

Multipurpose Drug from *RouwolfiaSerpentina* and *Nigella sativa* : A herbal Approach to Treat Hypertension and Hyperlipidemia in Experimental Rodent Model

Abstract :

Introduction

Hypertension refers to the condition where the pressure of blood towards the artery walls is constantly too excessive. Estimated 17.9 million lives each year is taken by this. Its key etiological factors consist of genetic predisposition, unhealthy lifestyle choices, including immoderate salt intake, obesity, lack of physical activity, and chronic situations like kidney disease or hormonal imbalances. Focusing on herbal medications for this will be safer, and affordable alternatives of unwanted risk and negative effects of synthetic medications.

Methods and materials

Six groups of mice were taken. In group 2-5 high fat diet and 3% NaCl was given to induce disease. Group 3,4 and 5 was treated by the ethanolic extract of *Nigella sativa*, *RouwolfiaSerpentina* and mixture of *Nigella sativa* and *RouwolfiaSerpentina* respectively.

Results and discussion

It has been observed that in all group hypertension condition was restored significantly ($p < 0.05$). In the treatment groups *RouwolfiaSerpentina* has displayed best efficiency. The mixture of *RouwolfiaSerpentina* and *Nigella sativa* showed 2nd most efficiency. On the otherhand, *Nigella sativa* showed comparatively less but statistically significant ($p < 0.05$) efficacy than others. *Nigella sativa* significantly ($p < 0.05$) restored disturbed lipid profile (Low density lipoprotein, High density lipoprotein and Triglyceride).

Conclusion:

As The mixture of the twoplant extracts gives both anti-hypertensive and anti-hyperlipidemic activity, it has high potential to be used in hypertension management specially when it is exacerbated by hyperlipidemia and this demands for further study.

Keywords: Hypertension; *Nigella sativa*; *Rauwolfia serpentina*; rat; anti-hypertensive activity; lipid profile, hyperlipidemia,

1. Introduction

Cardiovascular diseases (CVDs) are one of the leading causes of death globally which include coronary heart disease, cerebrovascular disease and rheumatic heart disease. It takes estimated 17.9 million lives each year. One of the main cause behind it is hypertension, also referred as 'silent killer'. According to WHO (World Health Organization) 1.3 billion people faces hypertension from age range 30-79 years old[1][2]. There are many aetiologies involved behind it like renin-dependency, arteriosclerosis, sympathetic-activity, salt-sensitivity, hyperhomocysteinemia, hyperlipidemia etc[3]. Among all of them hyperlipidaemia is one of the extremely common reason especially in the Western hemisphere. Due to this arteries become narrowed with cholesterol plaque and calcium, the heart has to strain much harder to pump blood through them which creates hypertension. There are lot of synthetic drugs available in the market for both conditions. Though those wonderfully help by decreasing blood pressure and LDL values, yet they have some adverse effects besides being expensive. For example some frequently used anti-hypertensive drugs are Diuretics (e.g. Thiazide), ACE inhibitors (eg. Captopril) and Calcium channel blockers (eg: Nicardipine). Calcium channel blockers which works by relaxing blood vessels can cause swelling of legs, heartburn and nausea.[4]. Also ACE inhibitors which works via decreasing angiotensin production might cause hypotension[5,6] and diuretics which increases urination might cause electrolyte imbalance, dehydration etc [7]. In case of Hyperlipidemia the first line drugs are the Statins (eg. Lovastatin) which is highly effective yet has adverse effects like damaging muscle and liver[8]. Also, it causes hyperglycemia[9], neurological, gastrointestinal, skin problems[10]. Niacin (eg. Niaspan) is another most commonly used anti-hyperlipidemic drug which shows adverse effects like flushing[11]. However natural drugs may show lesser adverse effects than these synthetic drugs. Medicinal plants are huge source of remedies. As a plant consists of different chemical substances, it is possible to acquire different pharmacological activities from a single plant extract.

Whereas from synthetic medicines we can get specific treatment from specific APIs. Moreover, it is noticeable that, for getting specific treatment from plants we can increase desired action or decrease undesired action via genetic modification.[12].

Basil, parsley, celery seeds, garlic, thyme, cinnamon, ginger, cardamom etc. many kinds of medicinal plants are used for treating hypertension. Among many of these hypertension-reducing medicinal plants one of the most commonly used is *Rauwolfia Serpentina* or Shorogondha[13]. It is also known as Indian snakeroot, found in Indian subcontinent and East Asia. Though Shorogondha has numerous health benefits but it is proclaimed as a best remedy for hypertension. The root of the plant is used in high blood pressure, mental agitation, insomnia and sedative. Its root extract is considered to be the best medicine for high blood pressure and has been adapted by the medical fraternity in most countries. *Rauwolfia serpentina* possesses the alkaloid reserpine that is major agent to treat the blood pressure and other neurological diseases. Reserpine produces its antihypertensive effect by binding to the catecholamine in the nerve cells and depletes the catecholamine. Angiotensin-converting enzyme (ACE) is inhibited

by Rescinnamine to halt the conversion of angiotensin I, resulting in a decrease of plasma angiotensin II then lowering blood pressure by relaxing the blood vessels and regulating nerve function in the muscles leading to the heart, Sarpagandha brings down high blood pressure and facilitates the circulation of blood[14]. Moreover R. Serpentina is the potential inhibitor of HMGCR which exerts hyperlipidemic effect due to the presence of high concentration of phenolic compounds. Also it has analgesic and antipyretic properties.[15]. On the other hand, *Nigella sativa* or Black cumin (also known as Kalonji, Roman coriander) is considered as one of the medicine for hyperlipidemia. It is widely available in the Southwestern Asia, Parts of the Mediterranean and Africa. FDA classified it as Generally Recognized as Safe (GRAS) for use as a spice, natural seasoning, flavouring agent. *Nigella sativa* mainly lowers systolic and diastolic blood pressure at the same time helps to reduce triglycerides, Low density lipoprotein and total cholesterol levels. *N. sativa* oil reduces blood pressure via calcium channel blockage, diuretic activity, increased cardiac heme oxygenase-1 activity, prevention of loss of plasma nitric oxide, antioxidant activity, reduction of angiotensin-converting enzyme and cardiac depressant activity[16]. Additionally it is used as antidiabetic, anticancer, anti-oxidant, hepatoprotective, neuro-protective, analgesic, anti-microbial, gastroprotective and immunomodulator agent.[17].

Our main objective of this study is to understand the effect of these two plant extracts individually and combinedly in case of hypertension and hyperlipidemia. If it only reduces cholesterol and thus lowers blood pressure so it will prove that it only has antihyperlipidemic activity otherwise it will prove it reduces hypertension. In the study we have used the combination of sodium chloride (NaCl) and high fat diet in Swiss albino rat where sodium chloride increases blood pressure and high fat diet increases cholesterol level. To reduce blood pressure from every aspect both of these activities are needed. These results can further lead us to work with more specific isolated compounds in future in vigorous ways.

2. Materials and methods

2.1. Plant Collection and extract preparation

Rouwolfia serpentina was obtained from medicinal plant garden of University of Dhaka and *Nigella sativa* seeds were bought from Shantinagar Bazar, Dhaka. These were authenticated by Dr Md. Shah Amran, chairperson of the Department of pharmaceutical chemistry, University of Dhaka. Shade dried them at 40°C in hot air oven and turned into fine powder.

Took **800** gram of *Nigella sativa* in **2000** ml of ethanol and **800** gm of *Rouwolfia Serpentina* roots in **2000** ml of ethanol in separate conical flasks and soaked them in 70% ethanol for 7 days. Following this collected the filtrate with Wattman filter paper in separate beaker and took them for rotary evaporation and collected the dried extract. Repeated the evaporation process

three times with same solvent. Finally collected the dry extract powder and kept them in separate air tight container for further use[18,19].

2.2 Drugs and Chemicals

Sodium chloride (NaCl) and ethanol were bought from the sigma company, USA. Captopril, a common hypertension medication, was received as a gift sample from Incepta Pharmaceutical Ltd. Ketamine, and Lipid profile test kit.

2.3 Creation of Hypertension Rat model and giving them anti-hypertensive treatment

Total 36 male Swiss Albino rats weighing between (125-200)gm was purchased from Jahangirnagar University, Savar, Dhaka, Bangladesh. They were divided into 6 groups and each group contained 6 rats. In every group there were 2 rats weighing between (126-150) gm, 2 rats weighing between (151- 175) gm, and 2 rats weighing between (176-200) gm. They were all housed in a carefully monitored setting (temperature $25 \pm 3^{\circ}\text{C}$, relative humidity $55 \pm 5\%$, and 12h light/dark cycle) at the Institute of Nutrition & Food Science at the University of Dhaka and treated according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals[20].

All of them except group 1 had gone through high fat diet to induce lipid profile so that hypertension can occur. High fat diet consists of 40% lipid (Milk powder 10%, ghee 30%, mutton fat 40%, coconut oil 10%, and butter 10%), 25% protein (dry powdered prone 40%, dry boiled mutton 20%, cheese 20%, and egg 20%), and 35% carbohydrate (boiled corn 2% and fructose 98%)[18,21].

Table 1: treatment plan across the different groups of rats

Group No.	Group specifications	Stress Chemical	Treatment Species	Dose (mg/kg)
1	Negative control	N/A	N/A	N/A
2	Positive control	3%NaCl+High fat diet	N/A	5
3	Standard drug	3%NaCl+High fat diet	Captopril	N/A
4	Test group	3%NaCl+High fat diet	<i>Nigella sativa</i> extract	1000mg/kg
5	Test group	3%NaCl+High fat diet	<i>RouwolfiaSerpentina</i> extract	1000mg/kg
6	Test group	3%NaCl+High fat diet	<i>Nigella sativa</i> extract+	500 mg/kg+500

			<i>RouwolfiaSerpentina</i> extract	mg/kg
--	--	--	------------------------------------	-------

2.4 Evaluation of Anti-Hypertensive Activity

Thirty rats were randomly picked and equally divided into six groups. 3% sodium chloride plus high fat diet was given to the rats regularly till 42 days. The blood pressure of the rats was evaluated by the tail cuff method [22].

After bringing the rats into the lab from the animal house, they were left in the lab for up to 5 minutes so that they can adjust with the ambient of the lab. Then Ketamine was injected through the inter peritoneal route as an anaesthetic agent according to the dose of 0.75 mg/kg body weight [23]. While waiting until the rats are completely anaesthetized, the incubation chamber of the machine is turned on to reach 37°C. Then each rat was placed into the hollow tube of the tail cuff machine and then the tube was closed off with the rear hatch and the tail was placed outside of the tube through the shutters of the tube. Inside the holder the nose cone was adjusted in such a way that it limits the movement of the animal. Then the holder was placed on the designated position of the warming platform. After that the tail was thread through the occlusion cuffs where the cuff was placed as close to the base of the tail as possible without force. Then the VPR sensor cuff was put in place for acquiring the reading. Then after turning on the machine, the values of systolic pressure, diastolic pressure and the mean blood pressure of the specimen were attained.

2.5 Evaluation of Anti-hyperlipidemic activity:

For evaluating the lipid profile of the rats, after getting the blood pressure readings and waiting for the rats to become stable, they were sacrificed and the blood was collected. The collected blood was centrifuged at 5000 rpm and the serum was collected from it. Finally, the serum was used for detecting the Lipid profile via the Humalyzer 3000 [24].

2.6 Statistical Analysis

All of our results (raw data) were accumulated and analysed on a large sheet using the MS Excel program and were divided into unique classes according to diverse studies criteria. Descriptive data were implemented to the data, and the findings were presented as mean \pm SD. To calculate the statistical importance of the inter-group heterogeneity on the idea of numerous biological characteristics, we used the "One Way ANOVA Test" function of the SPSS 1600 software. Though the "p" value that turned into identified as less than 0.05 ($P < 0.05$), we consider the occurrences to be statistically significant.

3. Results

Table 2. Measurement of lipid profile

Group	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)
Negative control	93.16±7.19	73.34±5.42	39.82±4.51	63.21±6.59
Disease control	182.45±13.62	51.40±4.30	82.46±11.60	104.42±12.20
<i>Nigella sativa</i>	119.35±11.21	66.68±6.40	54.41±8.20	74.48±10.41
<i>Rauwolfia serpentina</i>	180.33±11.56	57.34±7.51	78.21±8.41	100.40±9.32
<i>Nigella sativa</i> + <i>Rauwolfia serpentina</i>	131.71±9.53	61.83±6.10	68.30±5.49	87.40±8.31

Table 3. Measurement of blood pressure of control

Group	Mean blood pressure (mmHg)	Systolic (mmHg)	Diastolic (mmHg)
Negative control	81.40±7.95	105.49±9.32	69.35±7.26
Disease control	117.63±9.87	141.56±12.14	105.67±8.73
<i>Nigella sativa</i>	97.04±9.67	117.63±9.18	86.74±9.50
<i>Rauwolfia serpentina</i>	93.55±8.91	113.42±10.19	83.62±8.28
<i>Nigella sativa</i> + <i>Rauwolfia serpentina</i>	93.10±9.14	114.46±9.53	83.41±8.94

4. Discussion

We divided the rats in six groups including negative control group and five disease induced groups which includes the Positive control, Standard drug group (treated with Captopril), group 3 (treated with *Nigella sativa*), group 4 (treated with *Rauwolfia serpentina*) and group 5 (treated with *Nigella sativa* both *Rauwolfia serpentina*).

As we see from table 2, when mean blood pressure, systolic blood pressure and diastolic blood pressure were measured in group 3, 4 and 5 significant decreases ($p < 0.05$) had been observed compared to the disease control group. The result imparts that both *Nigella sativa* and *Rauwolfia serpentina* have anti-hypertensive activity as they both can control elevated systolic and diastolic

blood pressure. Another notable matter is that *Rauwolfia serpentina* has the highest anti-hypertensive activity among these groups. The second highest activity is imparted by the combination of *Nigella sativa* and *Rauwolfia serpentina*. Even though *Nigella sativa* demonstrated significant activity, it showed the least degree of activity among group 3, 4, and 5. This comparison denotes that *Rauwolfia serpentina* has more potency than *Nigella sativa* or the combination of *Nigella sativa* and *Rauwolfia serpentina*.

We used Humalyzer 3000 analysis to analyze the lipid profile by assessing the serum levels of triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol level which is demonstrated in table 1. In group 3 and 5, serum total cholesterol, low density lipoprotein and triglyceride level decreased significantly where $p < 0.05$, when compared with disease control group. Whereas group 4, *Rauwolfia serpentina* shows very little activity in controlling serum total cholesterol, low density lipoprotein and triglyceride level when compared with disease control group. It is also observed that *Nigella sativa* imparts the highest level of activity among the groups. Though combination of *Nigella sativa* and *Rauwolfia serpentina* demonstrated significant activity, still showed comparatively less activity than *Nigella sativa*. This comparison suggests that *Nigella sativa* can impart anti-hypertensive activity by restoring the normal physiological condition by decreasing serum low density lipoprotein, triglyceride and total cholesterol level. This indicates *Nigella sativa* has significant anti-lipemic activity whereas *Rauwolfia serpentina* does not have notable anti-lipemic activity.

We can conclude that both drugs showed significant anti-hypertensive activity. *Nigella sativa* imparts anti-hypertensive activity by controlling abnormal lipid profile. Thus, in terms of lipid profile measurement, *Nigella sativa* is more potent. On the other hand, *Rauwolfia serpentina* being more potent by demonstrating the highest level of anti-hypertensive activity.

5. Conclusion:

From the study it could be concluded that all of the three preparation has significant high blood pressure lowering ability. However, *Rauwolfia serpentina* has higher potential to reduce blood pressure among the three extracts. On the contrary, in case of the lipid profile, *Nigella sativa* extract had significant activity in restoring this pathological state while the effect of *Rauwolfia serpentina* was not significant. On the other hand, the mixture of the two extracts showed significant results in normalizing the lipid profile. From this we can point out that the mixture of the *Rauwolfia serpentina* and *Nigella sativa* extract can simultaneously show anti-hypertensive

activity and anti-hyperlipidemic activity. As a result, it might be a good candidate in managing hypertension of a patient who also has an abnormal lipid profile as their hypertensive condition is escalated by the hyperlipidemic condition. According to this preliminary research, further experimental studies are warranted to isolate and identify the phytochemical constituents of these plants and using the extract mixture to develop an experimental treatment for hypertension exacerbated by high lipid levels, which holds promise for success and might open a new doorway for hypertension management.

Ethical Approval

All ethical standards mentioned within the Declaration of Helsinki 2013, were observed at the same time as accomplishing the experiments. The Swiss Academy of Sciences and the Swiss Academy of Medical Sciences' guiding concepts were followed when handling and being concerned for animals. The Guidelines for the Euthanasia of Animals: 2013 version were followed for euthanizing animals.

References

1. World Health Organization. (2023, March 16). *Hypertension*. World Health Organization.
2. Ames, R. P. (1991). Hyperlipidemia in hypertension: causes and prevention. *American Heart Journal*, 122(4 Pt 2), 1219–1224.
3. Maideen, N. M. P., Balasubramanian, R., & Ramanathan, S. (2021). *Nigella sativa* (Black Seeds), a potential herb for the pharmacotherapeutic management of hypertension - A Review. *Current Cardiology Reviews*, 17(4).
4. Calcium channel blocker :Russell, R.P., 1988. Side effects of calcium channel blockers. *Hypertension*, 11(3_pt_2), p.II42.
5. Na Takuathung, M., Sakuludomkan, W., Khatsri, R., Dukaew, N., Kraivisitkul, N., Ahmadmusa, B., Mahakkanukrauh, C., Wangthaweasap, K., Onin, J., Srichai, S. and Buawangpong, N., 2022. Adverse Effects of Angiotensin-Converting Enzyme Inhibitors in Humans: A Systematic Review and Meta-Analysis of 378 Randomized Controlled Trials. *International journal of environmental research and public health*, 19(14), p.8373.
6. Agustí, A., Bonet, S., Arnau, J.M., Vidal, X. and Laporte, J.R., 2003. Adverse effects of ACE inhibitors in patients with chronic heart failure and/or ventricular dysfunction: meta-analysis of randomised clinical trials. *Drug safety*, 26, pp.895-908.
7. Sica, D.A., 2004. Diuretic- related side effects: development and treatment. *The Journal of Clinical Hypertension*, 6(9), pp.532-540

8. Chaipichit, N., Krska, J., Pratipanawat, T. and Jarernsiripornkul, N., 2015. Statin adverse effects: patients' experiences and laboratory monitoring of muscle and liver injuries. *International Journal of Clinical Pharmacy*, 37, pp.355-364.
9. Seshadri, S., Rapaka, N., Prajapati, B., Mandaliya, D., Patel, S., Muggalla, C.S., Kapadia, B., Babu, P.P., Misra, P. and Saxena, U., 2019. Statins exacerbate glucose intolerance and hyperglycemia in a high sucrose fed rodent model. *Scientific reports*, 9(1), p.8825.
10. Thompson, P.D., Panza, G., Zaleski, A. and Taylor, B., 2016. Statin-associated side effects. *Journal of the American College of Cardiology*, 67(20), pp.2395-2410.
11. Kamanna, V.S., Ganji, S.H. and Kashyap, M.L., 2009. The mechanism and mitigation of niacin- induced flushing. *International journal of clinical practice*, 63(9), pp.1369-1377.
12. Gómez-Galera, S., Pelacho, A.M., Gené, A., Capell, T. and Christou, P., 2007. The genetic manipulation of medicinal and aromatic plants. *Plant cell reports*, 26, pp.1689-1715.
13. Shah, S. M., Naqvi, S. A., Munir, N., Zafar, S., Akram, M., & Nisar, J. (2020). Antihypertensive and antihyperlipidemic activity of aqueous methanolic extract of *Rauwolfia serpentina* in Albino Rats. *Dose-Response*, 18(3), 155932582094207.
14. Lobay, D., 2015. *Rauwolfia* in the treatment of hypertension. *Integrative Medicine: A Clinician's Journal*, 14(3), p.40.
15. Jaarin, K., Foong, W. D., Yeoh, M. H., Kamarul, Z. Y., Qodriyah, H. M., Azman, A., Zuhair, J. S., Juliana, A. H., & Kamisah, Y. (2015). Mechanisms of the antihypertensive effects of *Nigella sativa* oil in L-name-induced hypertensive rats. *Clinics*, 70(11), 751–757.
16. Deeg, R., & Ziegenhorn, J. (1983). Kinetic enzymic method for automated determination of total cholesterol in serum. *Clinical Chemistry*, 29(10), 1798–1802.
17. BURSTEIN, M., SCHOLNICK, H. R., & MORFIN, R. (1970). Rapid method for the isolation of lipoproteins from human serum by precipitation with Polyanions. *Journal of Lipid Research*, 11(6), 583–595.
18. Jannath, S., Jannat, B., Ishraat, S. T., Sadat, M., Nirzana, S. A., Tahsin, M. R., Aktar, F., Chowdhury, J. A., Kabir, S., Chowdhury, A. A., & Amran, M. S. (2023). An assessment of antihypertensive & anti-hyperlipidemic activity of *Allium sativum* and *Rauwolfia serpentina* in hypertension and high fat induced rat model. *Asian Journal of Advanced Research and Reports*, 17(11), 185-192.
19. Zhang, Q.-W., Lin, L.-G., & Ye, W.-C. (2018). Techniques for Extraction and Isolation of Natural products: a Comprehensive Review. *Chinese Medicine*, 13(1).

20. Institute of Laboratory Animal Resources (US). Committee on Care and Use of Laboratory Animals, 1986. *Guide for the care and use of laboratory animals* (No. 86). US Department of Health and Human Services, Public Health Service, National Institutes of Health.
21. Alessandro Cosenzi, Bernobich, E., Bonavita, M., Bertola, G., Trevisan, R., & Bellini, G. (2002). Antihypertensive Treatment With Enrasentan (SB217242) in an Animal Model of Hypertension and Hyperinsulinemia. *Journal of Cardiovascular Pharmacology*, 39(4), 488–495.
22. Wilde, E., Aubdool, A.A., Thakore, P., Baldissera Jr, L., Alawi, K.M., Keeble, J., Nandi, M. and Brain, S.D., 2017. Tail- cuff technique and its influence on central blood pressure in the mouse. *Journal of the American Heart Association*, 6(6), p.e005204.
23. Van Pelt, L., 1977. Ketamine and xylazine for surgical anesthesia in rats. *Journal of the American Veterinary Medical Association*, 171(9), pp.842-844.
24. Qayyum, R. and Asif, H., 2017. Effect of crude extract of *Grewia asiatica* L. on lipid profile and coagulation parameters of rats and mice.