WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 7, 1149-1164. Research Article ISSN 2277– 7105

SOLUBLE GUANYLYL CYCLASE ACTIVATOR BAY 41-2272 IMPROVES VASCULAR ACTIVITY OF THORACIC AORTA ISOLATED FROM DOCA HYPERTENSIVE MALE WISTAR RATS

ShahidPrawez*[,1], Azad A Ahanger[2], Kathirvel Kandasamy[3], Thakur U Singh[4], Santosh K Mishra[4] and Souvendra N Sarkar[4]

[1]Department of Pharmacology & Toxicology, Faculty of Veterinary & Animal Sciences, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005 (UP), India.
[2]Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu of Kashmir, Shuhama, Alustang, Srinagar-190006, India.
[3]Post-Doctoral Research Trainee, Department of Physiology, University of Tennessee Health Science Centre, 894 Union Avenue, Memphis, Tennessee-38163, USA.
[4]Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar (UP), Pin 243122, India.

Article Received on
22 July 2014,

Revised on16 August 2014,
Accepted on 10 Sept 2014

***Correspondence for Author**
Dr.ShahidPrawez
Department of Pharmacology & Toxicology, Faculty of Veterinary & Animal Sciences, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005 (UP), India.

ABSTRACT

Hypertensionis the most common cardiovascular disease and of great concern to public health. Bay41-2272 a soluble guanylylcyclase activator induces vasodilation and reduces mortality. DOCA hypertensive group envisaged administration of Deoxycorticosterone acetate salt in soyabean oil in unilaterally nephrectomised male wistarrats @25mg/kg b. wt. subcutaneously twice in a week for 4-weeks with a provision of drinking water containing sodium chloride (1%) *ad lib*. Sham control unilateral-nephrectomised male wistar rats were treated with soyabean oil providing normal tap water. After completion of four weeks, rats were anaesthetized with pentobarbital sodium to evaluate Mean Arterial Pressure (MAP) and found significant increase ($P<0.05$)of MAP in DOCA hypertensive rats (160.3±3.02mm of Hg, n=9) compared to sham control(114.6±2.34mm of Hg, n=5). Also *in vitro* effect of Bay 41-2272 was studied on isolated thoracic aorta. In Bay pre-treated aortic rings of DOCA group, a significant decrease in noradrenaline maximal contraction (Emax0.61±0.03g; n=7) was found as compared to un-incubated aorta (Emax0.99±0.05g; n=5). A significant decrease in acetylcholine relaxation was found in aorta from DOCA hypertensive (E_{max}=61.19±3.43%, n=7) compared to sham control (E_{max}=111.10±1.61%; n=6). Bay 41-2272 pre-treatedaorta of DOCA rats, revealed significant

increase in acetylcholine relaxation (E_{max}=97.81±1.68%; n=6) as compared to un-incubated. Result of present study clearly indicates that Bay41-2272 pre-treatment significantly improves the vascular endothelial functions of aorta of DOCA hypertensive rats.

KEYWORDS: DOCA-salt, Hypertension,Mean Arterial Pressure, Bay 41-2272,vascular activity, thoracic aorta, male, wistar rats.

INTRODUCTION

Non-infectious disease hypertension called silent killer is major health concern in both developed and developing country like India. It progresses to functional and structural disparity in different tissues and physiological systems (Cebovaet al., 2006). Rise of arterial pressure produces changes such as cardiac hypertrophy, vascular smooth muscle cells hypertrophy, vascular remodelling and endothelial dysfunction, whichresults in heart failure, renal insufficiency, myocardial infarction or stroke (Pechanova et al., 2006; Simko, 2007). Lifestyle and heredity are the important factors which determine who will develop hypertension (Meyer et al., 2009). According to World Health Organization report 2002, the cardiovascular disease will be the largest cause of death and disability by 2020 in India. Therefore silent killer hypertension has to control by using relevant measures.

Treatment of hypertension depends on the etiology of disease, diet, body weight, exerciseand pharmacological interventions. Bay 41-2272 a potent nitric-oxide independent soluble guanylylcyclase (sGC) activator induces vasodilation, antiplatelet activity, thus reduces mortality (Parker, 1989; Stamler, 1994). Besides these, Bay 41-2272 possess anti-inflammatory activity and improves glomerular filtration rate (Ahluwalia et al., 2004). Keeping the prevalence of hypertension in view, present study was designed to examine the *in vitro* effect of Bay 41-2272 on vascular activity of thoracic aorta isolated from DOCA hypertensive rats.

MATERIALS AND METHODS

Animals

Healthy adult male wistar rats (150-200g) were housed in animal house having a provision for an alternative period of 12 hour light/dark cycle. The experimental animals were procured from Laboratory Animal Resource Section, Indian Veterinary Research Institute, Izatnagar (UP), India. Research work was conducted after approval by the Institute Post-Graduate study committee and also as per Animal Ethics Committee guidelines. After acclimatization, rats

were divided into two experimental groups namely Sham control and DOCA hypertensive group.

Hypertensioninduction and experimental design

Healthy adult male wistar rats were unilaterally nephrectomised by removing left kidney under anaesthetics ketamine (75mg/kg, i.m.) and xylazine (10mg/kg, i.m.). Three days after nephrectomy, deoxycorticosterone acetate (DOCA) salt dispersed in soyabean oil was administered subcutaneously at dose rate of 25mg/kg body weight twice in a week for four weeks (Chan et al., 2006). Drinking water provided *ad lib* contained sodium chloride (1%) and potassiumchloride (0.2%). Whereas unilateral nephrectomised sham control rats were treated with vehicle (soyabean oil) with a provision of plain drinking water. After completion of four weeks of DOCA salt administration, rats were anaesthetized with pentobarbital sodium (60mg/kg, intra-peritoneally). Anaesthetic was used to restrain the rats to measure the MAP by connecting to data acquisition system possessing pressure transducer (Model MLT0380/D, AD Instrument). After measuring the MAP of rats of both the groups, thoracic aorta was isolated and kept in ice cold Modified Krebs Henseleit Solution (4^0C, MKHS) for further processing of tissue. Ice cold MKHS was poured in petri-plate and surrounding fat tissue was removed (Kandasamy et al., 2011). Aorta rings was made (3-4cm) and mounted between stainless steel wire hooks under a resting tension of 1.5g in a thermostatically controlled (37^0C±0.1^0C) organ bath (Data Acquisition System, UGO Basile, Italy) of 10mL capacity containing MKHS and continuously bubbled with medical gas (74%N_2+21%O_2+5%CO_2). After attainment of equilibration (about an hour), aortic rings were contracted using potassium chloride depolarizing solution (80mM). Tissue contraction indicates its viability and also acts as reference standard to compare with vasoconstrictors under experiment. Once contraction plateau of potassiumchloride solution was reached, washing (MKHS) was done to produce baseline. Arterial rings again werecontracted withnoradrenaline to record cumulative contractile effect. Further to evaluate the effect of Bay 41-2272 on vascular functions,aortic rings from of DOCA hypertensive rats were incubated (pre-treated) with Bay 41-2272 (0.3µM) for 30min.On pre-treated aortic rings,contraction and relaxation responses were performed using noradrenaline (0.1nM-30µM) and acetylcholine/sodium nitroprusside (1nM-30µM),respectively.

Statistical analysis

Vascular activity (contraction & relaxation) was expressed in both E_{max} (maximal response) and EC_{50}/pD_2 (concentration producing 50% of maximal response) values. Value of vascular activity obtained was analyzed by nonlinear regression analysis (Sigmoidal dose response with variable slope) using Graph Pad Prism version 4.00 (San Diego, California, USA). Also experimental data was analyzed by Student Newman Keuls method for multiple group analysis. Concentration dependent response data obtained was analyzed by two way Analysis of Variance (ANOVA) followed by Bonferroni post hoc test (Snedecor and Cochran, 1989). Differences in values were considered statistically significant at $p<0.05$.

RESULTS

Effects of DOCA salt on Mean Arterial Pressure of treated rats.

A significant increase in MAP was found in DOCA salt treated uninephrectomised rats (160.3±3.02mm of Hg, n=9) compared to sham control (114.6±2.34mm of Hg, n=5) (Fig.1 & fig. 2.a & b).

Effects of Bay 41-2272 on Noradrenalineelicited contraction of aorta of DOCA hypertensive rats.

Aorta isolated from DOCA hypertensive rats were incubated with Bay 41-2272 (0.3µM) for 30 minutes (Fig. 3&4.A-C). After incubation, noradrenaline (0.1nM-10µM) was added cumulatively with an increment 0.5 log unit to observe the differences in contraction elicited in Bay incubated aorta isolated from rats of both the groups.A significant increase ($P<0.05$) in potency (pD2) of noradrenaline induced contraction was found in DOCA hypertensive rats (pD2=8.17±0.19; n=5) in comparison to sham control (pD2=7.65±0.06, n=9). Bay 41-2272 pre-treatment (0.3µM for 30 min) significantly decreases ($p<0.05$) the noradrenaline induced maximal response elicited in (Emax=0.61±0.03g; n=7) aortic rings of DOCA hypertensive rats in comparison to un-incubated rings from same DOCA hypertensive rats (Emax=0.99±0.05 g; n=5).

Effects of Bay 41-2272 on Phenylephrineelicited contraction of aorta of DOCAhypertensive rats.

Aorta isolated from DOCA hypertensive rats was incubated with Bay 41-2272 (0.3µM) for 30 minutes to see the effect of Bay on phenylephrine induced contraction (Fig. 5 and fig. 6.A-C). Phenylephrine (1nM-10µM) added cumulatively with an increment of 0.5 log unit concentration to contract the aortic rings of both the groups. A non-significant change of

phenylephrine induced potency (pD_2) and maximal response (E_{max}) was found in Bay incubated aorta isolated from DOCA hypertensive rats in comparison to un-incubated aortic rings. Also a non-significant change in phenylephrine induced potency and E_{max} values was found in aortic rings of DOCA hypertensive rats in comparison to sham control.

Effects of Bay 41-2272 on Acetylcholine induced relaxation on phenylephrine precontracted aorta from DOCA hypertensive rats.

Acetylcholine was (1nM-10µM) added cumulatively with an increment of 0.5 log unit concentration to produce relaxationof aortic rings of both the groups (fig.7 and fig. 8.A-C). A significant ($p<0.05$) decrease in ACh induced maximal relaxation was found in aortic rings of DOCA hypertensive rats (E_{max}=61.19±3.43%, n=7) in comparison to sham control (E_{max}=111.10±1.61%; n=6). Similarlya significant decrease ($p<0.05$) in relaxation potency wasfound in DOCA hypertensive rats (pD_2=6.91±0.19; n=7) in comparison to sham control (pD_2=7.58±0.05; n=6). Aorta isolated from DOCA hypertensive rats incubated with Bay 41-2272 (0.3µM) for 30 minutes significantly increases both ACh induced maximal relaxation response and potency (E_{max}=97.81±1.68%; pD_2=7.76±0.07, n=6) in comparison to unincubated rings of same rats (E_{max}=61.19±3.43%; pD_2=6.91±0.19; n=7).

Effects of Bay 41-2272 on SNP induced relaxation on phenylephrine precontrated aorta from DOCA hypertensive rats.

Vasodilating potency of SNP (fig.9 and fig. 10.A-C) was significantly decreases in thoracic aorta isolated from DOCA hypertensive rats (pD2=7.73±0.05; n=6) in comparison to sham control (pD2=7.96±0.05; n=7). However, a non-significant change in maximal relaxation was found in aortic ring from DOCA hypertensive rats. Aortic rings of DOCA hypertensive rats were incubated with Bay 41-2272 (0.3µM) for 30min, showed a non-significantchange in maximal relaxation response to SNP in comparison to Bay un-incubated rings of same groups. However, a significant increase of potency was found in Bay 41-2272 incubated rings of DOCA rats (pD2=8.36±0.09; n=5) as compared to un-incubated rings of same groups (pD2=7.73±0.05; n=6).

Effects of DOCA salt treatment on body weight and water consumption in DOCA hypertensive rats.

A non-significant change of body weight (fig.11) of DOCA hypertensive rats (219.17±6.76g, n=6) was found in comparison to sham control (203.33±9.28g, n=6). However, a significant

increase of water intake per daywas found in DOCA hypertensive rats (1593.40±102.21mL, n=5) in comparison to sham control (424.60±82.57mL, n=5).

DISCUSSION

Despite variety of pharmacological intervention, the trend of hypertension remains unabated. Recent reports showed Bay 41-2272 improves the condition to a significant level (Stamler, 1994). DOCA salt treatment impairs the vascular activity in DOCA induced hypertensive rats (Lima et al., 2009). Bay 41-2272 a soluble guanylylcyclase activator ameliorate the vascular activity (Teixeira et al., 2006). The present study was planned with the concept to study *in vitro* effect of Bay 41-2272 on thoracic aorta isolated from DOCA hypertensive rats.

DOCA salt treatment significantly increases the MAP of DOCA hypertensive rats in comparison to sham control. Increased MAP in mineralocorticoid (DOCA salt) induced hypertension by enhancing sodium intake followed by water retention increases extracellular fluid (Gomez-Sanchez *et al*., 1996). Increase in extracellular fluid volume suppressesthe plasma rennin activity or through Endothelin-1 dependent pathway (Gomez-Sanchez et al., 1996; Watts et al., 2002). Change in both contractile and relaxing vascular activity (*in vitro*) was found. Similar to present study, increase in potency of noradrenaline contraction was reported in DOCA hypertensive rats (Watts et al., 2002). However a non-significant changein E_{max}value was found in DOCA hypertensive rats. The said change in maximal response maybe because of decrease in contractile property elicited by ET-1 (endothelin-1) was found in DOCA hypertensive rats (Watts et al., 2002). Further decrease in maximal response to NA contraction was noted in Bay 41-2272 incubated aorta of DOCA hypertensive rats in comparison to un-incubated aorta from the same rats. On Bay incubated aorta, decrease in maximal response of contraction to noradrenalinewas found by increasing cGMP level through cGMP-dependent pathway (Stasch et al., 2001; Hobbs and Moncada, 2003).

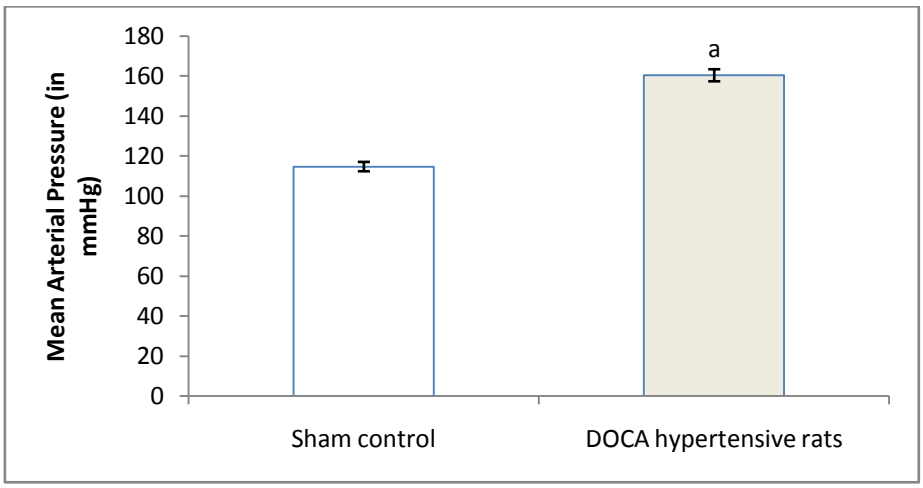
Vascular relaxing activity to acetylcholine, a significant decrease in aortic relaxation was found in DOCA hypertensive rat comparison to sham controlindicating the effect of DOCA salt on vascular activity. Similar significant decrease in aortic relaxation was reported in DOCA hypertensive rats (Callera et al., 2003; Callera et al., 2006; Lima et al., 2009).The mechanism which decreases the aortic relaxation was explained by Callera et al. (2006) and Limaet al. (2009) showed an increase of O-Glc-N-Acylation or decrease in phosphorylated eNOS activity results in decrease of vascular activity. Also supported by a significant decrease in basal cGMP level was found in aorta ofDOCA hypertensive rats. On Bay

incubation of aorta of DOCA hypertensive rats, a significantly increase in ACh relaxation was found. Indicating Bay improves the vascular activity by increasing cGMP level (Otsukaet al., 1988) or through calcium dependent efflux mechanism or ameliorates oxidative stress condition (Teixeira et al., 2006; Priviero et al., 2009).

A significant decrease of SNP relaxation potency was found in DOCA hypertensive rats. DOCA salt treatment decreases the potency by decreasing the cGMP level in aorta (Otsuka et al., 1988).Decrease in cGMP level produces decrease in vascular relaxation confirmed by incubating the aorta of DOCA rats using Bay which increases the potency (Stasch et al., 2001; Hobbs and Moncada, 2003).

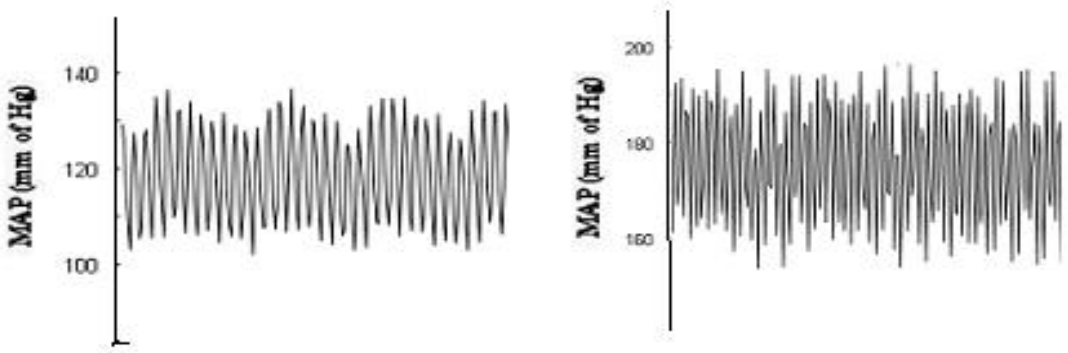
Interestingly a significant increase in water intake was found in DOCA hypertensive rats. Increased water intake in DOCA treated ratsindicates an increase in sodium load followed by augmentation of water intake (Gomez-Sanchez et al., 1996; Thunhorst et al., 2007) which results in hypertension.The motivating finding of present study was that Bay 41-2272 (0.3µM) incubation significantly decreases the vascular contraction elicited by noradrenaline in comparison to un-incubated aorta of same ratsof DOCA hypertensive rats. After Bay incubation of aortaof DOCA hypertensive rats a significantly improvement in acetylcholine relaxation potency (pD_2) and maximal response (E_{max}) was found in comparison to un-incubated aorta of same rats.Hence sGC activator Bay 41-2272 improves the vascular endothelial functions mediated through cGMP dependent or independent pathways or by decreasing reactive oxygen species generation.

Fig.1: Showing the effects of DOCA salt on MAP of DOCA hypertensive male wistar rats.



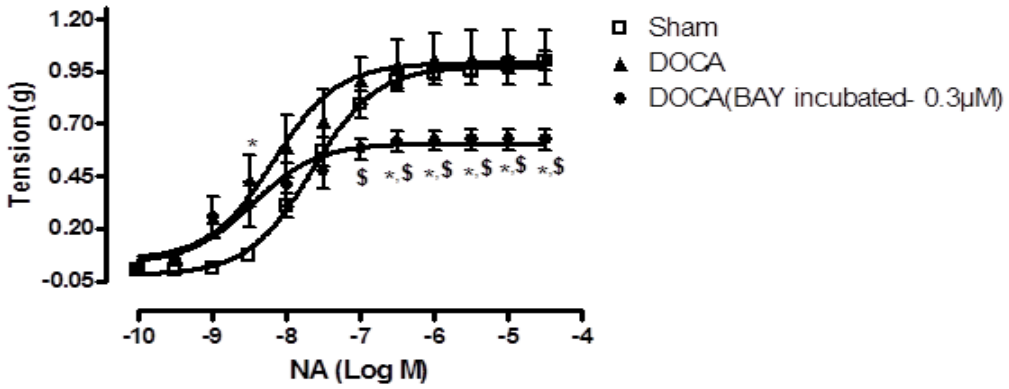
At $p^a<0.05$ indicate level of significance in comparison to sham control

Fig.2: Tracing depicting the effect of DOCA salt on MAP of DOCA hypertensive male wistar rats.



a Pentobarbital-Na (Sham control) **b.Pentobarbital-Na (DOCA rats)**

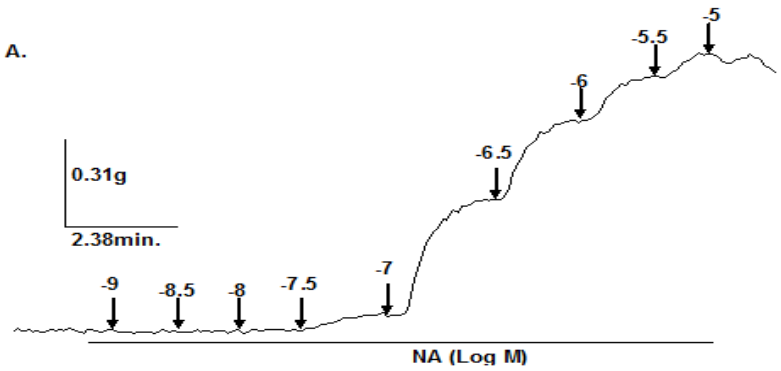
Fig.3: Showing the effect of Bay 41-2272 (0.3µM) on noradrenaline induced contraction of thoracic aorta of DOCA hypertensive rats.



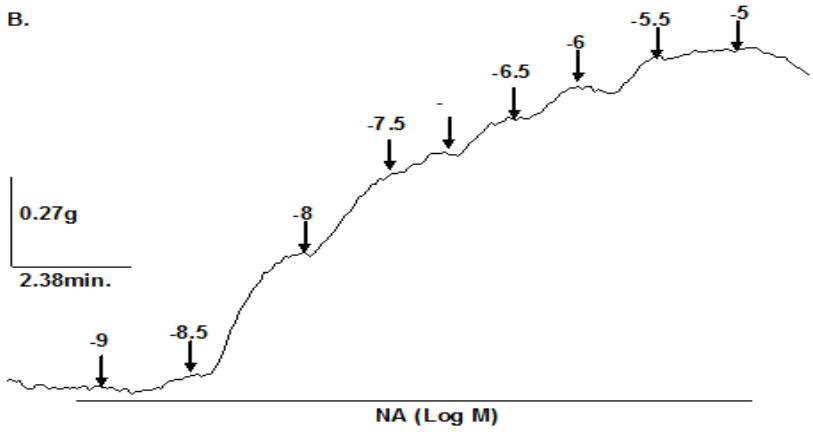
At p*<0.05 indicate level of significance in comparison to sham control

At p$<0.05 indicate level of significance in comparison to DOCA hypertensive rats

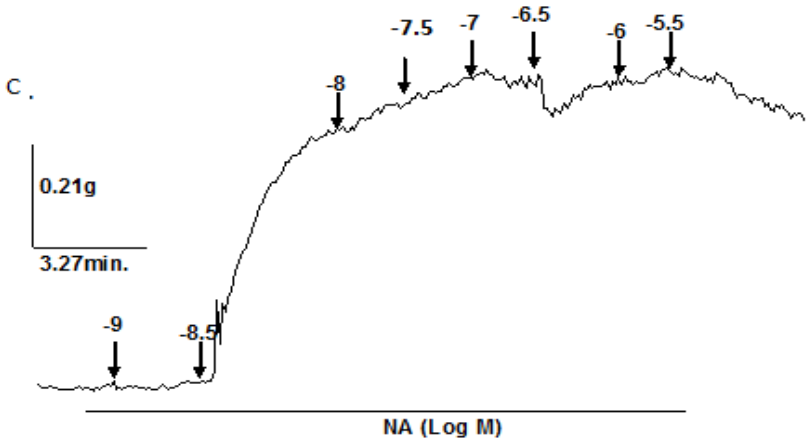
Fig.4: Tracing depicting the effect of Bay 41-2272 (0.3µM) on noradrenaline induced contractile response of thoracic aorta of DOCA hypertensive rats.



A. Bay unincubated aorta of Sham control



B. Bay unincubated aorta of DOCA hypertension



C. Bay incubated aorta of DOCA hypertension

Fig.5: Showing the effect of Bay 41-2272 (0.3µM) on Phenylephrine induced contraction of thoracic aorta of DOCA hypertensive rats.

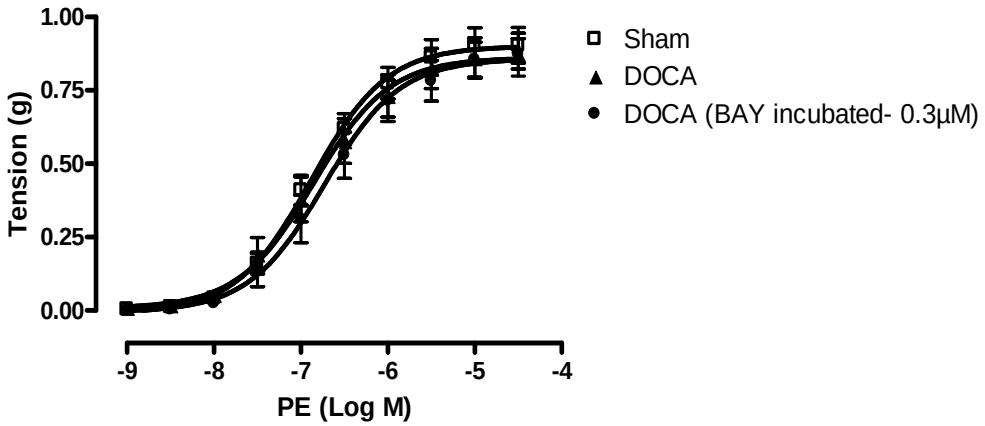
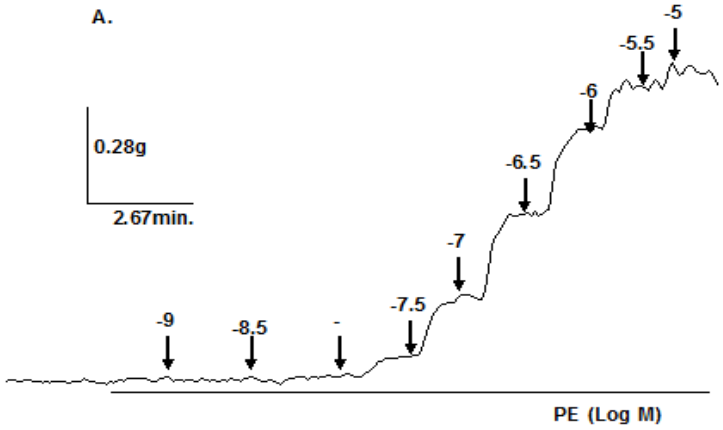
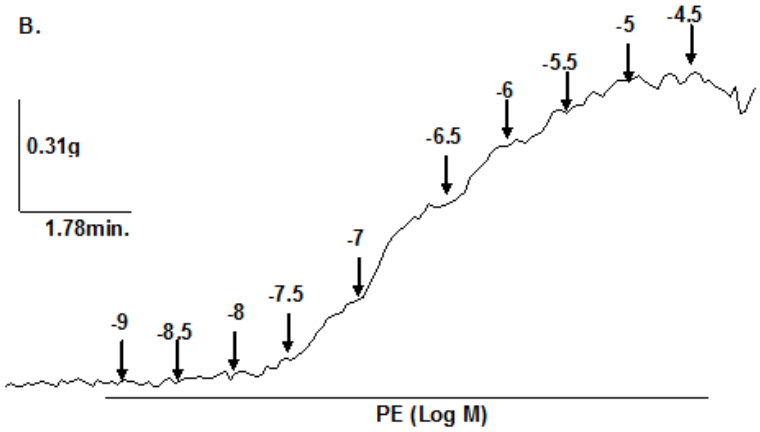


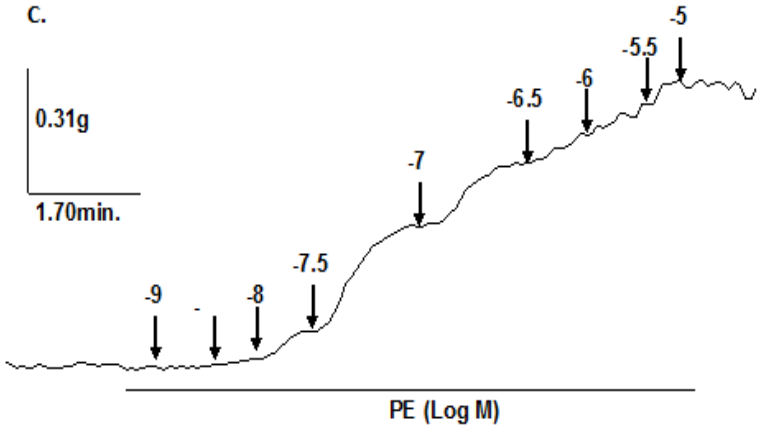
Fig.6: Tracing depicting the effect of Bay 41-2272 (0.3µM) on phenylephrine induced contractile response of thoracic aorta of DOCA hypertensive rats.



A. Bay unincubated aorta of Sham control,

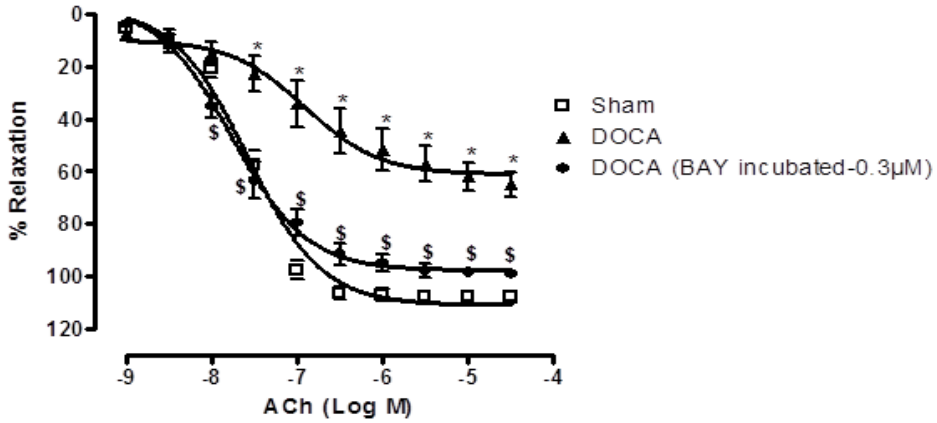


B. Bay unincubated aorta of DOCA hypertension



C. Bay incubated aorta of DOCAhypertension

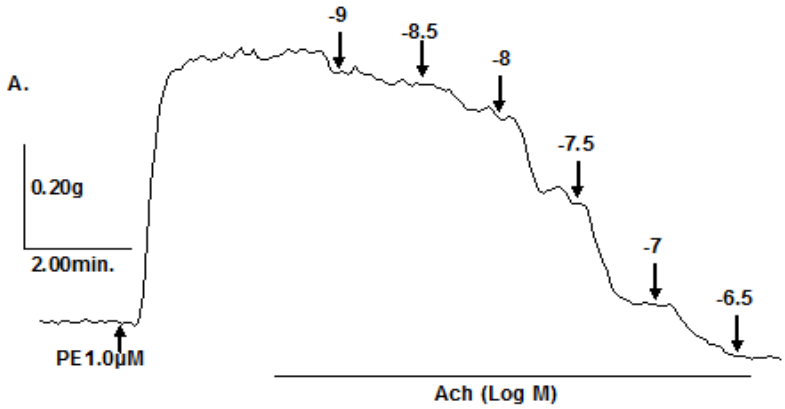
Fig.7: Showing the effect of Bay 41-2272 (0.3µM) on ACh induced relaxation on phenylephrine precontracted thoracic aorta of DOCA hypertensive rats.



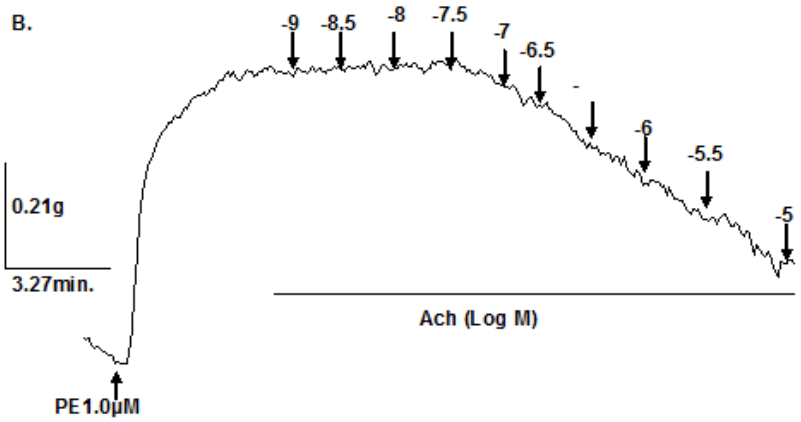
At p*<0.05 indicate level of significance in comparison to sham control

At p$<0.05 indicate level of significance in comparison to DOCA hypertensive rats

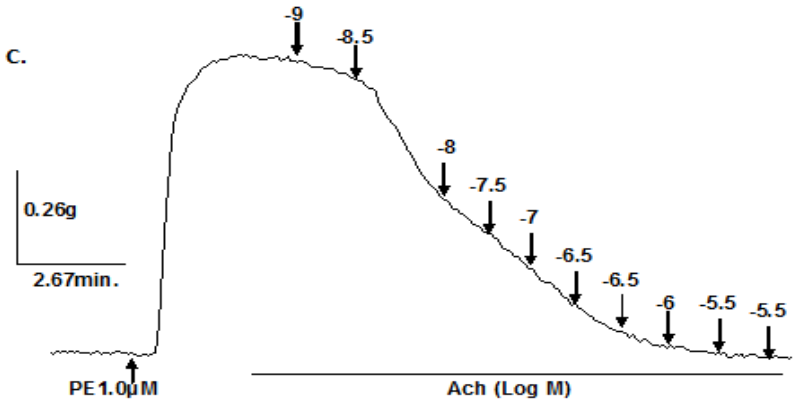
Fig.8: Tracing depicting the effect of Bay 41-2272 (0.3µM) on ACh induced percentage relaxation on phenylephrine precontracted thoracic aorta of DOCA hypertensive rats.



A. Bay unincubated aorta of Sham control,

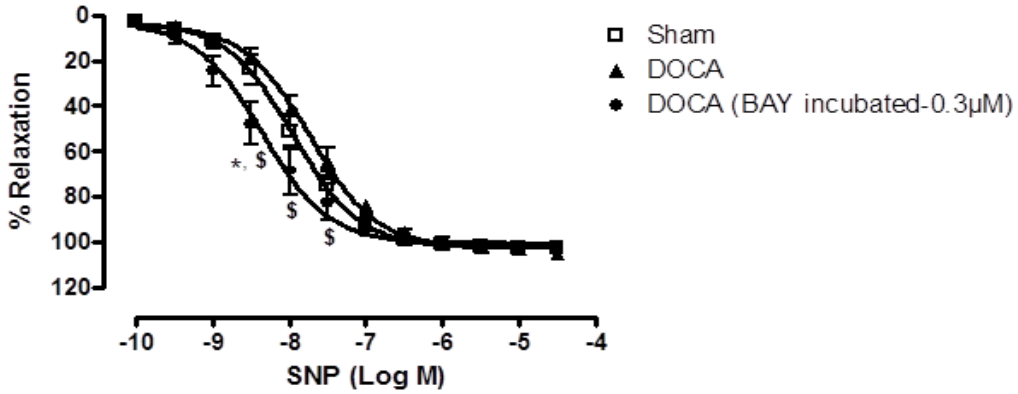


B. Bay unincubated aorta of DOCA hypertension



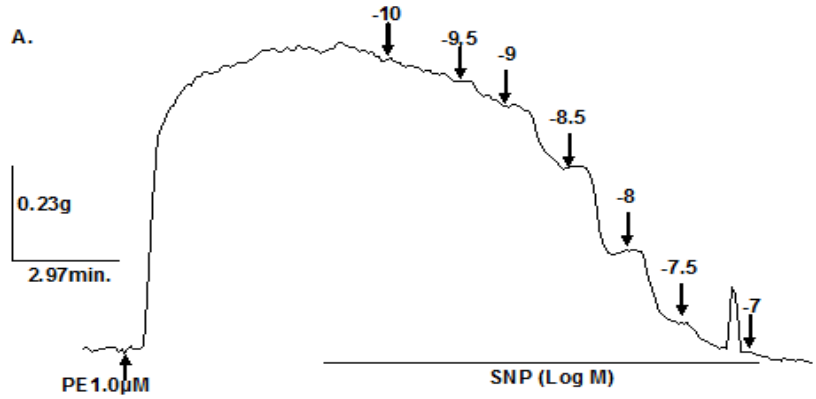
C. Bay incubated aorta of DOCA hypertension

Fig.9: Showing the effect of Bay 41-2272 (0.3µM) on SNP induced relaxation in phenylephrine precontracted thoracic aorta of DOCA hypertensive rats.

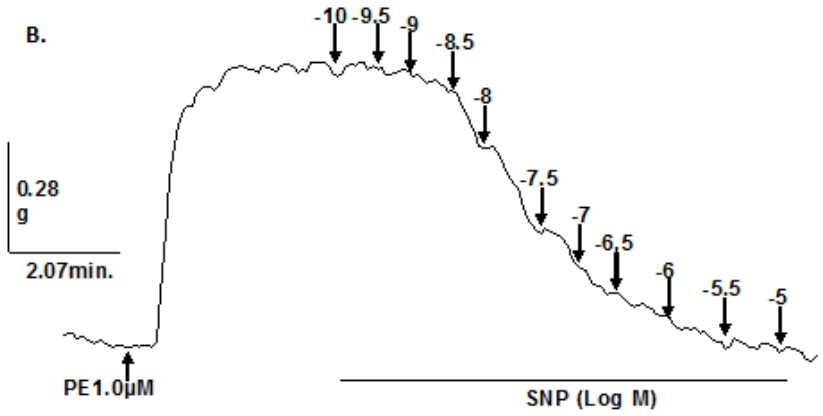


At p*, $<0.05 indicate level of significance in comparison to Sham & DOCA hypertensive rats, respectively.

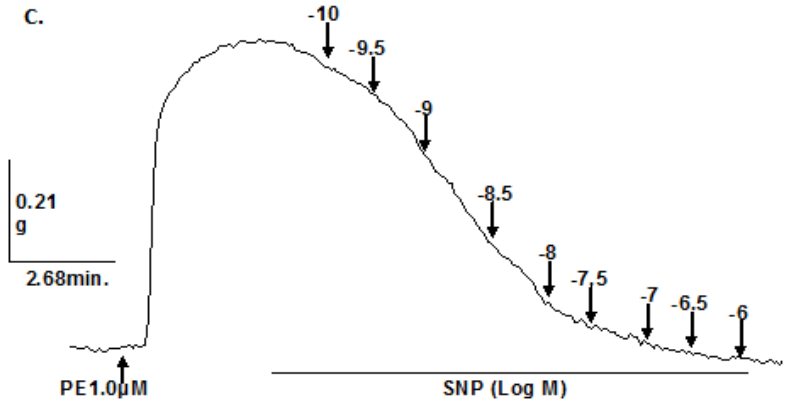
Fig.10: Tracing depicting the effect of Bay 41-2272 (0.3µM) on SNP induced percentage relaxation on phenylephrine precontracted thoracic aorta of DOCA hypertensive rats.



A .Bay unincubated aorta of Sham control,

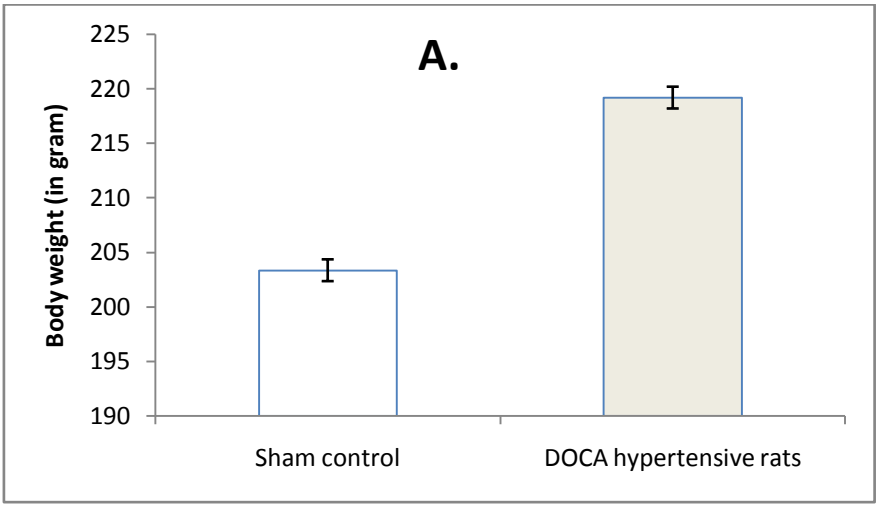


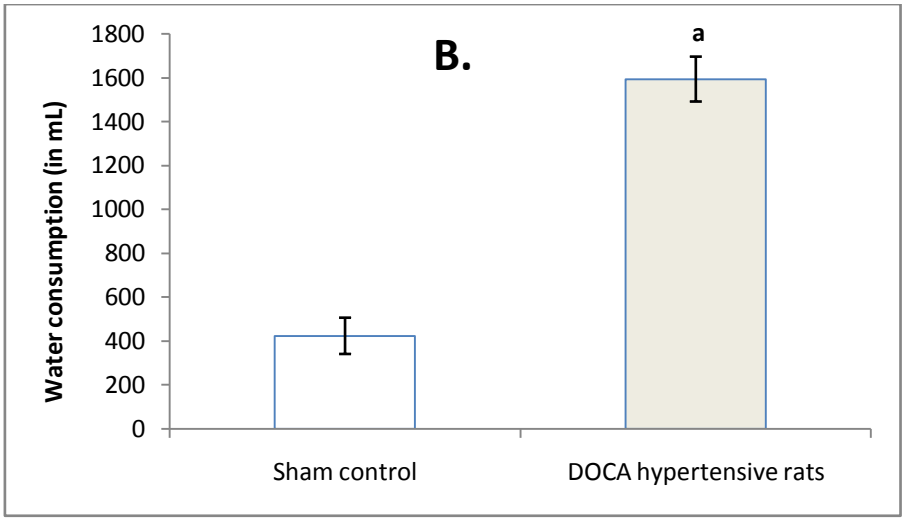
B. Bay unincubated aorta of DOCA hypertension



C. Bay incubated aorta of DOCA hypertension

Fig.11: Showing the effects of DOCA salt on (A) body weight and (B) water consumption of DOCA hypertensive rats.





At $p^a<0.05$ indicate level of significance in comparison to sham control.

ACKNOWLEDGEMENTS

I am extremely thankful to Division of Pharmacology & Toxicology, Indian Veterinary Research Institute, Izatnagar, Bareilly-243144 (UP) for providing research facilities and creating homely atmospheres to facilitate the research work.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

FUNDING

The research work is PhD work.

REFERENCES

1. Ahluwalia A, Foster P, Scotland RS, McLean PG, Mathur A, Perretti M, et al. (2004) Antiinflammatory activity of soluble guanylatecyclase: cGMP-dependent down-regulation of P-selectin expression and leukocyte recruitment. *Proceeding of the National Academy of Sciences* 101: 1386-1391.
2. Callera GE, Touyz RM, Teixeira SA, Muscara MN, Carvalho MHC, Fortes ZB, et al. (2003) ETA Receptor Blockade Decreases Vascular Superoxide Generation in DOCA-Salt Hypertension. *Hypertension* 42: 811-817.
3. Callera GE, Tostes RC, Yogi A, Montezano AC and Touyz RM (2006) Endothelin-1-induced oxidative stress in DOCA-salt hypertension involves NADPH-oxidase-independent mechanisms. *Clinical Science* 110: 243-253.

4. Cebova M, Kristek F and Kunes J (2006) Differential remodelling of carotid artery in spontaneously hypertensive and hereditary hypertriglyceridemic rats. *Physiological Research* 55: S81-S87.
5. Chan V, Hoey A and Brown L (2006) Improved cardiovascular function with aminoguanidine in DOCA-salt hypertensive rats. *British Journal of Pharmacology* 148: 902-908.
6. Gomez-Sanchez EP, Zhou M and Gomez-Sanchez CE (1996) Mineralocorticoids, salt and high blood pressure. Steroids 61: 184-188.
7. Hobbs AJ, Moncada S (2003) Antiplatelet properties of a novel, non-NO-based soluble guanylatecyclase activator, BAY 41-2272. *Vascular Pharmacology* 40: 149-154.
8. Kandasamy K, Prawez S, Choudhury S, More AS, Ahanger AH, Singh TU, et al. (2011) Atorvastatin prevents vascular hyporeactivity to norepinephrine in sepsis: role of nitric oxide and α-1adrenoceptor mRNA expression. *Shock* 36: 76-82.
9. Lima VV, Giachini FRC, Choi H, Carneiro FS, Carneiro ZN, Fortes ZB, et al. (2009) Impaired Vasodilator Activity in Deoxycorticosterone Acetate Salt Hypertension Is Associated With Increased Protein O-GlcNAcylation. *Hypertension* 53: 166-174.
10. Meyer TE, Shiffman D, Morrison AC, Rowland CM, Louie JZ, Bare LA, et al. (2009) GOSR2 Lys67Arg is associated with hypertension in whites. *American Journal of Hypertension* 22: 163-168.
11. Otsuka Y, DiPiero A, Hirt E, Brennaman B and Lockette W (1988) Vascular relaxation and cGMP in hypertension. *American Journal of Physiology* 254: H163-169.
12. Parker JO (1989) Nitrate tolerance in angina pectoris. *Cardiovascular Drugs and Therapy* 2: 823-829.
13. Pechanova O, Rezzani R, Babal P, Bernatova I and Riantsitohaina R (2006) Beneficial effects of ProvinolsTM: cardiovascular system and kidney. *Physiological Research* 55: S17-S30.
14. Priviero FBM, Zemsem SM, Teixeira CE and Webb RC (2009) Oxidative Stress Impairs Vasorelaxation Induced by the Soluble GuanylylCyclase Activator Bay 41-2272 in Spontaneously Hypertensive Rats. *American Journal of Hypertension* 22: 493-499.
15. Simko F (2007) Is NO the king? Pathophysiological benefit with uncertain clinical impact. *Physiological Research* 56: S1-S6.
16. Snedecor GW, Cochran WG (1989) Statistical Methods (8th edition). The Iowa State University Press.

17. Stamler JS (1994) Redox signalling: nitrosylation and related interactions of nitric oxide. *Cell* 78: 931-936.
18. Stasch JP, Becker EM, Alonso-alija C, Apeler H, Dembowsky K, Feurer A, et al. (2001) NO-independent regulatory site on soluble guanylatecyclase. *Nature (London)* 410: 212-215.
19. Teixeira CE, Priviero FB, Todd J Jr and Webb RC (2006) Vasorelaxing effect of BAY 41-2272 in rat basilar artery: involvement of cGMP-dependent and independent mechanisms. *Hypertension* 47: 596-602.
20. Thunhorst RL, Beltz TG and Johnson AA (2007) Glucocorticoids increase salt appetite by promoting water and sodium excretion. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 293: R1444-R1451.
21. Watts SW, Fink GD, Northcott CA and Galligan JJ (2002) Endothelin-1-induced venous contraction is maintained in DOCA-salt hypertension; studies with receptor agonists. *British Journal of Pharmacology* 137: 69-79.