



INVESTIGATION OF ANTIHYPERTENSIVE MECHANISM OF *CURCULIGO ORCHIOIDES* IN DOCA SALT INDUCED HYPERTENSIVE RATS

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ABSTRACT

To investigate antihypertensive mechanism of methanolic extract of *Curculigo orchioides* root (MECO) in DOCA salt induced hypertensive rats. MECO of authenticated sample was prepared using soxhlet extraction using methanol as an extracting solvent. Antihypertensive mechanism of MECO was investigated using DOCA salt induced hypertension in wistar rats. Nephrectomy were performed and hypertension were induced by DOCA salt (25 mg/kg, s.c.) then MECO (600 mg/kg, i.p.); and enalapril (standard) were administered then hemodynamic parameters like Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MABP) and pulse pressure (PP) were measured using tail-cuff apparatus with AD instrument powerlab to find out mechanism of antihypertensive effect. SBP, DBP, MABP and PP were significantly decreased in MECO treated rats as compared to disease control group ($p < 0.001$ / $p < 0.01$ / $p < 0.05$). MECO possess antihypertensive activity with ACE inhibitor mechanism similar as enalapril in DOCA salt induced hypertensive rats.

Keywords: MECO, Antihypertensive effects, Nephrectomy, DOCA salt, AD powerlab.

INTRODUCTION

Hypertension is the most common cardiovascular illness and is a major public health issue in developed as well as in developing countries. Hypertension (HTN) or high blood pressure, sometimes called arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated. Normal blood pressure at rest is within the range of 100-140mmHg systolic and 60-90mmHg diastolic. High blood pressure is said to be present if it is persistently at or above 140/90 mmHg (Susanta Kumar Rout, 2010).

World Health Organization (WHO) has carried out epidemiological studies in India in between 1995 to

2006. According to this, prevalence of hypertension is 29.3% in men and 25.2% women has been found at the end of 2006. In India cardiovascular diseases cause 1.5 million deaths annually. Deaths due to hypertension arise from cerebrovascular and cardiovascular complications such as stroke, end-stage renal disease, congestive heart failure, myocardial infarction and cardiac arrest. Hypertension is directly responsible for 57% of all stroke deaths and 24% of all coronary heart disease deaths. This fact is important because hypertension is a controllable disease and a population wide 2 mmHg decrease in BP can prevent 151,000 stroke and 153,000 coronary heart disease deaths (Gupta Rajeev and Gupta VP, 2009).

Hypertension involves mainly two types, essential and secondary. The pathogenesis of essential hypertension is multi factorial and highly complex which will be caused by increase in sympathetic nervous system activity, increase in production of sodium-retaining hormones and vasoconstrictors, deficiencies of

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vasodilators such as prostacycline and nitric oxide, inappropriate or increased renin secretion and genetic predisposition while pathogenesis of secondary hypertension will be caused by chronic kidney disease, renovascular disease, Cushing's syndrome, pheochromocytoma, drugs such as nonsteroidal antiinflammatory drugs and oral contraceptives (Susanta Kumar Rout, 2010).

Symptoms associated with high blood pressure can include, shortness of breath (dyspnea), fatigue, dizziness or fainting spells (syncope), chest pressure or pain, bluish colour to lips and skin (cyanosis), racing pulse or heart palpitations, headache and nosebleeds (Susanta Kumar Rout, 2010).

Normally, hypertension is diagnosed by physical history, laboratory tests, sphygmomanometer and digital blood pressure monitor. Although, many antihypertensive allopathic remedies are readily available to prevent and manage the hypertension, but many of these drugs cause serious and life threatening adverse effects. Synthetic antihypertensive like diuretics cause muscle cramps, dehydration, extreme tiredness, skin rash, blurred vision and abnormal heart rate, ACE inhibitors cause cough, kidney failure, skin rash and fever, calcium channel blockers cause fatigue, skin rash, constipation and edema, β -blockers cause bronchospasm, Reynaud's syndrome, heart failure and postural hypotension, as β -blockers cause bronchospasm so contraindicated in asthma, others like centrally acting drugs cause sexual dysfunction. In addition, all antihypertensive drugs are contraindicated during pregnancy except methyldopa. Other major drawback of synthetic anti-hypertensive is that most of these drugs are very costly (Gupta Rajeev and Gupta VP, 2009).

Use of appropriate herbals causes least or no side effects with multiple actions and cost is also relatively low. Scientific studies suggest different lifestyle changes and use of appropriate herbal medicine use in the management of hypertension. *Rauwolfia serpentine*, *Ginkgo Biloba*, *Allium sativum* and *Crocus sativus* are the most popular herbs are used for management of hypertension today. The way they work is that they improved blood circulation by dilating the arteries (Gupta Rajeev and Gupta VP, 2009).

Modern science has already, accepted the potential of the herbs as a source of new bio-active constituents. There are numerous plants-derived drugs of unknown chemical structure that have been found clinically useful in different alternative system of medicine, including Ayurveda, Homeopathy and Unani system of medicine. The recent development of science of

phyto-pharmaceuticals has generated new enthusiasm in herbal drug research to discover new medicines in various diseases (Patel SS and Saluja AK, 2010)

Looking at the dire need of a new, safe and economical antihypertensive molecule, we resolved to investigate beneficial effect of *Curculigo orchoides* commonly known as kali musli belongs to family Amaryllidaceae responsible for its antihypertensive activity. In Ayurvedic literature, root of *Curculigo orchoides* is reported for its beneficial effect in hypertension (Anonymous 1). This drug also act as Ayurvedic rasayana because it contain many of therapeutic phytochemical constituents (i.e. glycosides, alkaloids, flavanoids, tannin, resins etc.) with no adverse effects (Daniel M, 2006; Gupta M, 2005; Wu Q, 2005; Misra TN, 1984). *Curculigo orchoides* root/rhizome also possess, anticancer (Balamurugan G *et al.*, 2009), antioxidant (Singh J *et al.*, 2006), antiasthmatic (Patil J *et al.*, 2008), immunostimulatory (Bafna AR and Mishra SH, 2006), hypoglycemic, antibacterial (Nagesh KS and Shanthamma C, 2009) and estrogenic (Vijayanarayana K *et al.*, 2006) activities without any side effects.

Curculigo orchoides has no significant adverse effects as compare to other antihypertensive herbals, they do not interfere with synthetic drugs during hypertension treatment and any other disease treatment, it also prevents cardiovascular complications which accelerate hypertension additionally both drugs are also available at low costs.

MATERIALS AND METHODS

Collection and authentication of plant

The *Curculigo orchoides* roots were collected from the medicinal garden of R. K. College of Pharmacy, Rajkot. Then were authenticated by Department of Botany, Christ College, Rajkot.

Preparation of plant extract

The collected roots of *Curculigo orchoides* were subjected to dry to brittle material at 60°C in hot air oven to remove moisture. These dried roots were subjected for size reduction using mixer grinder and comminuted to very fine powder. Methanolic extracts of *Curculigo orchoides* was prepared using methanol as a solvent in soxhlet apparatus.

Selection of animals

Either sex Wistar albino rats (n=6) of weighing 220-300 g were used for the present study. The animals were procured from animal house, Department of Pharmacology, R. K. College of Pharmacy, Rajkot, India. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk

as bedding. Animals were housed at a temperature of $24\pm 20^{\circ}\text{C}$ and relative humidity of 30 – 70 %. A light and dark cycle was followed. All animals were fed on standard balance diet and provided with water *ad libitum*. All the experimental procedures and protocols used in study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of R. K. College of Pharmacy and care of laboratory animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

***In vivo* anti-hypertensive study to evaluate mechanism using DOCA salt induced hypertensive rats (Non-invasive method)**

Method to induce hypertension by DOCA salt

1. Surgical removal of kidney (Nephrectomy)

A small patch of skin above the right kidney was shaved and disinfected. Using a pair of alcohol-sterilized rat toothed forceps and scissors, two 1 cm incisions were made: one on the skin and other through the body cavity parallel to the second or third last rib thereby exposing the chest cavity. A pair of retractors was used to incision open. One end of surgical thread was tied to pair of wide-tipped forceps and single loop of forceps was placed over the forceps tip. This arrangement was allow a worker to slip a loop over the kidney once the kidney is pinched with the forceps. Failure to use this or similar method was required an addiction worker to tie off the ureter and renal blood vessels. A second knot should be made to secure the initial knot. The kidney was cut off above the surgical thread. The ureter and renal blood vessels should be made to secure with animal blood loss. Finally the two incisions were sewn up. The rat was replaced in its cage and covered with cotton to minimize the heat lost (Leo V. Pavone and Rudy Boonstra, 20008).

DOCA salt administration

All operated rats receive an injection of ampicillin (10 mg/kg, i.m.) daily for 5 days and local application of neosporin-H to prevent infection after nephrectomy. Rats weighing about 200-300g are kept on a diet high in sodium chloride and drinking water was replaced by 2% sodium chloride solution *ad libitum*. After they attain a weight of about 250-300 g, they were given DOCA dissolved in sesame seed oil at a dose of 25 mg/kg was injected nephrectomised rats subcutaneously, twice weekly for 43 days. To confirm the induction of hypertension, different hemodynamic parameters were measured by using Non-invasive tail cuff method with AD instrument power lab (Rathod SP *et al.*, 1997; Santhoshkumari KS and Devi KS, 1991).

Trial design (Groups and recievers)

Control: Water (as a vehicle)

Disease control: DOCA salt (25 mg/kg, s.c. twice weekly for 43 days)

Standard: Enalapril inj. (48 mg/kg, i.p.)

Test : Methanolic extract of *C. orchioides* (600 mg/kg i.p)

Method to measure hemodynamic blood pressure parameters using AD instrument Powerlab with tail cuff apparatus

After administration of dose to animal, blood pressure was measured by Non-invasive Tail cuff method using pressure meter. Measurement was done after 1 hour and 3 hour time interval intensive stepwise by using AD instrument powerlab (The ML125/R NIBP system with ML125 NIBP Controller and MLT125/R Pulse Transducer) (Fig. 9). The tail was cleaned with moist cotton to remove the dirty matter and talcum powder was sprayed on tail to make its surface smooth. Tail cuff and pulse transducers were fixed around the tail. Initially animal shows particular pulse level, when the pulse rate is within the normal range. ‘STRAT’ switch was put on and the recorder records the blood pressure as SBP and DBP were displayed on monitor. The pressure can be easily read from the pre-calibrated monitor and other hemodynamic parameters like MABP and PP were calculated using equations $[(\text{SBP}-\text{DBP})/3] + \text{DBP}$ and $\text{SBP}-\text{DBP}$ (Gupta SK, 2004) respectively. Once all the values were displayed, the recorder was switched off and for next measurement was done (Vogel GH and Vogel WH, 1997).

Statistical analysis

To check the significance of data, following statistical tests were performed:

ANOVA: to see the variability within all the groups.

INSTAT software: to derive all the statistical terms like Standard Error of Mean (SEM), ANOVA, *p* – value, Degree of freedom, Standard deviation, etc.

RESULTS

25 mg/kg s.c. Deoxycorticosterone acetate (DOCA) was injected into nephrectomised rats produced moderate hypertension. When all rats were put in tail-cuff apparatus with AD instrument, they were displayed Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) according to respective treatment in normal control, disease control, standard (enalapril) and test groups (*Curculigo orchioides*) respectively, which were observed. (Fig. 1, Fig. 2, Fig. 3 and Fig. 4)

SBP in normal control, disease control, standard (enalapril) and test groups (*Curculigo orchioides*) were measured. There were significant reduction in SBP were

found in standard (enalapril) and test groups (*Curculigo orchioides*) as compared to diseases control group. (Table 1) DBP in normal control, disease control, standard (enalapril) and test groups (*Curculigo orchioides*) were measured. There were significant reduction in DBP were found in standard (enalapril) and test groups (*Curculigo orchioides*) (Table 2)

Mean Arterial Blood Pressure (MABP) and

Pulse Pressure (PP) normal control, disease control, standard (enalapril) and test groups (*Curculigo orchioides*) were calculated by $[(SBP-DBP)/3] + DBP$ and $SBP-DBP$ equations respectively. There were significant reduction in MABP and PP was found in standard (enalapril) and test groups (*Curculigo orchioides*) as compared to diseases control group. (Table 3 and Table 4)

Table 1. Systolic blood pressure in DOCA induced hypertensive rats

Group	Systolic Blood Pressure (mmHg)	Reduction in SBP (mmHg)
Normal Control	139.13 ± 0.76	-
Disease Control	163.26 ± 0.83 [#]	-
Enalapril	139.70 ± 0.84***	23.56
<i>Curculigo orchioides</i>	149.35 ± 0.53***	13.91
<i>F</i>	65.626	-
<i>df</i>	29 (4, 25)	-
<i>p</i>	< 0.001	-

Table 2. Diastolic blood pressure in DOCA induced hypertensive rats

Group	Diastolic Blood Pressure (mmHg)	Reduction in DBP (mmHg)
Normal Control	98.46 ± 0.68	-
Disease Control	113.8 ± 1.51 [#]	-
Enalapril	99.02 ± 0.77***	14.78
<i>Curculigo orchioides</i>	102.87 ± 1.31***	10.93
<i>F</i>	36.365	-
<i>df</i>	29 (4, 25)	-
<i>p</i>	< 0.001	-

n = 6 and results were shown as mean ± SEM. [#] indicate significant difference in the data compared to control group and the level of significance was $p < 0.05 \approx$ significant. *** indicate significant difference in the data disease compared to control group and the level of significance was $p < 0.001 \approx$ highly significant.

Table 3. Mean arterial blood pressure in DOCA induced hypertensive rats

Group	Mean arterial blood pressure (mmHg)	Reduction in MABP (mmHg)
Normal Control	112.02 ± 0.48	-
Disease Control	130.28 ± 1.08 [#]	-
Enalapril	112.58 ± 0.51***	17.42
<i>Curculigo orchioides</i>	118.36 ± 0.82***	11.92
<i>F</i>	87.195	-
<i>df</i>	29 (4, 25)	-
<i>p</i>	< 0.001	-

Table 4. Pulse pressure in DOCA induced hypertensive rats

Group	Pulse Pressure (mmHg)	Reduction in PP (mmHg)
Normal Control	40.67 ± 1.1	-
Disease Control	49.46 ± 1.62 [#]	-
Enalapril	40.68 ± 1.27**	8.78
<i>Curculigo orchioides</i>	46.48 ± 1.59*	3.2
<i>F</i>	6.286	-
<i>df</i>	29 (4, 25)	-
<i>p</i>	< 0.05	-

n = 6 and results were shown as mean \pm SEM. # indicate significant difference in the data compared to control group and the level of significance was $p < 0.05 \approx$ significant. *** indicate significant difference in the data disease compared to control group and the level of significance was $p < 0.001 \approx$ highly significant. ** indicate significant difference in the data disease compared to control group and the level of significance was $p < 0.01 \approx$ moderately significant. * indicate significant difference in the data compared to disease control group and the level of significance was $p < 0.05 \approx$ significant.

Fig. 1. SBP and DBP recording in control rats

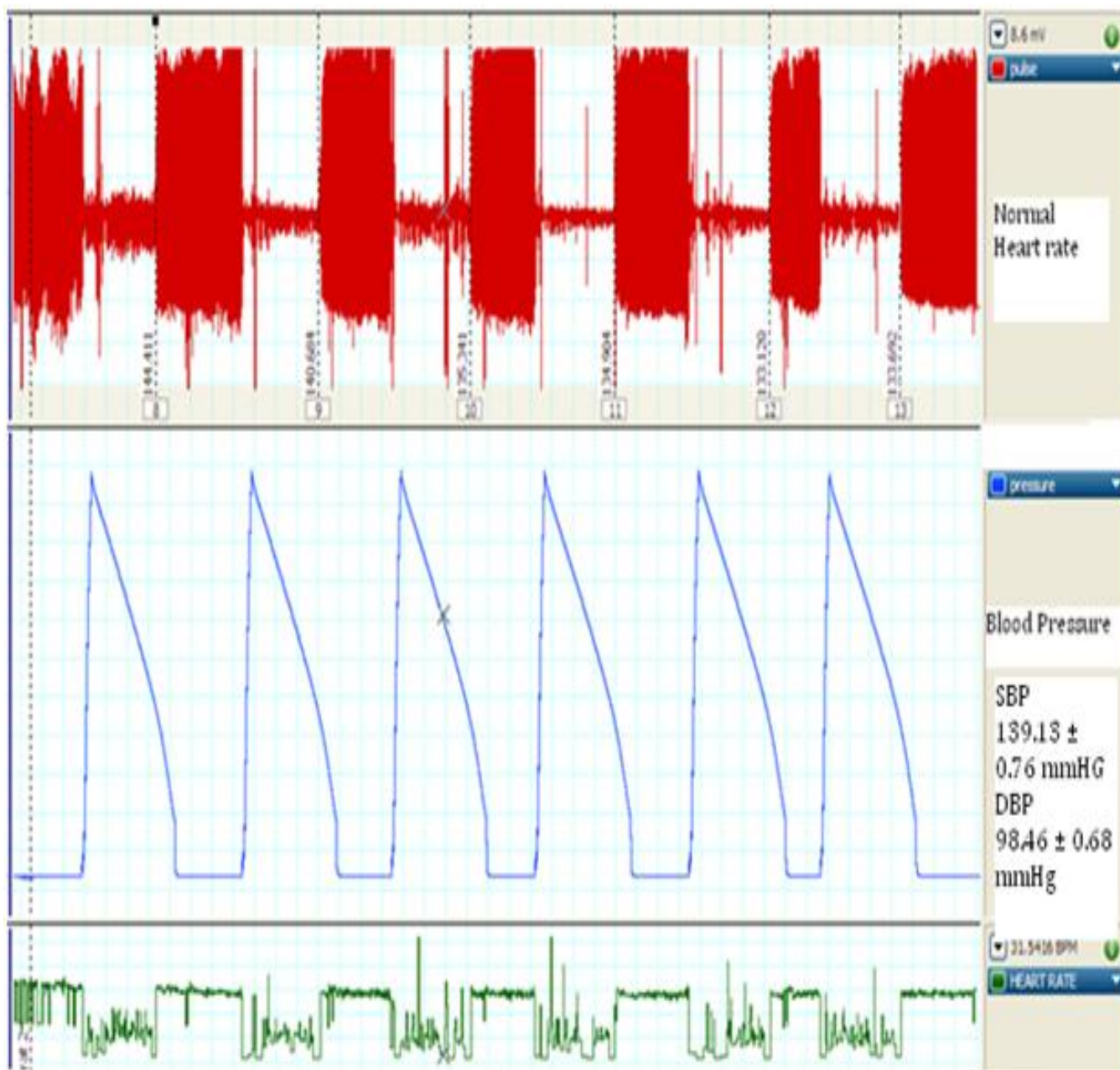


Fig. 2 SBP and DBP recording in disease control with DOCA salt induced hypertensive rats

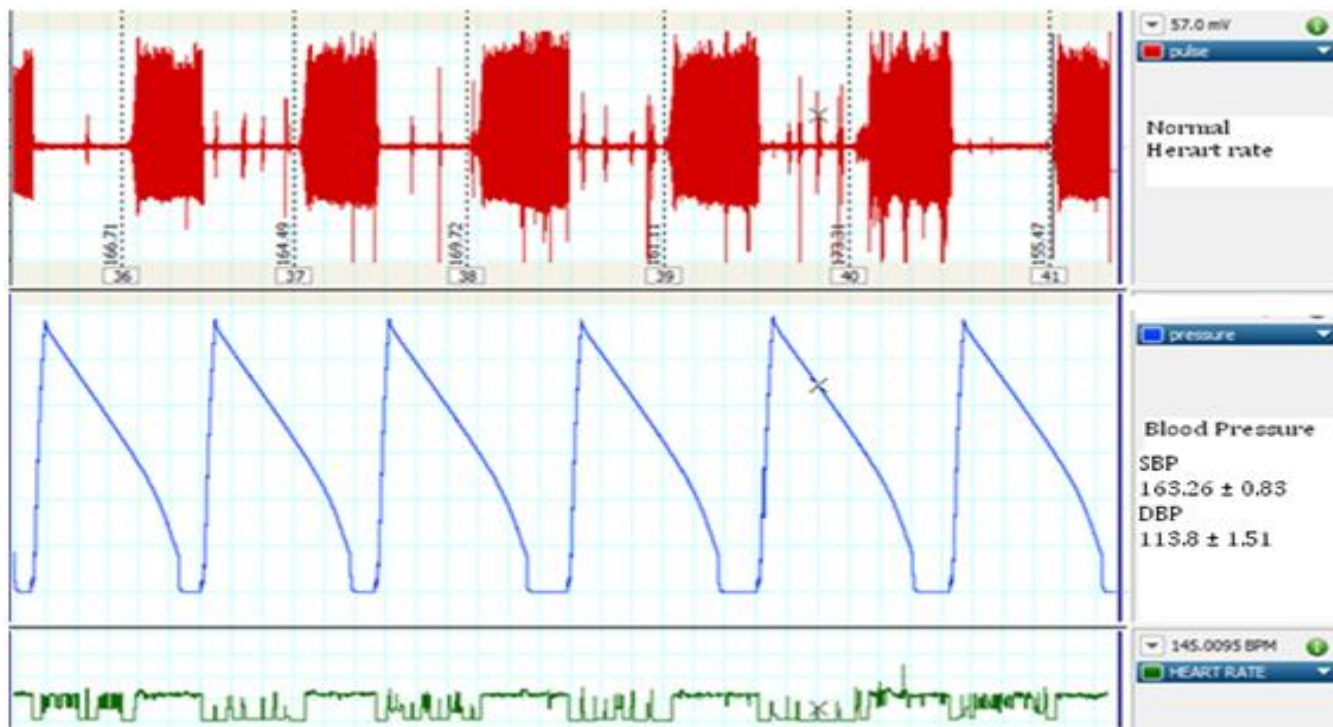


Fig. 3 SBP and DBP recording in standard (Enalapril) with DOCA salt induced hypertensive rats.

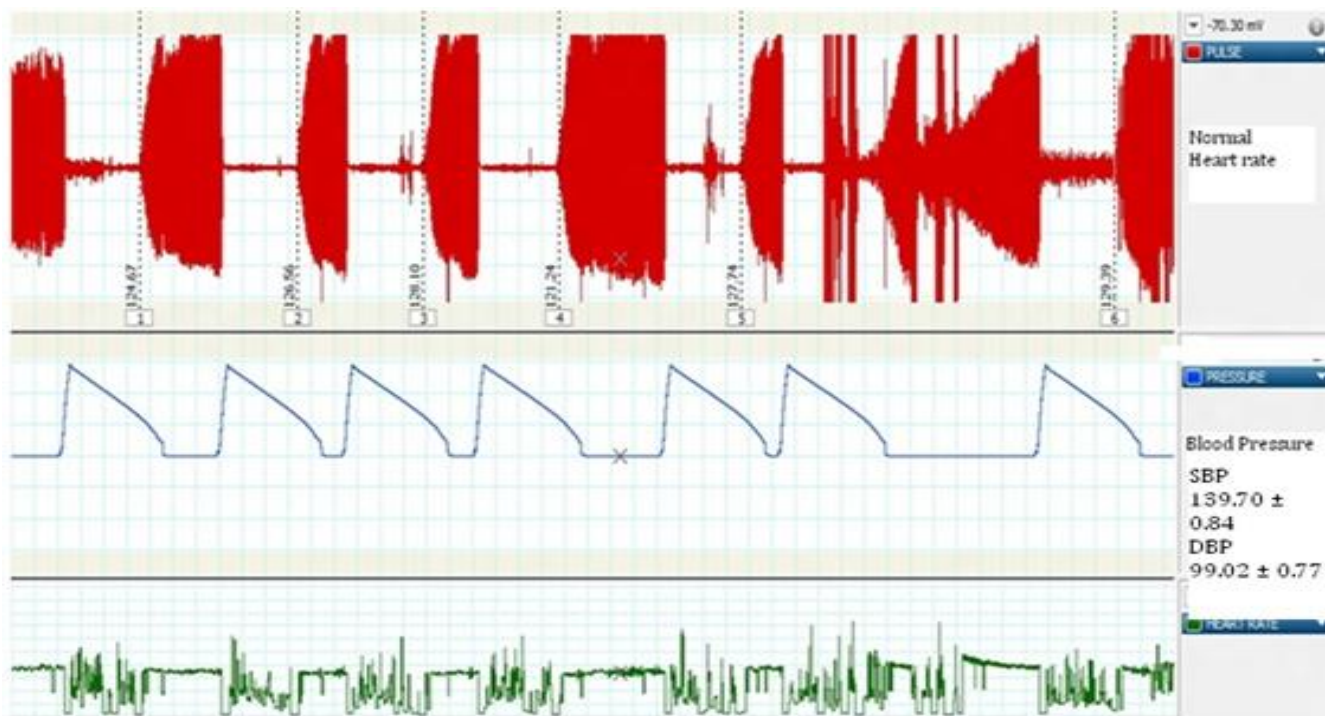
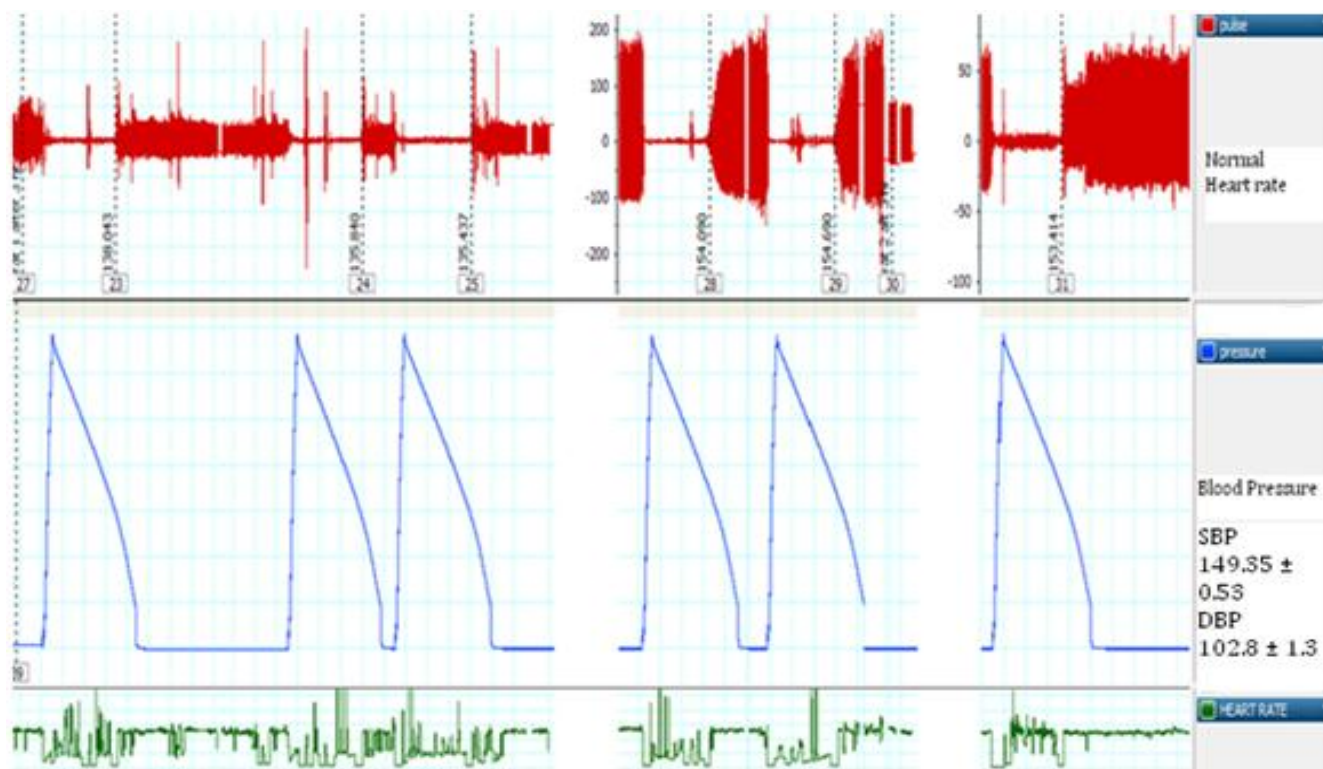


Fig. 4 SBP and DBP recording in test (*Curculigo orchoides*) with DOCA salt induced hypertensive rats.



DISCUSSION

Hypertension is common debilitating illness among the people in both developed and developing countries. Community surveys in industrialized countries have shown a prevalence of 15-33 % in people aged 30 years. The disease continues to be a leading cause of morbidity and mortality from coronary artery disease and stroke. Fortunately, antihypertensive drugs are available to reduce blood pressure to normal level which is necessary to manage cardiovascular disease, coronary heart disease and other cardiovascular related complication. In this respect, herbal drugs are helpful and render encouraging results in comparison to synthetic drugs due to their fewer or no side effects and easy availability (Susanta Kumar Rout, 2010; Gupta Rajeev and Gupta VP, 2009).

The screening of various plants according to their traditional uses and nutritional value based on their therapeutic value leads to discovery of newer and safer alternative for management of hypertension. One of such plant of medicinal value is *Curculigo orchoides* root, belongs to family Amaryllidaceae which is commonly known as kali musli. This drug also acts as ayurvedic rasayana because it contains many of therapeutic phytochemical constituents (i.e glycosides, alkaloids, flavanoides, tannin, resins etc.) with no adverse effects.

Pilot in vivo study suggested that the antihypertensive activity was highest at dose of 600 mg/kg in methanolic extract the plant. Antihypertensive mechanism of *Curculigo orchoides* root was evaluated by using DOCA salt induced hypertensive model. In present study hypertension was induced by 25 mg/kg s.c. Deoxycorticosterone acetate (DOCA) injection into nephrectomised rats produced moderate hypertension. Nephrectomy was performed because it potentiates effect of DOCA salt on RAAS system for activation of renin release.

Hypertension was induced in nephrectomised rats by DOCA salt because of RAAS alteration. DOCA salt induces reabsorption of salt and water leading to increased blood volume and hence increased blood pressure. There was also increased secretion of vasopressin leading to water retention and vasoconstriction. More potential effect is to alter activity of Renin Angiotensin Aldosterone System (RAAS) leads to increased sympathetic activity by activating ACE enzyme which converts angiotensin-I to angiotensin-II leads to vasoconstriction result in increase in blood pressure (Rathod SP *et al.*, 1997; Santhoshkumari KS and Devi KS, 1991).

SBP and DBP were increased persistently in DOCA salt treated nephrectomised rats as compared to normal rats in tail-cuff with AD instrument; simultaneously derived hemodynamic parameters like mean arterial blood pressure (MABP) and pulse pressure (PP) were also increased in DOCA salt treated nephrectomised rats. Due to previous mechanism of DOCA salt, angiotensin converting enzyme (ACE) inhibitor- enalapril was used as standard in this study to evaluate antihypertensive mechanism of plant extracts (methanolic extracts of *Curculigo orchioides* root) (Vogel GH and Vogel WH, 1997).

Results data were suggested that blood pressure (i.e. SBP, DBP, MABP and PP) were decreased in standard group and in plant extract as compared to disease control group. During whole study no significant effect on heart rate (pulse rate) was observed in nephrectomised hypertensive rats.

CONCLUSION

The present study revealed that methanolic

extracts of *Curculigo orchioides* root possessed profound antihypertensive activity. The antihypertensive effect has been found due to ACE inhibitor mechanism of *Curculigo orchioides* root extract because this extract lower the blood pressure as similar to enalapril which is a ACE enzyme inhibitor in DOCA salt induced hypertensive model. Additionally, *Curculigo orchioides* root did not interfere with pulse rate (i.e. normal heart rate). The intake of *Curculigo orchioides* root extract as medicine might have potential benefits in management of hypertension. Concomitant administration of the extracts might be helpful in better management of hypertension along with available anti-hypertensive without any interference.

Further research is required on isolation, characterization and purification of active constituent which is responsible for antihypertensive activity. Future aspects of this study include preparation of suitable formulation as well as bulk manufacturing of same at industrial scale.

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