Evaluation of Anti-Hypertensive & Cardioprotective activity of Dawa ul Motadil Misk : An age old Unani Preperation

Thesis submitted in partial fulfillment of the requirement for the degree of Master of Pharmacy

Under the guidance of

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Certification

This is to certify that Sk Zeeshan Ali, (Examination Roll No: 002011402015, Registration No: 154276 of 2020-2022) has carried out the project work on the subject entitled "*Evaluation of Anti-Hypertensive & Cardioprotective activity of Dawa ul Motadil Misk : An age old Unani Preperation*" under the supervision of Prof. Sanmoy Karmakar, Department of Pharmaceutical Technology. This project work submitted by him in partial fulfillment of the requirements for the degree of Master of Pharmacy (Pharmacology) of Jadavpur University. He has carried out his work independently and with proper care and attention to our entire satisfaction. The ideas put into effect were original and are not a copy of or similar to any other thesis submitted/published elsewhere.

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DECLARATION OF THE ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains literature survey and original research as part of my work on "*Evaluation of Anti-Hypertensive & Cardioprotective activity of Dawa ul Motadil Misk : An age old Unani Preperation*". All information in this document have been obtained and presented in accordance with academic rules and ethical conduct. I also declare that as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

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CONTENTS

INTRODUCTION

Providing a comprehensive range of health services for hypertension, from early identification to control and increasing care quality, is crucial to living a healthy life. Furthermore, it is poorly controlled due to a lack of understanding about hypertension , improper primary care, and lack of follow-up. Nearly 63 percent of all deaths in India are caused by non-communicable diseases, with cardiovascular disease accounting for 27 percent of all deaths in the 40–69 age range. The Fourth National Family Health Survey investigated hypertension in a large population-based sample (n = 799,228) and found that 13.8 percent of men and 8.8 percent of women (a total of 11.3 percent) between the ages of 15 and 54 reported hypertension. According to the Global Burden of Diseases study, hypertension caused 1.63 million deaths in India in 2016, up from 0.78 million in 1990 (+108 percent). Dawa-ul-Misk Motadil Sada is an Unani herbal tonic. It is said to strengthen heart, liver, brain. However, pharmacological evaluation is required to validate these claims. So in this study we tried to evaluate for probable anti-hypertensive and cardio-protective potential of this unani preparation.

LITERATURE SURVEY

Hypertension is an important cardiovascular risk factor. High blood pressure per se is not a disease but a hemodynamic alteration associated with vascular disease. In general, two haemodynamic forces regulate normal blood pressure: cardiac output and total peripheral vascular resistance. The product of cardiac output and systemic vascular resistance is blood pressure. As a result, patients with arterial hypertension may experience an increase in cardiac output, systemic vascular resistance, or both.Many ideas exist to explain the pathogenesis of essential hypertension. High catecholamine levels in the blood, increased blood volume (volume hypertension) and arteriolar constriction (vasoconstrictor hypertension), increased cardiac output, and altered renin responsiveness.

Hypertension produced by renal diseases is called renal hypertension. When the kidneys sense diminished effective arterial blood volume or reduced pressure in afferent blood arteries or arterioles in the glomerulus, renin is released by the macula densa of the juxtaglomerular apparatus (via β adreno-receptors). Renin catalyses the conversion of angiotensinogen, which is produced by the liver, to angiotensin I once it enters the circulation. Angiotensin I is converted to the active moiety angiotensin II when it comes into contact with the angiotensin-converting enzyme, which is found on the surface of the vascular endothelium. Angiotensin II exerts its physiologic function by binding to the high-affinity AT1 receptor site in tissues. The fight-or-flight response is dependent on the endocrine RAAS's acute reaction to changes in systemic arterial pressure.The hemodynamic and metabolic problems that lead to endothelial dysfunction are exacerbated by inappropriately activated systemic and local tissue renin angiotensin aldosterone systems (RAAS). An increasing body of research suggests that RAAS activation, excessive generation of reactive oxygen species, and HTN are all linked. RAAS blockage improves cardiovascular and renal outcomes from a therapeutic standpoint.Renin stimulates the release of angiotensin II, which causes direct vasoconstriction in resistance

vessels. Renal hypertension can be caused by an obstruction of a major renal artery, interstitial nephritis, diabetic nephropathy, eclampsia, polyarteritis nodosa, and fibromuscular dysplasia of the renal artery, as well as other forms of glomerulonephritis, pyelonephritis, amyloidosis, polycystic kidney disease and renin-producing tumours. Systolic hypertension can also occurs in the upper part of the body due to narrowing of a short section of aorta.

The endothelial cell may produce a range of molecules that modulate pulmonary flow of blood and vascular resistance, including NO and endothelin.The endothelial cell may produce a range of molecules that modulate pulmonary flow of blood and vascular resistance, including NO and endothelin.The vascular endothelium's dysfunction is a common early sign of cardiovascular illness, and it's linked to clinical outcomes in individuals with atherosclerosis and hypertension.

Endothelial dysfunction refers to endothelium-derived relaxing substances including nitric oxide (NO) and prostacyclin having a reduced vasodilator function. For example, endothelial dysfunction is marked by a malfunction in the L-arginine-NO pathway in essential hypertension.The generation of oxygen free radicals, which promote NO breakdown and so reduce NO availability, appears to be the primary mechanism behind this change.

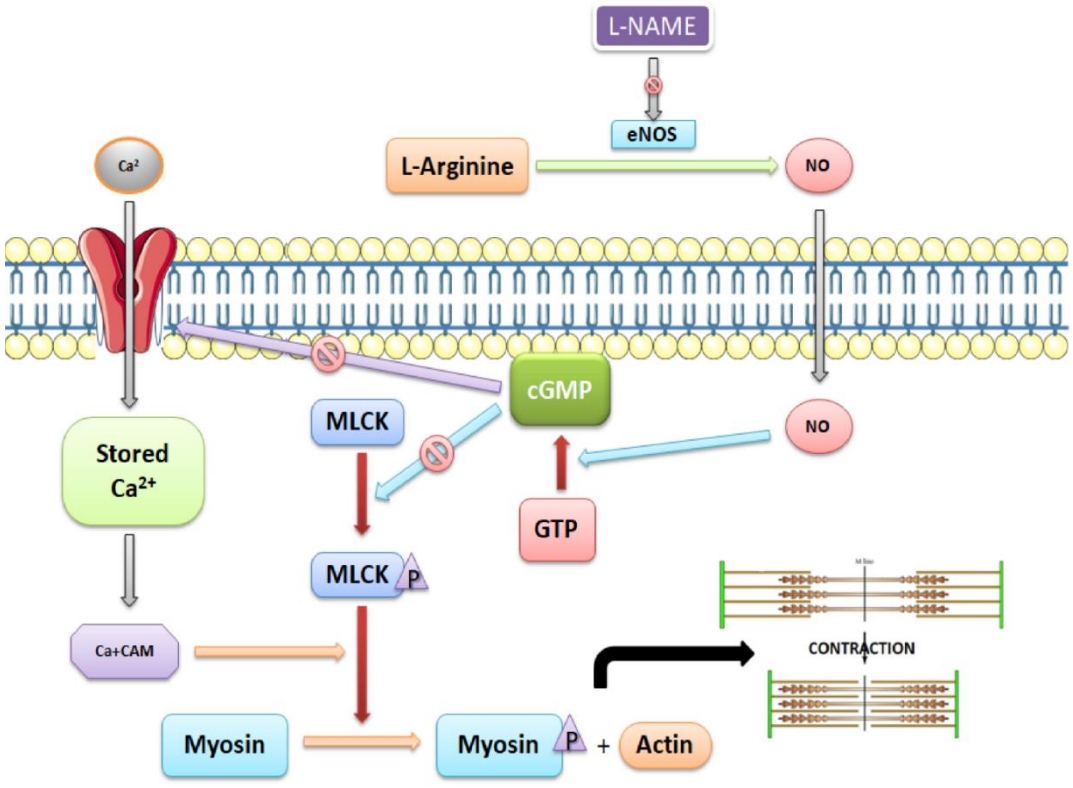
A chemical named as L-NAME (N(G)-Nitro-L-arginine methyl ester) is responsible for the inhibition of NO formation by inhibition of eNOS (Endothelial nitric oxide synthase)

ET-1 is a strong vasoconstrictor peptide that was first obtained from endothelial cells.Endothelial dysfunction leads to an increase in the synthesis and physiological activity of endothelin (ET)-1, a strong vasoconstrictor & pro-inflammatory peptide.DOCA-salt rats, angiotensin II-infused rats, Dahl salt-sensitive rats, two-kidney one-clip hypertensive rats, one-kidney one-clip Goldblatt rats, nitro-L-arginine methyl ester chronically treated rats or transgenic (mREN2)27 rats, are all examples of experimental hypertension models.ET-1 was shown to be overexpressed in the vessel walls in hypertension models where ET-1 serves as a vasoconstrictor role. Endothelin receptor antagonists also reduced vascular expansion and inflammation, as well as improving endothelial dysfunction, in these experimental animal models.

The renin-angiotensin system (RAS) is well-known for its role in blood pressure regulation, electrolyte balance, and vascular remodelling. The RAS's Angiotensin Converting Enzyme

(ACE) is a critical component. Although ACE is usually found in pulmonary capillaries, it can also be found in endothelial and renal epithelial cells. The amount of body fluids is primarily controlled by this hormone system. Angiotensin I is made by renin cleaving six amino acids from angiotensinogen. ACE then hydrolyzes angiotensin I, resulting in activated angiotensin II. Angiotensin II also induces an increase in blood pressure by promoting salt reabsorption by the kidney by inducing the adrenal cortex to release aldosterone. ACE inhibition has become a viable technique for therapeutic targeting in the treatment of cardiovascular illnesses such as hypertension since ACE plays a vital role in regulating RAS.

Stretch-induced hypertrophic response is mediated in part by Ang II release. Ang II is involved in blood pressure regulation and plays a key function in hypertension. Myocardial infarction, hypertension, and left ventricular hypertrophy are all increased by variations in the Ang II gene.



RATIONALE AND OBJECTIVE

The aim and objectives of the work was to:

1. Investigate any potent anti-hypertensive activity of Dawa-ul-Misk-Motadil (DMM) in Wistar albino rats.

2. Investigate any cardiac protective activity of Dawa-ul-Misk-Motadil in Wistar albino rats.

MATERIALS AND METHODS

Chemicals and Drugs:

L-NAME (Sigma aldrich) was purchased from Sigma Aldrich.Isoproterenol (TCI Chemicals (India) Pvt. Ltd.). DMMwas a kind donation from The Calcutta Unani Medical College and Hospital, 8/1,Abdul Halim Lane, Kolkata, West Bengal 700016 . Na+, K^+ and Cl^- (ELYTE-3) estimation Kit. Griess Reagent (SRL) .

Animal Husbandry and Maintenance:

Healthy adult male Wistar rats weighing 150-180 gm were procured from M/S Chakraborty Enterprise, 3/1D Girish Vidyaratna Lane, Narkeldanga, Kolkata -700011, and used for the study.

Animals were maintained in polypropylene cages, each containing a maximum of four animals. Animals were kept in the departmental animal house under controlled conditions of temperature (12 hr light and dark cycle, temperature of 25 ± 2^0C and 50± 20 % relative humidity).

The study was conducted in accordance with the Institutional Ethical Committee (Constituted under the Guidelines Committee for the Purpose of Control and Supervision of Experiments on Animals).

Development of Hypertension in rat model

Adult male Wistar rats weighing 150–180 g were utilized in the anti-hypertensive study. The animals had free access to food and water, and they were kept on a 12-hour light-dark cycle. In this study, L-NAME (N-nitro-l-arginine methyl ester) was administered intraperitoneally at 185 mmoles per kg body weight for 7 consecutive days.

Development of Hypertrophy in rat model

Adult male Wistar rats weighing 150-180 g were utilized in the anti-hypertensive study. The animals had free access to food and water, and they were kept on a 12-hour light -dark cycle. In this study, Isopreterenol was administered subcutaneously in a dose of 5.25 mg per kg body weight on 13^{th} day 8.5 mg per kg body weight on 14^{th} day.

Blood Pressure Measurement using Non-Invasive method & ECG measurements

The BIOPAC–MP36 device was used to assess systolic blood pressure in a non-invasive manner (Biopac Syste, Inc., USA) and ECG. The cuff was placed around the animal's tail and held in an animal restrainer. We kept the animals in a constant temperature of 37°C. For data augmentation, we utilized NIBP-200A and MP-36 software. (IP injections, subcutaneous and oral dosing were given following standard protocols.)

Animals were restrained in an animal holder before recording the blood pressure using Non invasive blood pressure.

Before recoding the ECG, the rats were anaesthetized using Ketamine (50 mg /kg) and muscle relaxant Xylazine (10 mg/kg).

Effect of Dawa ul Misk Motadil (DMM) on L-NAME induced Hypertensive rats

The animals were separated into three groups (n = 3), each with three animals. The first group was designated as the Control group, and they were given L-NAME (IP) for 7 days before being left untreated for another 7 days. The second group received L-NAME for the first 7 days and then L-NAME + DMM (low dose, i.e., 1000mg/kg b.w.) until day 14. Finally, the third group received L-NAME + DMM (high dose, i.e., 2000mg/kg b.w) in the same way as the second group. Finally, on days 0, 7, and 14, systolic blood pressure was monitored using NIBP (non-invasive blood pressure) and the R-R interval was recorded. In addition, we drew retro-orbital blood (1.5ml) on day 0 (i.e., before treatment), day 7, and day 14. Serum was separated from the collected blood and the concentrations of Na^+, K^+, and Cl^- concentrations were analyzed . The same serum was also further used to calculate the nitrite levels.

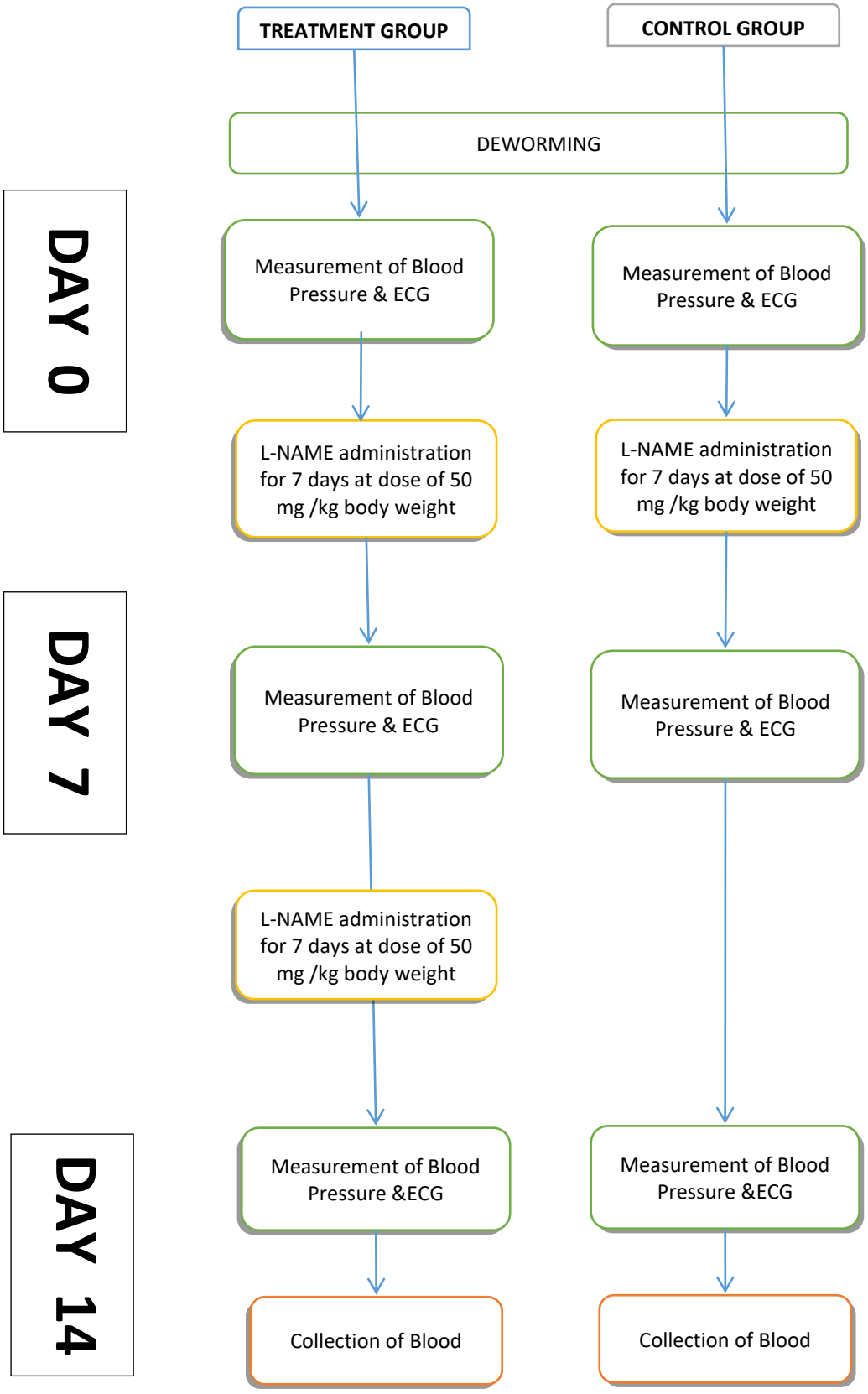
Estimation of heart weight/tail length ratio

In each group, heart weight/tail length ratio was measured on the day of sacrifice as a parameter of cardiac hypertrophy. Tail length was measured by using a centimeter (cm) scale. Heart weight (gm) was measured after keeping the heart in ice cold saline (0.9% w/v) and blotting out the heart with tissue paper to remove adhering liquid in folds and gaps.

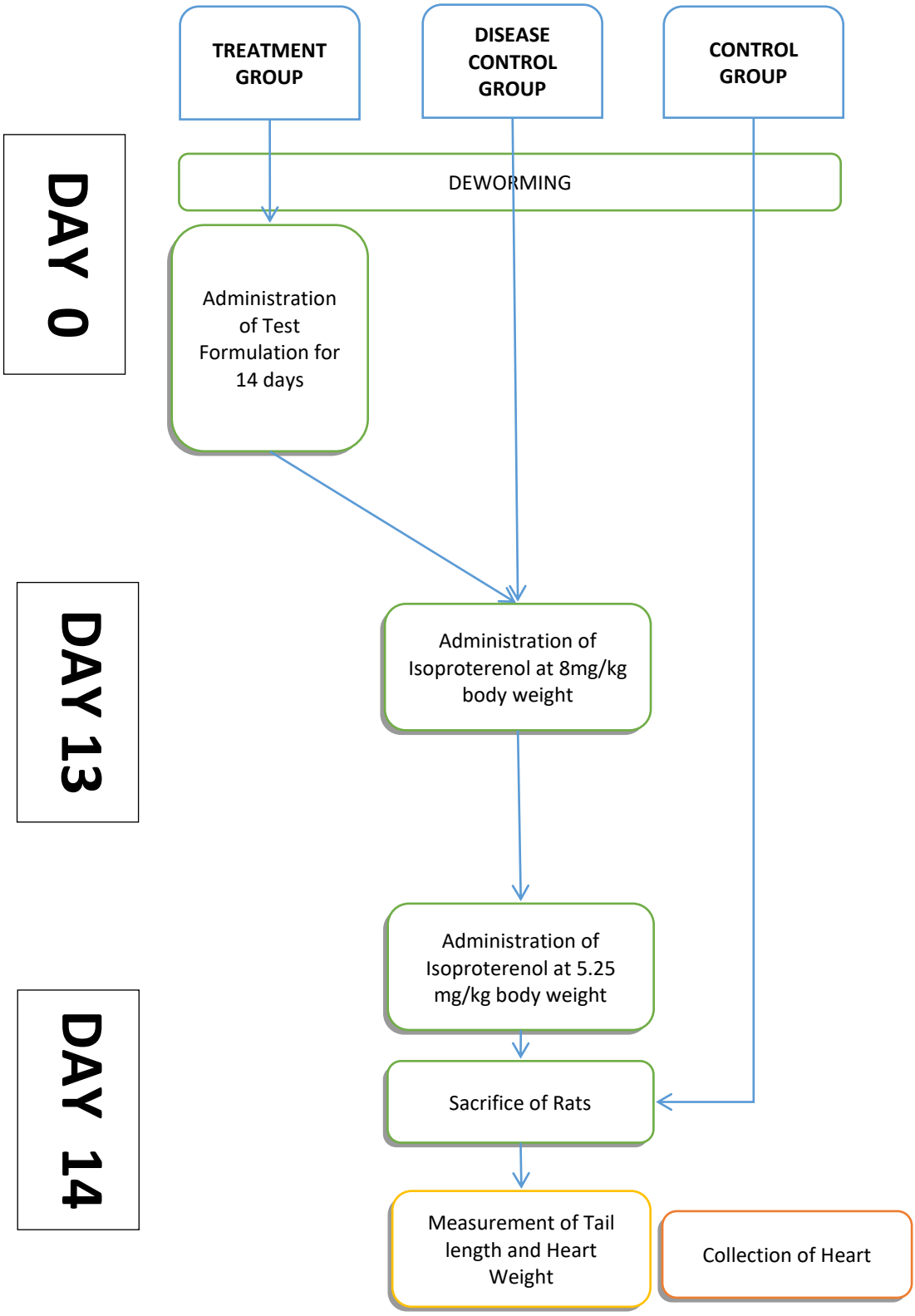
Statistical analysis:

All data were given as Mean ± SEM in the charts.

PLAN OF WORK (Anti-Hypertensive L-Name Model)



PLAN OF WORK (Anti-Hypertrophy Isoproterenol Model)



RESULT AND DISCUSSION

1. Effect of DMM on Systolic Blood Pressure.

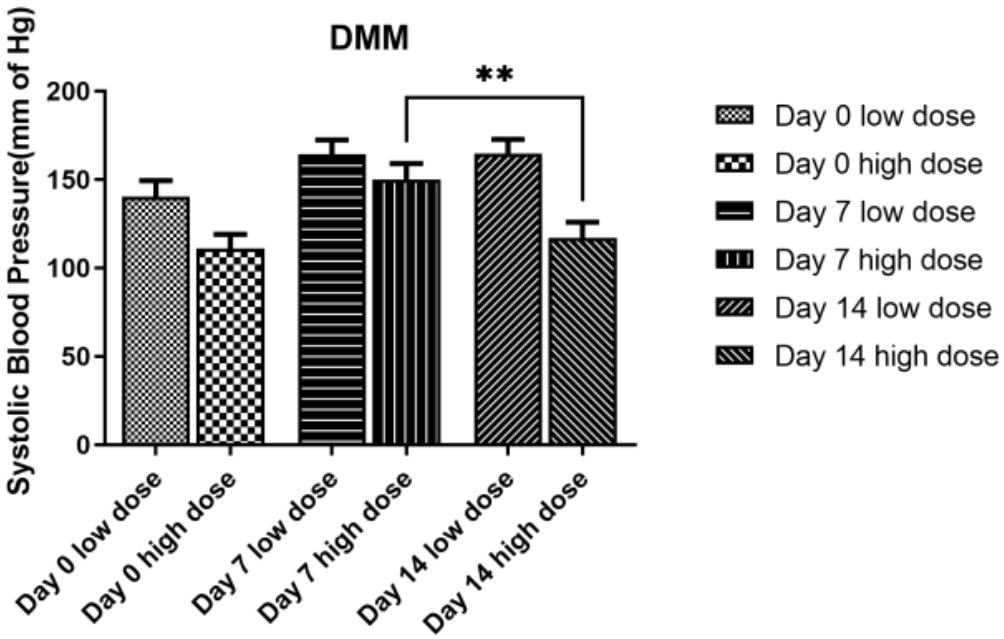


Fig 1: Systolic blood pressure (Non-invasive)

L-Name significantly able to developed hypertension within 7 days in all the groups.

It is evident for Fig 1 that on day 14 blood pressure decreases in the treated group with high dose. However blood pressure did not decreased significantly in the treated group with low dose.

2. Effect of DMM on Systolic Blood Pressure.

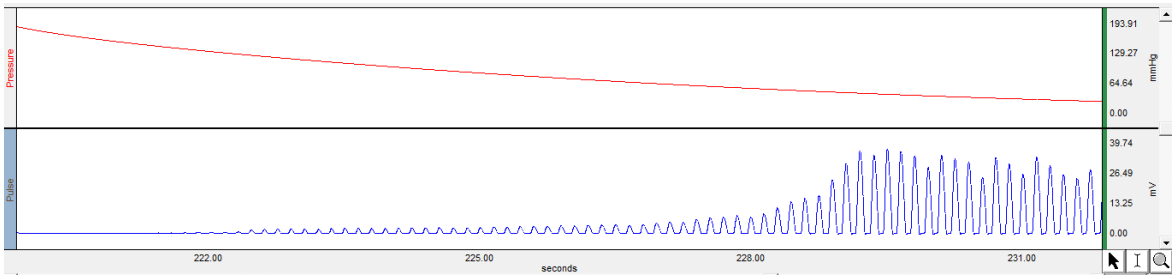


Fig 6: Systolic Blood Pressure Day 0

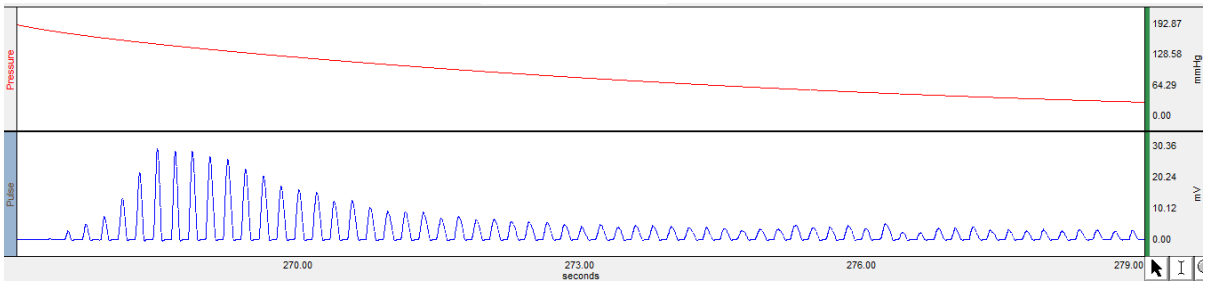


Fig 7: Systolic Blood Pressure Day 7

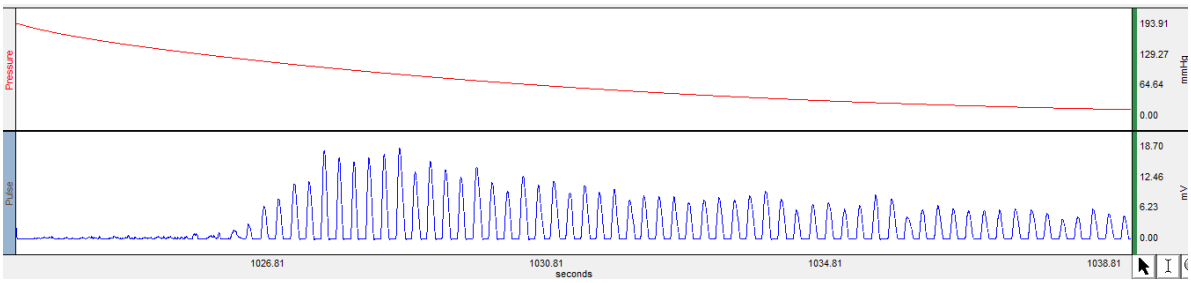


Fig 8: Systolic Blood Pressure Day 14

3. Effect of DMM on RR interval.

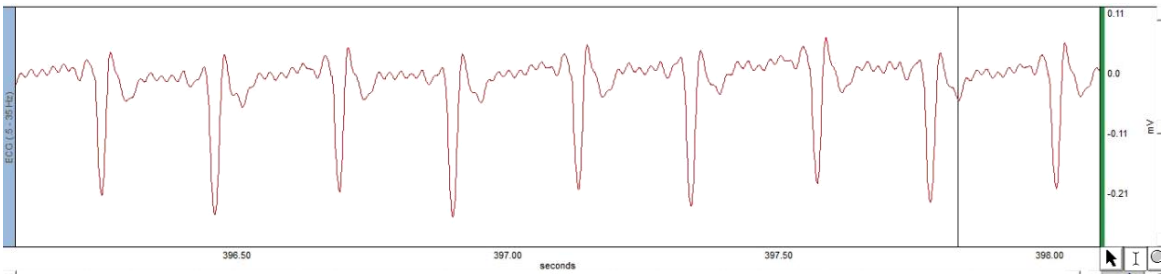


Fig 9: RR interval Day 0

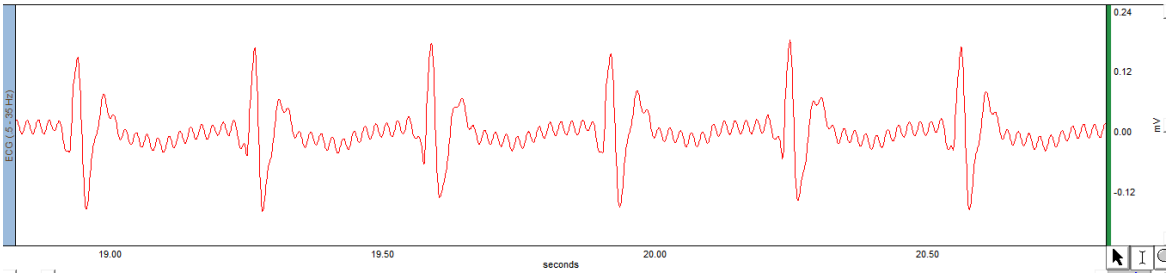


Fig 10: RR interval Day 7

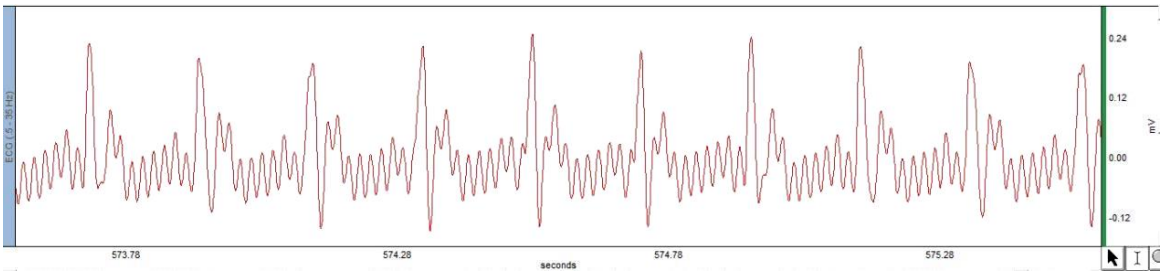


Fig 11: RR interval Day 14

The systolic blood pressure was observed to change at high doses of DMM. However there was marginal changes in systolic blood pressure in the group which was administered with low dose of DMM.

Conventionally if the blood pressure increases then the heart rate should decrease, so as to maintain the cardiac output. After L-NAME treatment, we observed that there wan an increase in the blood pressure with a decrease in heart rate. After treatment with DMM, the blood pressure decreases and accordingly the heart rate increased. This not only complies with accepted CVS conventionbut also indicates that there is probable an anti-hypertensive activity of DMM evident only in high dose, at least in our study.

4. Effect of DMM on Serum Nitrite Level.

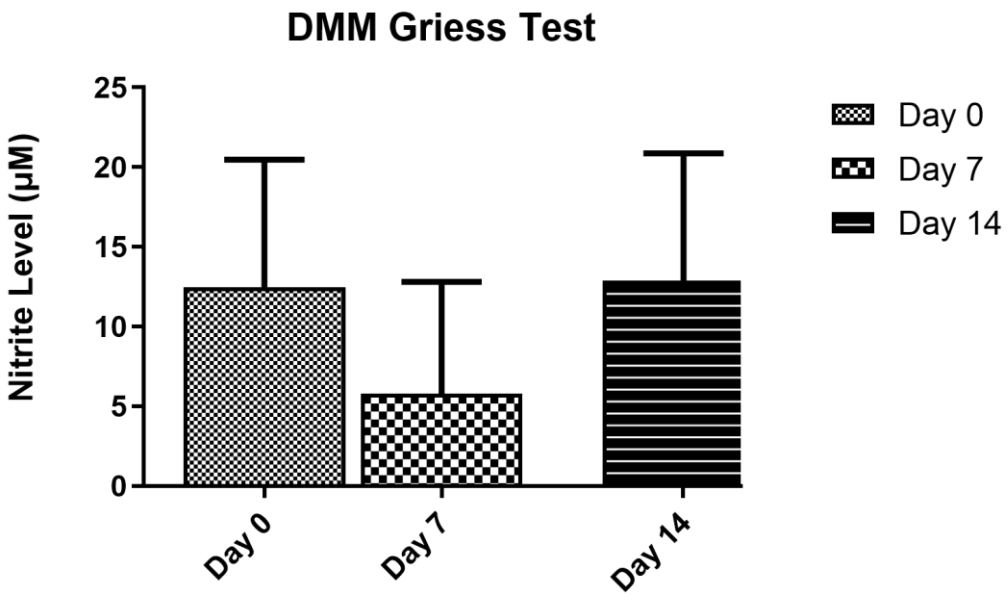


Fig 5: Serum Nitrite Level

In addition, L-NAME treated rats showed a decreased in serum nitrite level. This is probably due to its inhibition of eNOS. But, after treatment with DMM for 7 days, the serum nitrite level increased. The observed blood pressure lowering action of DMM may be related to increased heart rate and also with increased NO levels.

5. Effect of DMM on Serum Sodium, Chloride and Potassium concentrations

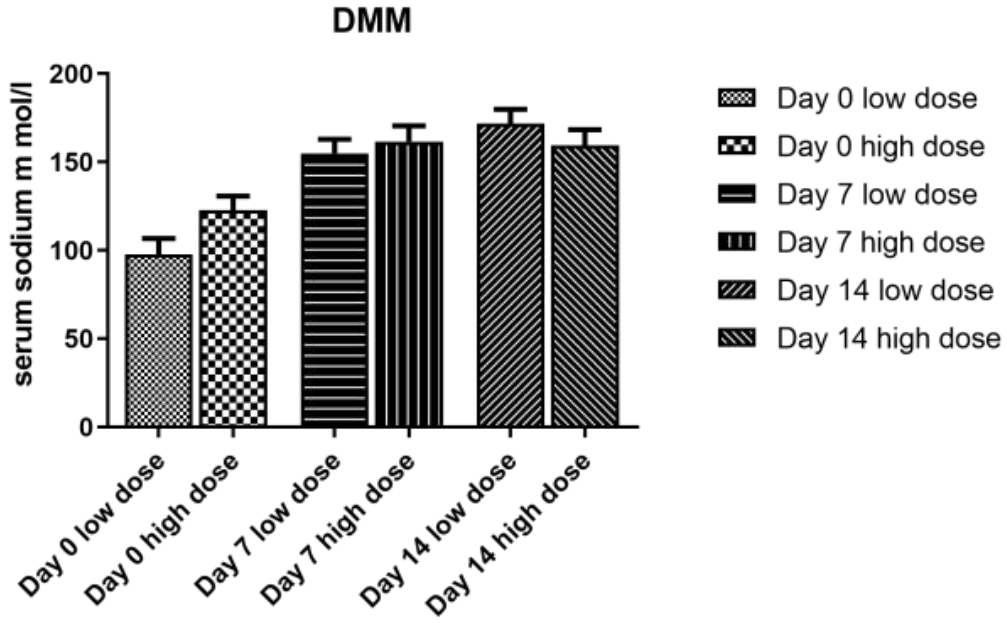


Fig 2: Serum Sodium level

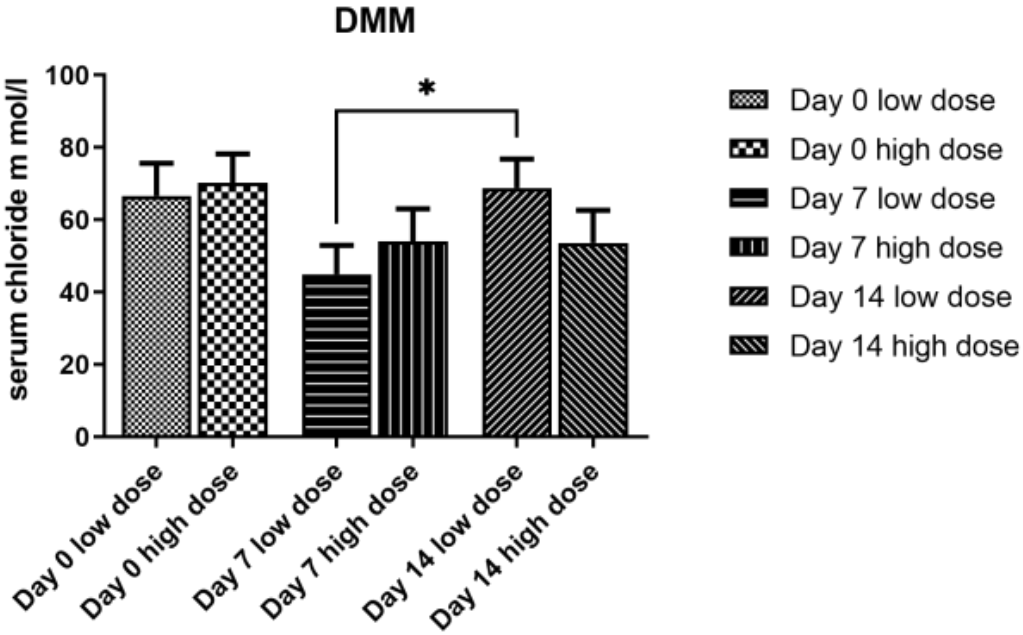


Fig 4: Serum Chloride level

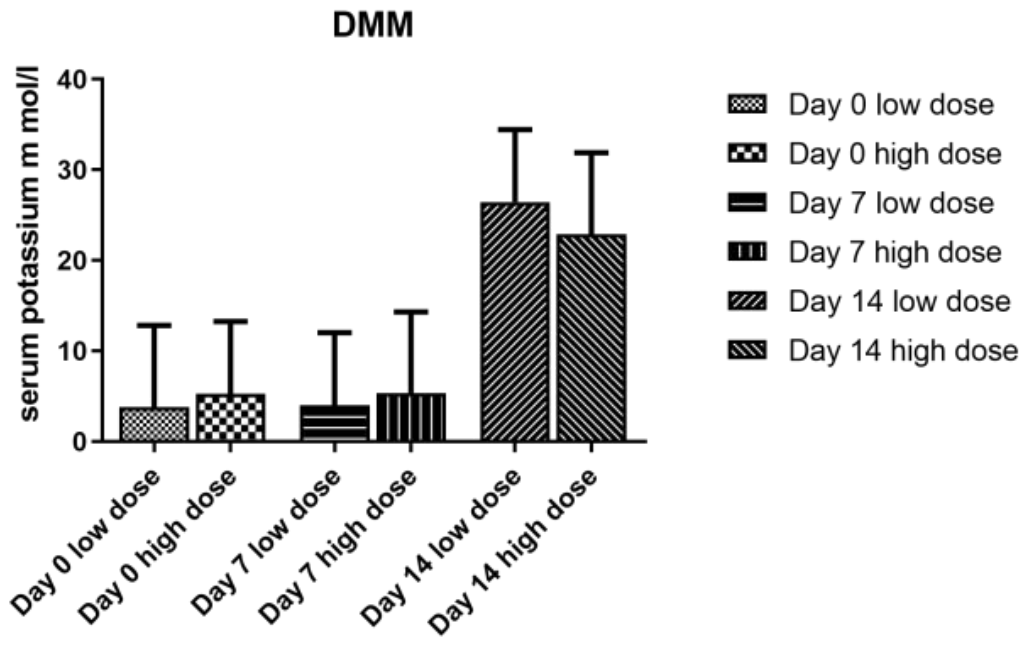


Fig 3: Serum Potassium level

The sodium level did not change appreciably from day 7 to day 14, accompanied by an increase inserum potassium level, indicating that the formulation may not have diuretic activity. This observation is also associated by the fact that there was no significant decrease in the animals' body weight from Day 7 to Day 14.

Unlike sodium, potassium is vasoactive; for example, it is seen that when potassium is infused into the arterial supply of a vascular bed, blood flow increases. Most likely the vasodilation results from hyper-polarization of the vascular smooth muscle cell.

The increase in heart rate might be attributed to our earlier observation that potassium level increases in the treated group. The relation between potassium and heart rate can be explained from the fact that potassium probably helps in filling the repolarising reserve. Creating excess repolarization reserve can cause APD shortening by hyperkalemia and initially decreases the effective refractory period (ERP), hence increased heart rate.

6. Effect of DMM on Weight of Heart in Rats with Isoproterenol Induced Hypertrophy .

Isoproterenol (ISO), a synthetic β-adrenoceptor agonist, is reported to induce myocardial injury in rat as a result of disturbance in physiological balance between production of free radicals and antioxidative defense system. The oxidative metabolism of catecholamines produce quinones which react with oxygen to produce superoxide anions (O2-.) and H2O2. The catecholamines, however, are important under stress conditions but may have damaging effects due to the generation of reactive oxygen species (ROS) and formation of oxidation products. ROS are involved as causative factors in many diseases, therefore, the generation of ROS by catecholamines may also contribute to this process. Isoproterenol (ISO) when administered to rats in two doses so as to evaluate their beta-adrenergic and toxicological actions in terms of lipid peroxidation (LPO) and the changes in the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione (GSH) content in heart, liver and kidney. ISO treatment have shown to caused LPO in tissues, however, the heart initially showed decreased LPO. This is attributed to the condition of hypertrophy by which the heart can protect itself to a limited extent against oxidative stress. The second dose of ISO, administered 24 h after the first treatment, showed toxic effects resulting in a higher increase in LPO. It is the acute condition of myocardial necrosis which caused cardiac dysfunctions, increased lipid peroxidation, altered activities of cardiac enzymes and antioxidants. The pathophysiological and morphological changes observed in ISO-treated rats have been similar to those observed in human MI and finally lead to a certain degree of hypertrophy.

To quantify the amount of hypertrophy we came upon a **Heart tail index**which was calculated by dividing the heart weight by tail length as devised by Chowdhury et al., 2013.

Fluctuations in body weight occurs with aging,thus body weight can’t be reliable reference for normalizing heart weight. Accordingly heart weight normalized by tail length, appears to be authentic. Since it remains constant even after maturity. Thus, in conditions where body weight changes, cardiac hypertrophy can be more accurately quantified by relating heart weight to tail length than to body weight.

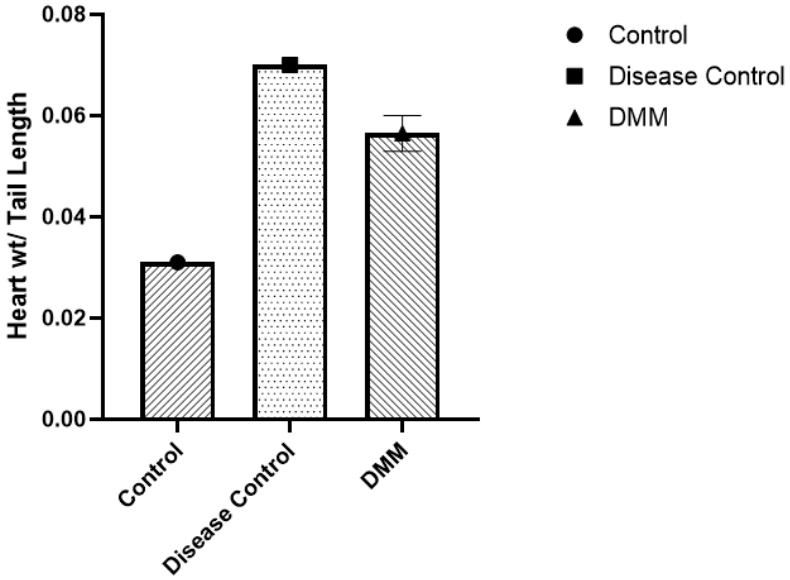


Fig 12: Comparison of Ratio of Heart weight to Tail Length between Control and Isoproterenol Induced Treated & Untreated rats.

It is evident from the graph that the ratio of Heart weight and tail length is significantly greater in disease control compared to the treated group and control. Also heart weight to tail lengthwere significantly increased in the ISO groupas compared to the control group. Treatment of DMM significantly decreasedheart weight to tail length ISO+DMM group compared to disease control (Figure 12).

CONCLUSION:

Ancient Unani Literature revels that DMM is a cardiac stimulant and helps to increase reduced blood pressure.Our observation indicates an Anti-hypertensive action at least against L-NAME-induced hypertension in experimental rats. This peculiar observation might be due to enhanced restoration of NO levels and augmented serum potassium levels which ultimately leads to increase in repolarisation reserve. Any probable hypotensive action due to diuretic effect of DMM (which is often observed with available herbal therapies) can be countered due to unaltered concentration of sodium levels, which was further co-related with no change in body weight of the experimental animals.

However extensive research needs to be conducted to relate the findings with other direct markers like Angiotensin I and II, Endothelin 1(ET-1), C-Reactive Protein (CRP), Angiotensin Converting Enzyme (ACE) which are all strongly linked with Hypertension.

It also appears to have a beneficial effect against isoproterenol induced hypertrophy. However the mechanism underlying such effect is yet to be uncovered. It can be concluded that DMM can mitigate the cardiotoxic effect of ISO in rat heart.

References:

1. Vasan RS, Evans JC, Larson MG, Wilson PW, Meigs JB, Rifai N, Benjamin EJ, Levy D. Serum aldosterone and the incidence of hypertension in nonhypertensive persons. N Engl J Med. 2004;351:33– 41. 17. Wang TJ, Evans JC, Meigs JB, Rifai N, Fox CS, D'Agostino RB, Levy D, Vasan RS. Low-grade albuminuria and the risks of hypertension and blood pressure progression. Circulation. 2005;111:1370 –1376.).

2. Brasier AR, Recinos A III, Eledrisi MS. Vascular inflammation and the renin-angiotensin system. Arterioscler Thromb Vasc Biol. 2002;22: 1257–1266. 52. Wang CH, Li SH, Weisel RD, Fedak PW, Dumont AS, Szmitko P, Li RK, Mickle DA, Verma S. C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. Circulation. 2003;107:1783–1790.)

3. Lip GY, Blann AD. Does hypertension confer a prothrombotic state? Virchow's triad revisited. Circulation. 2000;101:218 –220

4. Poli KA, Tofler GH, Larson MG, Evans JC, Sutherland PA, Lipinska I, Mittleman MA, Muller JE, D'Agostino RB, Wilson PW, Levy D. Association of blood pressure with fibrinolytic potential in the Framingham offspring population. Circulation. 2000;101:264 –269.

5. Wall U, Jern C, Bergbrant A, Jern S. Enhanced levels of tissue-type plasminogen activator in borderline hypertension. Hypertension. 1995; 26:796 – 800.

6. Lip GY, Blann AD. Does hypertension confer a prothrombotic state? Virchow's triad revisited. Circulation. 2000;101:218 –220.

7. Huvers FC, de Leeuw PW, Houben AJ, De Haan CH, Hamulyak K, Schouten H, Wolffenbuttel BH, Schaper NC. Endothelium-dependent vasodilatation, plasma markers of

endothelial function, and adrenergic vasoconstrictor responses in type 1 diabetes under near-normoglycemic conditions. Diabetes. 1999;48:1300 –1307.

8. Brown NJ, Nakamura S, Ma L, Nakamura I, Donnert E, Freeman M, Vaughan DE, Fogo AB. Aldosterone modulates plasminogen activator inhibitor-1 and glomerulosclerosis in vivo. Kidney Int. 2000;58: 1219 –1227.

9. Weiss, J. N., Qu, Z., & Shivkumar, K. (2017). Electrophysiology of Hypokalemia and Hyperkalemia. *Circulation. Arrhythmia and electrophysiology*, *10*(3), e004667.

10. Anderson TJ. Assessment and treatment of endothelial dysfunction in humans. J Am Coll Cardiol 1999; 34:631–638

11. Loscalzo J. Endothelial dysfunction in pulmonary hypertension. N Engl J Med 1992; 327:117–11

12. Felix Böhm, John Pernow, The importance of endothelin-1 for vascular dysfunction in cardiovascular disease, Cardiovascular Research, Volume 76, Issue 1, October 2007, Pages 8–18.

13. Ernesto L. Schiffrin, Role of endothelin-1 in hypertension and vascular disease, *American Journal of Hypertension*, Volume 14, Issue S3, June 2001, Pages 83S–89S.

14. Taddei, Stefano; Virdis, Agostino; Ghiadoni, Lorenzo; Sudano, Isabella; Salvetti, Antonio Endothelial Dysfunction in Hypertension, Journal of Cardiovascular Pharmacology: November 2001 - Volume 38 - Issue - p S11-S14.

15. The Renin Angiotensin Aldosterone System in Hypertension: Roles of Insulin Resistance and Oxidative Stress,Medical Clinics of North America,Volume 93, Issue 3,2009,Pages 569-582.

16. Sealey JE, Laragh JH: The renin–angiotensin–aldosterone system for normal regulation of blood pressure and sodium and potassium homeostasis, in Laragh JH, Brenner BM (eds):

Hypertension: Pathophysiology, Diagnosis, and Management, Vol. 1. Raven Press, New York, 1990, p 1287.

17. Timmermans PB, Wong PC, Chiu AT, et al: Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol Rev 1993;45:205–251.

18. Dzau VJ, Sasamura H, Hein L: Heterogeneity of angiotensin synthetic pathways and receptor subtypes: physiological and pharmacological implications. J Hypertens 1993;11(suppl):S13–18.

19. Beevers G, Lip G Y H, O'Brien E. The pathophysiology of hypertension *BMJ* 2001; 322 :912

20. Hall, J.E., Granger, J.P., do Carmo, J.M., da Silva, A.A., Dubinion, J., George, E., Hamza, S., Speed, J. and Hall, M.E. (2012). Hypertension: Physiology and Pathophysiology. In Comprehensive Physiology, R. Terjung (Ed.).

21. Goyal, S., Siddiqui, M. K., Siddiqui, K. M., Arora, S., Mittal, R., Joshi, S., & Arya, D. S. (2010). Cardioprotective effect of 'Khamira Abresham Hakim Arshad Wala' a unani formulation in isoproterenol-induced myocardial necrosis in rats. *Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie*, *62*(1), 61–74.

22. Mohanty I, Arya DS, Dinda A, Talwark KK, Joshi S, Gupta SK. Mechanisms of cardioprotective effect of Withania somnifera in experimentally induced myocardial infarction. Basic Clin Pharmacol Toxicol 2004;94:184–90.

23. Rathore, N., John, S., Kale, M., & Bhatnagar, D. (1998). Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. *Pharmacological research*, *38*(4), 297–303. https://doi.org/10.1006/phrs.1998.0365