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PRECLINICAL LIPID PROFILE STUDIES OF AN AYURVEDIC PREPARATION NARADIYA LAKSMIVILASA RASA AFTER CHRONIC ADMINISTRATION TO MALE SPRAGUE-DAWLEY RATS

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ABSTRACT

Naradiya Laksmivilasa Rasa (NMB) is a classical Ayurvedic formulation markedly used in the treatment of sinusitis, chronic skin diseases, diabetes, fistula, obesity, rheumatoid arthritis, ascites, headache, gynecological disorders and urinary tract disorders. The present study is conducted to evaluate the effect of conventionally prepared NMB on different lipid profile parameters in experimental animals, for providing scientific data base for its logical use in clinical practice. Acute toxicity tests were conducted to determine the LD_{50} of the drug. To find out the effect of chronic administration of NMB on serum lipid profile it was administered chronically to the male Sprague-Dawley rats at a dose of 400 mg/kg for 43 days. A significant decrease in HDL cholesterol level (p=0.02, 25.98% decrease) was observed. The drug (NMB) did not affect triglyceride (TG), total cholesterol (TC), LDL, VLDL, and Non-HDL level significantly; thus leading to an insignificant change in atherogenic indices like Cardiac Risk Ratio (TC/HDL), Atherogenic Coefficient [(TC-HDL)/HDL)], Castelli's Risk Index-II (CRI-II) (HDL/LDL) and Atherogenic Index of Plasma (AIP) (log (TG/HDL)). This experimental data will help the clinician for the logical use of NMB in different disease conditions.

Keywords: Ayurvedic preparation, lipid profile, Cardiac Risk Ratio, Atherogenic Index of Plasma, Atherogenic Coefficient

INTRODUCTION

Ayurveda is one of the traditional medicinal systems originated in India more than 2000 years ago and relies heavily on herbal medicine products [1]. It is known as the "Mother of All Healing" [2]. Ayurveda is commonly referred as 'science of life' because the Sanskrit meaning of Ayu is life and *Veda* is science or knowledge. It focuses on bringing harmony and balance in all areas of life including mind, body and spirit [3]. Ayurvedic medicines are somewhat inexpensive and have wide acceptability among the general populace, particularly in rural areas. They have a good safety profile also [4]. Currently, the World Health Organization (WHO) has officially recognized and recommended large-scale use of

herbal (Unani and Ayurvedic) medicines, particularly in the developing countries, as an alternative system of medicine to provide health care services at the primary health care level [5]. The World Health Organization (WHO) estimates that 80% of the word's inhabitants still rely mainly on traditional medicines for their health care [6].

The Indian subcontinent is well-known to be one of the mega biodiversity centers with about 45,000 plant species [7]. This abundance of flora has contributed to its status as a reservoir of herbals throughout the history of mankind. Ayurveda has about 700 type of plants listed in its medicinal systems [8]. The use of such herbals is mentioned in the ancient Ayurvedic literature such as Chakara Samhita and Sushruta Samhita. In Ayurvedic system of medicine, the raw materials like plant, mineral, and metal resources are acquired from the natural surroundings. They have been used extensively for many centuries after thorough evaluation of the drug by traditional way. Ayurvedic medicines are divided into 2 major types: herbal-only and rasa shastra. Rasa shastra is an ancient practice of deliberately combining herbs with metals (eg, mercury, lead, iron, zinc), minerals (eg, mica), and gems (eg, pearl) [9, 10]. Rasa shastra experts claim that these medicines, if properly prepared and administered, are safe and therapeutic [9, 10]. But several cases of metal toxicity have been associated with the presence of lead, mercury, and arsenic in Ayurvedic traditional medicine. These include reports of lead poisoning in England, New Zealand, United States, and in India [11-15]. Experts in Ayurveda estimate that greater than 20% of the Ayurvedic medications contain at least one heavy metal [16-18].

Ayurveda as an ancient science of life has a long history, and its basic principles may be valid even today. But classical Ayurveda of the past cannot be blindly practiced without contemporary modifications. The use of herbal preparations with inadequate scientific studies on their safety profile has raised concerns on their toxicity. A well-designed rigorous scientific research on medicines and therapeutic practices of Ayurveda is necessary. Evidence-based Ayurveda needs appropriate blends of modern science, rigorous trial methods and observational studies. That is why; we designed our current experiment to observe the effect on lipid profile following chronic administration of NMB to Sprague-Dawley rats at a high dose. The objective is to have a better understanding of the potential toxicological profile of the drug. The study provides directions for further research as well. Nardiya Laxmivilas Rasa is a herbo-mineral ayurvedic product manufactured by Sri Kundeswari Aushadhalaya Limited. It is used in the treatment of sinuses, chronic skin diseases, diabetes, fistula, obesity, rheumatoid arthritis, ascites, headache, gynaecological disorders and urinary tract disorders [19-23]. It mainly contains *Cinnamomum camphora*, *Myristica fragrans*, *Argyreia speciosa*, *Dathura alba*, *Cannabis sativa*, *Pueraria tuberosa*, *Asparagus recemosa*, *Grewia populofolia*, *Abutilon indicum*, *Tribulus terrestris* and *Barringtonia acutangula*. Apart from these plant ingredients, it also contains Krsnabhra curna (calcined Mica), Parada (purified Mercury) and Gandhaka (purified Sulfur).

MATERIALS AND METHODS

Drugs, Chemicals and Reagents: For the toxicological study, NMB was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was purchased from ACI Pharmaceuticals Limited, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Human GmbH, Wiesbaden, Germany.

Experimental Animals: Eight-week old male Sprague-Dawley rats bred and maintained at the animal house of the Department of Pharmacy, Jahangirnagar University, were used in the toxicological experiment. These animals were apparently healthy and weighed 70-80 g. The animals were housed in a well-ventilated clean experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. They were fed with rat chow prepared according to the formula developed at Bangladesh Council of Scientific and Industrial Research (BCSIR). Water was provided ad libitum and the animals maintained at 12 hours day and 12 hours night cycle. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals approved by Ethical Review Committee, Faculty of Life Sciences, Department of Pharmacy, Jahangirnagar University.

Experimental Design

Acute toxicity study: The acute oral toxicity test was performed following the guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals with minor modification (OECD Guideline 425) [24]. Sixteen male mice (30-35 g body weight) were divided into four groups of four animals each. Different doses (1000 mg/Kg, 2000 mg/Kg, 3000 mg/Kg and 4000

mg/Kg) of experimental drug (NMB) were administered by stomach tube. The dose was divided into two fractions and given within 12 hours. Then all the experimental animals were observed for mortality and clinical toxicity signs (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 1, 2, 3 and 4 hours and thereafter once a day for the next three days following NMB administration.

Chronic toxicity studies: Prior to the experiment, rats were randomly divided into 2 groups of 10 animals each. One group was treated with NMB and another was used as a control. The control animals were administered with distilled water only as per the same volume as the drug treated group for 43 days. After acclimatization, Ayurvedic medicinal preparation was administered to the rats by intra-gastric syringe between the 10 am to 12 am daily throughout the study period. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. The experiment animals were marked carefully on the ear which helped to identify a particular animal. By using identification mark, responses were noted separately for a particular period prior to and after the administration.

Blood Samples Collection and Preparation of Serum: At the end of 43 days treatment, after 18 hours fasting, blood samples were collected from post vena cava of the rats anaesthetizing with Ketamine (500 mg/Kg body, intra peritoneal) and transferred into plain sample tubes immediately for serum generation. Blood was then centrifuged at 4,000 g for 10 minutes using bench top centrifuge (MSE Minor, England). The supernatant plasma samples were collected using dry Pasteur pipette and stored in the refrigerator for further analyses. All analyses were completed within 12 hours of sample collection.

Determination of Lipid Profile Parameters: Lipid profile studies involved analysis of parameters such as triglyceride (TG) level determined by GPO-PAP method [25]; total cholesterol (TC) level determined by CHOD-PAP method [26]; LDL-cholesterol level determined by CHOD-PAP method [27]; HDLcholesterol level determined by CHOD-PAP method [28]. The absorbance of all the tests was determined using Humalyzer, Model No-3500 (Human GmbH, Wiesbaden, Germany). Serum VLDL and LDL cholesterol concentrations were calculated using the Friedewald equation [29] as follows:

i. LDL cholesterol (mg/dl) = Total Cholesterol − (HDL cholesterol −Triglyceride / 5)
ii. VLDL cholesterol (mg/dl) = Triglyceride / 5.

While the serum non-HDL cholesterol concentration was determined as reported by Brunzell [30]:
Non-HDL cholesterol = Total cholesterol – HDL cholesterol.

The atherogenic indices were calculated as follows:
Cardiac Risk Ratio (CRR) =TC/HDL [31].
Castelli's Risk Index (CRI-II) = LDL-C/HDL [32].
Atherogenic Coefficient (AC) = (TC -HDLC)/ HDL [33].
Atherogenic Index of Plasma (AIP) = log (TG/HDL) [34].

Statistical Analysis: The data were analyzed using independent sample t-test with the help of SPSS (Statistical Package for Social Sciences) Statistics 11.5 package (SPSS Inc., Chicago Ill). All values are expressed as mean ± SEM (Standard error of the mean) and $p<0.05$, $p<0.01$, $p<0.001$ were taken as the level of significance.

RESULTS

Acute toxicity study: The drug (NMB) administered up to a high dose of 4000 mg/Kg produced no mortality of the experimental animals. Thus the LD_{50} value was found to be greater than 4000 mg/Kg body weight. The animals did not manifest any sign of restlessness, respiratory distress, general irritation or convulsion. Since NMB is in the clinical use for treatment of cardiovascular diseases for many years, a limit test was performed in acute oral toxicity study. According to the OECD test guideline 425 when there is information in support of low or nontoxicity and immortality nature of the test material, then the limit test at the highest starting dose level (4000 mg/Kg body weight) was conducted. There were no mortality and toxicity signs observed at 4000 mg/Kg body weight. Therefore, it can be concluded that NMB when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic lipid profile studies

Effect of NMB on lipid profile of male rats: There was a decrease in the triglyceride (p=0.07, 22.06 %) level, total cholesterol (p=0.66, 4.43 %) level, LDL level (p=0.11, 10.97 %) and VLDL level (p=0.07, 22.06 %) of the serum of the male rat, the decrease though not significant yet it was noticeable. There was also a significant decrease (p=0.02, 25.98 %] in

the HDL level. In case of Non-HDL level, there was an increase in the (p=0.92, 1.01 %) content, which was statistically not at all significant.

Effect of NMB on atherogenic indices of male rats: There was an increase in the Cardiac Risk Ratio (p=0.059, 27.43 %) and Atherogenic Coefficient (p=0.059, 34.06 %), the increase though not significant yet it was prominent. There was also an increase in the Castelli's Risk Index II (p=0.184, 16.34 %) and Atherogenic Index of Plasma (p=0.689, 2.27 %), which was statistically not at all significant.

DISCUSSION

A lipid profile or lipid panel is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglyceride. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease, certain forms of pancreatitis and other diseases [35]. A lipid profile measures TC, HDL, LDL and TG. High level of cholesterol, low-density lipoprotein cholesterol (LDL) and triglycerides (TG), and low levels of high-density lipoprotein cholesterol (HDL) are relevant risk factors for adverse cardiovascular events.

A high plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases [36] and is often related with hypertension [37], obesity and diabetes mellitus [38]. In this study, significantly lower serum triglyceride level was observed in the animals treated with NMB. Therefore NMB may have been responsible for the hypo-triglyceridemic effect.

While higher HDL levels are correlated with cardiovascular health, no medication used to increase HDL has been proven to improve health [39]. In other words, while high HDL levels might correlate with better cardiovascular health, specifically increasing one's HDL might not increase cardiovascular health. The remaining possibilities are that either good cardiovascular health causes high HDL levels, there is some third factor which causes both, or this is a coincidence with no causal link. Reduced serum HDL cholesterol is a risk factor for cardio-vascular disease [40] and is often found in hypertension [37, 41]. So, in the present study, the lower serum HDL cholesterol level, recorded for the treated groups is suggestive of the cardio-toxic effect of the drug.

High level of plasma LDL and VLDL cholesterol are risk factors for cardiovascular disease [42, 43] and often accompany hypertension [41] and obesity [44]. In this study, lower plasma LDL and VLDL cholesterol levels were observed in the animals treated with NMB.

Numerous studies have presented that non-HDL cholesterol is a better predictor of cardiovascular disease risk than is LDL cholesterol [45, 46]. Therefore, higher plasma non-HDL cholesterol level observed in the treated groups is indicative of the ability of the drug to increase cardiovascular risk.

Elevated serum total cholesterol level is a familiar and well-known risk factor for developing atherosclerosis and other cardiovascular diseases [47]. In this study, lower plasma total cholesterol level was observed in the animals treated with NMB.

In this study, NMB augmented all the atherogenic indices but all are not significant. Atherogenic indices are strong indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular problems and vice versa [31-34].

CONCLUSION

From the above experiment it can be concluded that NMB should not be administered chronically at a higher dose as it decreases HDL level significantly and increases all atherogenic indices. Further studies should be done by reducing the administered dose.

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Table 1: Name of the ingredients/herbs used in the preparation of Naradiya Laksmivilasa Rasa

Ingredient	Botanical name	Amount
1. 1. Krsnabhra curna (bhasma)	Mica (Calcined)	48 g
2. Ras (parada)	Mercury (purified)	24 g
3. Gandhaka	Sulfur (purified)	24 g
4. Camphor	*Cinnamomum camphora*	12 g
5. Jatiphala (Fruit)	*Myristica fragrans*	12 g
6. Jatikosa (Ar.)	*Myristica fragrans*	12 g
7. Vriddhadaru	*Argyreia speciosa*	12 g
8. Dhattura	*Dathura alba*	12 g
9. Bhanga	*Cannabis sativa*	12 g
10. Vidari mula	*Pueraria tuberosa*	12 g
11. Shatavari (Root)	*Asparagus recemosa*	12 g
12. Nagabala	*Grewia populifolia*	12 g
13. Atibala	*Abutilon indicum*	12 g
14. Gokshura	*Tribulus terrestris*	12 g
15. Nichula	*Strychnos nux vomica*	12 g
16. Pan	*Piper betle*	Quantity Sufficient

Table 2: Effect of Naradiya Laksmivilasa Rasa (NMB) on lipid profile of rat serum

Parameters	**CON**	**NMB**	***p*-Value**	**% Change**
TG	326.63±34.31	254.58±15.59	0.07	22.06 % Decrease
TC	75.84±7.25	72.48±2.43	0.66	4.43 % Decrease
LDL	125.85±6.61	112.05±4.72	0.11	10.97 % Decrease
VLDL	65.33±6.86	50.92±3.11	0.07	22.06 % Decrease
HDL	15.32±1.34	11.34±0.53	0.02	25.98 % Decrease
Non-HDL	60.53±6.97	61.14±2.59	0.92	1.01 % Increase

Independent sample t-test was performed to analyze this data set. All values are expressed as mean ± SEM and $p<0.05$, $p<0.01$, $p<0.001$ were taken as the level of significant

Table 3: Effect of Naradiya Laksmivilasa Rasa (NMB) on atherogenic indices of rat serum.

Parameters	**CON**	**NMB**	***p*-Value**	**% Change**
CRR	5.14±0.53	6.55±0.46	0.059	↑ 27.43 % Increase
CRI-II	8.69±0.78	10.11±0.68	0.184	↑ 16.34 % Increase
AC	4.14±0.53	5.55±0.46	0.059	↑ 34.06 % Increase
AIP	1.32±0.06	1.35±0.03	0.689	↑ 2.27 % Increase

Independent sample t-test was performed to analyze this data set. All values are expressed as mean ± SEM and $p<0.05$, $p<0.01$, $p<0.001$ were taken as the level of significant

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