Original Research Article

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 thiswill be more 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 groups 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 other hand Nigella Sativa showed comparatively lessbut statistically significant ($p<0.05$) efficacy than others.Nigella Sativa significantly ($p<0.05$) restoreddisturbed lipid profile (LDL, HDL and triglyceride) .

Conclusion:

As The mixture of the two plant extract gives both anti-hypertensive and anti hyperlipidemicactivity, 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, hyperlipidaemia,

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1.Introduction

Cardiovascular diseases (CVDs) are one of the leading causes of death globallywhich 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 faceshypertension from age range 30-79 years old **[1][2].**There are many aetiologies involvedbehindit likerenin-dependency,arteriosclerosis,sympathetic-activity, salt sensitivity,hyperhomocysteinemia, hyperlipidemiaetc. **[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 themwhich creates hypertension.There arelot of synthetic drugs available in the marketfor both conditions. Though those wonderfullyhelp by decreasing blood pressure and LDLvalues, yettheyhave some adverse effectsbesides being expensive. For example somefrequentlyused anti- hypertensive drugs are Diuretics (e.g. Thiazide),ACEinhibitors(eg.Captopril) and Calcium channel blockers (e.g: Nicardipidine). Calcium channel blockerswhich works by relaxing blood vesselscan cause swelling of legs , heartburn and nausea.[4]Also ACE inhibitorswhich works via decreasing angiotensin production might cause hypotension[5,6]anddiuretics which increases urination might cause electrolyte imbalance, dehydration etc [7]. In case of Hyperlipidaemiathe first line drugsare the Statins (e.g.Lovastatin) which is highly effective yet has adverse effects like damaging muscle and live[8]. Also it causeshyperglycaemia[9], neurological, gastrointestinal,skin problems [10]. Niacin(e.g. Nispasin) is another most commonly used anti-hyperlipidaemic drug which shows adverse effects like flushing [11]. However natural drugsmay showlesser 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.

Whereasfrom 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 fortreating hypertensions . Among many of these hypertension reducing medicinal plants one of the most commonly usedisRouwolfiaSerpentina or Shorpogondha**[13]**. It is also known as Indian snakeroot, found in Indian subcontinent and East Asia.Though Sorpogondha 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 mostcountries.Rauwolfia serpentina possesses the

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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 hyperlipidemiceffectdue 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 cummin(also known as Kalonji, Roman coriander) is considered as one of the medicine for hyperlipidemia.Itis 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, LDL 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, hepato-protective,neuro-protective,analgesic,anti-microbial,gastroprotectiveandimmunomodulator agent.**[17].**

Our main objective of thisstudy isto understand the effect of these two plant extracts individually and combinedly in case of hypertension and hyperlipidaemia.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 aspects 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

Rouwolfiaserpentinawas obtained from medicinal plant garden of University of Dhakaand Nigella sativa seeds were bought from Shantinagar Bazar, Dhaka. These were authenticated by Dr. Md. Shah Amran, chairpersonof theDepartment of pharmaceutical chemistry, University of Dhaka.Shade dried them at 40°C in hot air oven andturned into fine powder.

Took **800** gram of Nigella sativain**2000** ml of ethanol and **800** gm of RouwolfiaSerpentinaroots in **2000** ml of ethanol in separate conical flasks and soaked them in 70% ethanol for 7

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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].

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2.2 Drugs and Chemicals

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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.

2.3Creation of Hypertension Ratmodeland giving them anti -hypertensive treatment

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Total 36 male Swiss Albino rats weighing between (125-200)gm were 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 ± 3ºC, relative humidity 55 ± 5%, and 12h light/dark cycle) at the Institute of Nutrition & Food Science at the University of Dhakaand treated accordingto the National Institute of Health Guidelines forthe Care and Use of Laboratory Animals.

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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] [20].

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Table 1: treatment plan across the different groups of rats

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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	Nigella Sativa extract	1000mg/kg
5	Test group	3%NaCl+High fat diet	RouwolfiaSerpentina extract	1000mg/kg

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6	Test group	3%NaCl+High fat diet	Nigella Sativaextract+ RouwolfiaSerpentina extract	500mg/kg+500 mg/kg

2.4 Evaluation of Anti-Hypertensive Activity

30 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 were evaluated by the tail cuff method.After bringing the rats into the lab from the animal house, they were left in the lab for up to 5 minutes so that theycan adjust with the ambient of the lab. Then ketamine was injected through the interperitoneal route as an aesthetic agent according to the dose of 0.75mg/kg body weight.While waiting until the rats are completely anesthetized, the incubation chamber of the machine is turned on to reach 37℃. Then each rat was placed into the hollow tube of the tail cuff machine and then the tubewas 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 nosecone 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 pressureof the specimen were attained.

2.5 Evaluation of Anti-hyperlipidemicactivity:

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 rpmand the serum was collected from it. Finally the serum was used for detecting the Lipid profile viathe Humalyzer 3000.

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 ± 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.

2.7 Experimental Guideline

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All ethical standards mentioned withinside 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.

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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
Nigella sativa	119.35±11.21	66.68±6.40	54.41±8.20	74.48±10.41
Rauwolfia serpentina	180.33±11.56	57.34±7.51	78.21±8.41	100.40±9.32
Nigella sativa+ *Rauwolfiaserpentina*	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
Nigella sativa	97.04±9.67	117.63±9.18	86.74±9.50
Rauwolfia serpentina	93.55±8.91	113.42±10.19	83.62±8.28
Nigella sativa+ *Rauwolfiaserpentina*	93.10±9.14	114.46±9.53	83.41±8.94

4. DISSCUSSION

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We divided the rats in five groups including negative control group and four disease induced groups that are disease control, group 3 (treated with *Nigella sativa*), group 4 (treated with *Rauwolfia serpentina*)and group 5 (treated with *Nigella sativa* both *Rauwolfia serpentina*).

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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.

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 Rauwolfiaserpentina 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 -hyperlipidemicactivity. 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

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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.

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