A Study on the Antihypertensive Activity of MuktaVati (Ayurvedic Preparation) In Deoxycorticosterone acetate (DOCA) salt Induced Hypertension in Rats

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ABSTRACT

Mukta vati, an ayurvedic formulation is being routinely used to treat cardiovascular ailments like hypertension. However, so far its antihypertensive effect has not been validated in any animal model of hypertension. Therefore, the present study was performed to evaluate the antihypertensive effect of Mukta vati in Deoxycorticosterone acetate (DOCA) salt induced hypertension in rats. Hypertension was induced in male SD rats by administration of DOCA (10mg/rat,s.c) every alternate day and drinking water was replaced with 1% NaCl solution. The animals were kept for 6 weeks. The effects of Mukta vati (10mg/kg,p.o.) on hemodynamic parameters like systolic BP (SBP), diastolic BP(DBP), mean arterial pressure (MAP) and heart rate(HR), cardiac hypertrophy and oxidative stress (MDA andGSH levels) were studied. Preventive treatment with Mukta vati (10mg/kg,p.o.) for 6 weeks resulted in a significant reduction in SBP, DBP and MAP as compared to hypertensive group. However, there was no significant change in HR and cardiac hypertrophy parameters between the groups. Further, Mukta vati blunted hypertension induced oxidative stress as evident by the suppression of MDA level and upregulation of GSH levels.Our study corroborate the clinical observations that Mukta vati possess antihypertensive property and this property may be due to its anti oxidative action.

Key words: Mukta vati, DOCA, Oxidative stress, Hypertrophy.

1. INTRODUCTION

Hypertension is an important worldwide public-health challenge and remains a major cause of morbidity and mortality worldwide (Moser, 2013). About 26.4% of the world adult population in 2000 had hypertension and 29.2% were projected to have this condition by 2025 (Whelton, 2005). It is the primary cause of stroke, coronary artery disease and sudden cardiac deaths (Lawes, 2004). Conventional antihypertensive drugs are usually associated with many side effects and various studies have found a variety of alternative therapies to be successful in reducing high blood pressure (Sruthy, 2013). Traditional medicine, as an alternate medicine system, has gained wider popularity over the years, not only in the developing, but also in the developed nations (Sanjeeta, 2011).

Ayurveda, one of the ancient systems of medicine, is considered as the science of life and has been practiced in India since times immemorial (Sanjeeta, 2011). It describes ways to prevent and manage lifestyle disorders (Chandola, 2012). Importantly, the findings of various experimental and clinical studies conducted on single and compound ayurvedic preparations for their efficacy strongly emphasize ayurvedic therapy as a scientifically driven one and not simply unconventional (Balachandran, 2005). Various ayurvedic formulations like Abana (Verma, 1992), Serpina (Kamjorn et al, 1956), Arjuna (Sumita, 2009) etc. are in use for hypertension. Compare to allopathic system, ayurvedic medicines are economical, easily affordable by common people and effective (WHO, 2002).

Mukta vati is a herbomineral compound commonly advocated for the prevention and management of various heart diseases (Upadhyay, 2010). Each Mukta vati tablet mainly contains *Terminalia arjuna*, *Withania somnifera*, *Tinospora cordifolia*, *Centella asiatica* plants extracts in their optimum concentrations.

In a series of comprehensive clinical investigations, the antihypertensive significance of this compound has been already proved. However, there is not a single study in any animal model to verify its anti-hypertensive property. Also, there is no scientific explanation about its mechanism of action. Therefore this study was designed to validate its anti-hypertensive action and associated mechanism.

2. MATERIALS AND METHODS

2.1. Animals: The experiments were carried out with adult male Sprague-Dawley (SD) rats procured from the Laboratory Animal Services Division of CSIR-Central Drug Research Institute (CDRI), Lucknow, India. Experiments were performed according to internationally followed ethical standards and approved by Institutional Animal Ethics Committee (IAEC) CSIR- CDRI. Rats were maintained under standard housing conditions (room temperature 24–27 ◦C and humidity 60–65%) with a 12 h light and dark cycle. Food and water were available *ad libitum* where drinking water is replaced from 1% salt water.

2.2. Materials: The bio-chemicals DOCA, sodium chloride (NaCl), bovine serum albumin (BSA), 5,50-dithiobis(2-nitro-benzoic acid) (DTNB), TEP (Tetra ethoxy propane), and other chemicals were purchased from Sigma–Aldrich, USA. Mukta Vati (tablet formulation of Divya Pharmacy) was purchased from Yog Sadhna Mandir, Patanjali Chikitsalaya, Rishaldar park, Lucknow, India.

2.3. DOCA-Salt model of hypertension (DOCA) and experimental design: For the Deoxycorticosterone acetate (DOCA, Sigma, St Louis, MO, USA) - Salt model of hypertension, DOCA was dissolved in sesame oil. Male SD rats (250–280 g) received 10 mg DOCA/rat subcutaneously every other day for 6 weeks and drank 1% NaCl (Pietranera, 2006). The experimental rats were divided into three groups (n=4).

Group -1: Control group.
Group -2: DOCA salt hypertensive group.
Group -3: DOCA salt hypertensive + Mukta Vati 10 mg/kg treated group.

Drug administration: Ayurvedic formulation Mukta Vati was administered orally at 10 mg/kg for 6 weeks. Mukta Vati was suspended in 1% gum acacia before administration.

2.4. Measurements of Hemodynamic parameters: After 6 weeks, rats were anesthetized with urethane (1.25 gm/kg, i.p.) and placed on an isothermal pad to maintain normal body temperature during surgical procedures. The hemodynamic parameters i.e Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP) and heart rate (HR) were recorded by inserting fluid filled catheters into the carotid artery. The catheter was attached to the pressure transducer coupled to Data Acquisition System (DAS). All animals are allowed to stabilize for 20 min before the final readings. After taking hemodynamic parameters, blood was taken by cardiac puncture for biochemical estimation and hearts for evaluation of cardiac hypertrophy.

2.5. Evaluation of Cardiac Hypertrophy: Immediately after exsanguinations, the thorax was opened and the heart removed, dried with tissue paper, and weighed. The atria and right ventricle were then removed, and the left ventricle and septum were weighed. The HW/BW and LV+S/BW ratios were determined according to Yan Zhang et al (2012).

2.6. Biochemical Estimation:

2.6.1. Estimation of Malondialdehyde (MDA): Malondialdehyde (MDA), a marker of lipid peroxidation, was done in serum, according to the method of Colado et al. (1997). Serum was mixed with 30% TCA, 5 N HCl followed by the addition of 2% TBA in 0.5 N NaOH. The mixture was heated for 15 min at 90 °C and centrifuged at 12,000x g for 10 min. The pink colour of the supernatant was measured at 532 nm, using ELISA plate reader (BIOTEK, USA). MDA concentration was calculated by using standard curve prepared with Tetra ethoxy propane (TEP) and expressed as nmol/g serum.

2.6.2. Estimation of glutathione (GSH): Estimation of reduced glutathione (GSH), an antioxidant marker, was done according to the method of Sharma and Gupta (2001). The serum was mixed with 10% trichloroacetic acid (TCA) in a 1:1 ratio and then centrifuged at 1000 x g for 10 min at 4 °C to centrifuge out the proteins. To 0.01 ml of this supernatant, 0.2 ml of phosphate buffer (pH 8.4), 0.05 ml of 5, 5-dithiobis (2-nitro-benzoic acid), and 0.04 ml of double distilled water were added. Mixture was vortexed and the absorbance read at 412 nm within 15 min using ELISA plate reader (BIOTEK, USA). The concentration of glutathione was expressed as mg/g serum.

2.7. Protein estimation: Protein concentration was measured at 750 nm wavelengths by the method of Lowry et al. (1951) in all the brain samples using Bovine serum albumin (BSA) (1 mg/ml) as standard.

2.8. Statistical analysis: The results are expressed as Mean ± S.E.M using Graph Pad Prism software. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test where data of more than two groups were compared. Significance of difference between the two groups was determined by using *student's t* test.

3. RESULTS AND DISCUSSION

3.1. Effect of Mukta vati on hemodynamic parameters: As shown in Fig.1, after 6 weeks, hemodynamic parameters like systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were significantly higher in DOCA group as compared to control group respectively. Treatment with Mukta vati 10 mg/kg significantly attenuated the increase in SBP, DBP and MAP. However, there was no significant change in heart rate between the groups.

3.2. Effect of Mukta vati on cardiac hypertrophy: There was a slight increase in cardiac hypertrophy parameters in DOCA salt group (Fig. 2) but the increase was not significant as compared to control

group. Treatment with Mukta vati ameliorated this increase in cardiac hypertrophy.

3.3 Effect of Mukta vati on oxidative stress markers:

3.3.1. MDA level: Oxidative stress, marked by increased lipid peroxidation, is the main culprit in hypertension. Similarly, we also found DOCA salt group showed significant increase in MDA level in the serum as compared to the control group. Treatment of Mukta Vati significantly decreased MDA level in serum as compared to the DOCA salt group (Fig.3).

3.3.2. GSH level: GSH, an important antioxidant and ROS scavenger, is decreased in hypertension. In this study we also found the similar trend as there was a significant fall in the levels of GSH in the DOCA salt group as compared to the control group in serum. As evident in Fig.3, treatment of Mukta vati (10mg/kg) showed significant increase in the GSH level in serum as compared to DOCA salt group.

3.4 Discussion: This study is the first scientific study to validate the antihypertensive effect of Mukta vati in DOCA salt model of hypertension. This is in line with the clinical findings that Mukta vati exerts an antihypertensive effect in hypertensive patients. In this study, hemodynamic parameters like SBP, DBP and MAP were evaluated in control, DOCA salt hypertensive and DOCA salt + Mukta vati (10mg/kg) treated rats. Apart from hemodynamic parameters, its effect on oxidative stress was also assessed.

In the present study, hypertension induced by DOCA salt treatment resulted in significant increase in the hemodynamic parameters like SBP, DBP and MAP respectively. This increase in blood pressure was significantly prevented by the treatment with Mukta vati at the dose of 10mg/kg for 6 weeks. This antihypertensive effect of Mukta vati may be due to its ingredients like *Centella asiatica* which have already been reported to possess antihypertensive property (Muangnongwa, 2004). Similarly, Bhatia et al, (2000), demonstrated hypotensive and antioxidant potential of *Terminalia arjuna* in DOCA-salt hypertensive dogs.

Oxidative stress plays an important role in the pathogenesis of hypertension (Mujahid, 2011). Oxidative damage to biomolecules, cells and hyperactivation of ROS signaling pathways has been linked to the detrimental effects associated with hypertension. Oxidative stress is kept in check by natural antioxidant molecules, such as catalase, lipid peroxidase, superoxide dismutase, glutathione peroxidase, glutathione, vitamin E and vitamin C, acting as free radical scavengers (Aruoma, 1991). In our study we also found significant reduction in the antioxidant GSH levels in DOCA salt hypertensive group that was ameliorated by treatment with Mukta vati. Our observation is supported by the study of Pilani et al,(2009) who demonstrated the anti-oxidative effect of ethanolic extract of Acorus calamus in APAP (N-acetyl-p-aminophenol, Paracetamol) induced oxidative stress in rats. Moreover (Rajasankar, 2009) demonstrated that Withania *somnifera* an active ingredient of Mukta vati, increased the levels of anti-oxidants like superoxide dismutase, catalase and glutathione peroxidase.

MDA is a reliable marker of lipid peroxidation and oxidative tissue injury (Janero, 1990). It has been shown to be elevated in animal models of experimentally induced hypertension. The present study also corroborates with these studies as there was significant elevation in the MDA level in DOCA salt hypertensive group. Treatment with Mukta vati blunted this increase in MDA levels. This anti-lipid peroxidation effect of mukta vati may be due to active constituents, *Acorus calamus* (Muthuraman, 2011), *Lavandul stoechas* (Barakat, 2012), *Centella asiatica* (Fredrico, 2009), *Convolvulus pluricalis* (Mishra, 2010), *Terminalia arjuna* (Gauthaman 2001), *Onosma bracteatum* (Ekta 2011) , *Withania somnifera* (Jayanta, 1998), *Celastrus paniculatus* (Kumar, 2002) *and Tinospora cordifolia* (Stanley, 1999; Kumar P, 2011), known to possess anti oxidative and anti-inflammatory potentials.

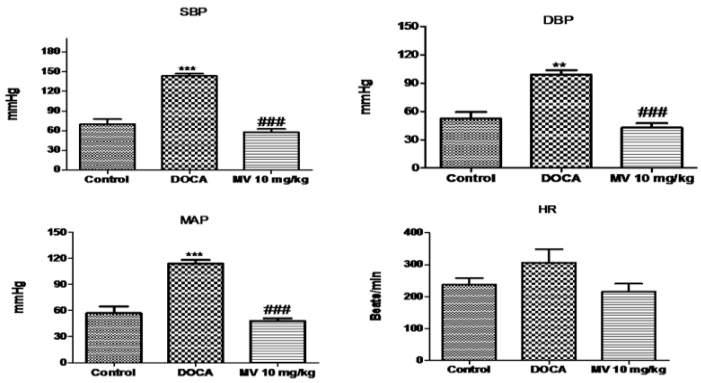

Figure.1. There was significant increase in the hemodynamic parameters like SBP, DBP and MAP in DOCA salt group as compared to control group. Treatment with Mukta vati significantly ameliorated this increase in hemodynamic parameters. However, HR increased in DOCA salt group but was not significant. SBP (systolic blood pressure), DBP (diastolic blood pressure), MAP (Mean arterial pressure) and HR (heart rate)

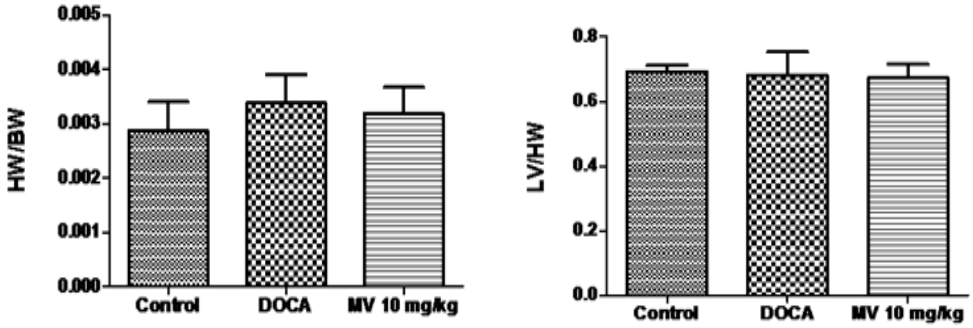

Figure.2.Cardiac hypertrophy parameters in Control, Doca Salt and Mukta vati treated group.

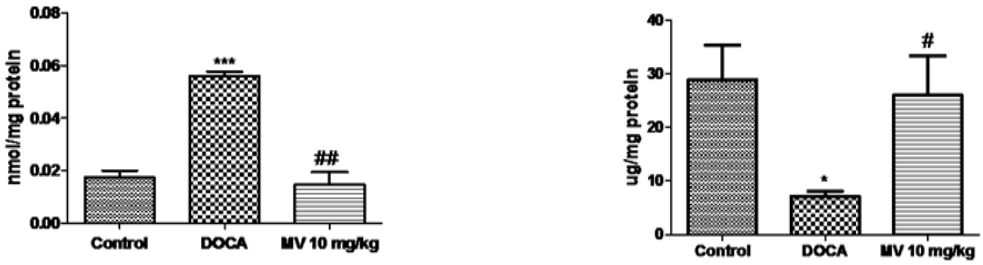

Figure.3.GSH and MDA level in Control, Doca Salt and Mukta vati treated group. p* denotes significant difference from control; p# denotes significant difference from DOCA salt group

4. CONCLUSION

This is the first scientific study that validated the anti hypertensive activity of Mukta vati in DOCA salt induced model of hypertension in rats. This antihypertensive effect may be due to its strong antioxidant property as evident from increased GSH levels and decreased MDA levels following the treatment with Mukta vati in hypertensive rats

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