive and calcium channel blocking activity. J Appl Pharm Sci 2011; 1(5): 109-13.
27. Ameer OZ, Salman IM, Sriramaneni RN. Vascular responsiveness to *Macrosolen cochinchinesis* extracts in isolated Rat Thoracic Aorta. Int J Pharmacol 2009; 5(3): 191-99.
28. Hallal AH, Amortegui JD, Jeroukhimov IM, Casillas J, Schulman CI, Manning RJ. Magnetic resonance Cholangio-pancreatography accurately detects common bile duct stones in resolving gallstone pancreatitis. J Am Coll Surg (2005); 200(6): 869-75.

