



PRECLINICAL BLOOD CHEMISTRY SAFETY PROFILE STUDIES OF NARADIYA LAKSMIVILASA RASA ON THE KIDNEY FUNCTION AFTER CHRONIC ADMINISTRATION TO MALE SPRAGUE-DAWLEY RATS

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ABSTRACT

Naradiya Laksmivilasa Rasa (NMB) is a classical Ayurvedic formulation, indicated for the treatment of sinusitis, chronic skin diseases, diabetes, obesity, rheumatoid arthritis, headache, gynaecological disorders and urinary tract infections. However, till date, no safety profile of this formulation has been reported. That is why, the present study was conducted to evaluate the effect of conventionally prepared NMB on different kidney profile parameters in experimental animals. Acute toxicity study was conducted to determine the median lethal dose (LD₅₀) of the drug. The LD₅₀ study of NMB recorded no death or any signs of toxicity even at the highest dose of 4000 mg/Kg body weight. To find out the effect of chronic administration of NMB on serum kidney profile, it was administered chronically to the male Sprague-Dawley rats at a dose of 400 mg/kg for 43 days. Naradiya Laksmivilasa Rasa significantly decreased albumin and A/G ratio and significantly increased globulin, urea and Blood Urea Nitrogen (BUN) level. BUN/Creatinine and Urea/Creatinine level were significantly increased in NMB treated rats when compared to normal control. The drug (NMB) did not affect total protein and creatinine level significantly. This experimental data will help the clinician for the logical use of NMB in different disease conditions.

Keywords: Naradiya Laksmivilasa Rasa, Ayurvedic preparation, Kidney profile, Albumin, Creatinine,

INTRODUCTION

Naradiya Laksmivilasa 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 infections [1-5]. It mainly contains *Cinnamomum camphora*, *Myristica fragrans*, *Argyrea speciosa*, *Dathura alba*, *Cannabis sativa*, *Pueraria tuberosa*, *Asparagus recemosus*, *Grewia populifolia*, *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).

Ayurveda which means 'Science of life' is derived from the Sanskrit words 'Ayur' meaning life and 'Veda' meaning knowledge. It focuses on bringing harmony and balance in all areas of life including mind, body and spirit [6]. Ayurveda aims to keep the structural and physiological entities in a state of equilibrium, which signifies good health. Any imbalance due to internal or external factors may cause disease [7]. Ayurvedic treatment aims to

restore the equilibrium through various techniques, procedures, regimens, diet and medicines. Ayurvedic treatment consists of drugs, diet, exercise and general mode of life. Ayurveda largely uses plants as raw material for the manufacture of drugs, though materials of animal and marine origin, metals and minerals are also used [8]. Ayurveda has about 700 type of plants listed in its medicinal systems [9]. 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. They have a good safety profile also [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 without any standard dosage along with inadequate scientific studies on their safety profile has raised concerns on their toxicity [19]. That is why; we designed our current experiment to observe the effect of 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 and to decide how justifiable the use of this drug is under the stated conditions.

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 research 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) [20]. 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 Biochemical Parameters: Biochemical analysis was carried out on serum to assess the state of the kidney [21]. Biochemical studies involved analysis of parameters such as Total Protein [22], Albumin by Bromacresol green method [23], Creatinine [24], Blood Urea Nitrogen (BUN) [25] and Uric Acid [26]. The absorbance of all the tests were determined using Humalyzer Model No-3500.

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 \pm 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 LD50 (Median Lethal Dose) 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 non-toxicity 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 ml/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.

Effect of NMB on Total Protein, Albumin, Globulin content and A/G ratio in male rats: After 43 days of chronic administration of the NMB preparation, the total protein, albumin content and the calculated ratio of albumin to globulins, termed the A/G ratio in serum were determined in the male rats. In the study, the total protein content in the serum was increased (0.15 %) in the NMB treated male rats. The increase in total protein was not statistically significant ($p=0.995$). On the contrary, the albumin content was decreased (34.31 %) in NMB treated male rats and it was statistically significant ($p=0.001$). The globulin content was significantly ($p=0.001$) increased (36.03 %) as a result the decrease (51.77%) in the Albumin / Globulin ratio was significantly different from their corresponding control values ($p=0.001$)

Effect of NMB on Creatinine, BUN and Urea level in male rats: Kidney function test was performed to measure the creatinine and blood urea nitrogen content in the serum. These two contents can provide information about how effective the kidney function is. There was a statistically insignificant decrease in the creatinine (2.67% decrease; $p=0.711$) content in the NMB treated male rats. On the other hand, a statistically significant ($p=0.001$) increase of blood urea nitrogen (BUN) (22.26%) level and urea (22.23%) level in the serum were noted in comparison to their control group. The increase in BUN/Creatinine ratio (27.76%) and Urea/Creatinine ratio (27.73%) were also statistically significant ($p=0.01$).

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	<i>Cinnamomum camphora</i>	12 g
5. Jatiphala (Fruit)	<i>Myristica fragrans</i>	12 g
6. Jatikosa (Ar.)	<i>Myristica fragrans</i>	12 g

7. Vriddhadaru	<i>Argyreia speciosa</i>	12 g
8. Dhattura	<i>Dathura alba</i>	12 g
9. Bhanga	<i>Cannabis sativa</i>	12 g
10. Vidari mula	<i>Pueraria tuberosa</i>	12 g
11. Shatavari (Root)	<i>Asparagus recemosa</i>	12 g
12. Nagabala	<i>Grewia populifolia</i>	12 g
13. Atibala	<i>Abutilon indicum</i>	12 g
14. Gokshura	<i>Tribulus terrestris</i>	12 g
15. Nichula	<i>Strychnos nux vomica</i>	12 g
16. Pan	<i>Piper betle</i>	Quantity Sufficient

Table 2: Effect of Naradiya Laksmivilasa Rasa on Total Serum Protein, Albumin, Globulin content and A/G ratio in male rats

Parameters	CON	NMB	p-Value	% Change
Total Protein (TP)	6.72±6.72	6.73±0.06	0.995	0.15 % Increase
Albumin	4.08±0.15	2.68±0.14	0.001	34.31 % Decrease
Globulin	2.97±0.14	4.04±0.12	0.001	36.03 % Increase
A/G ratio	1.41±0.09	0.68±0.05	0.001	51.77 % Decrease

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 on Creatinine, BUN, BUN/Creatinine ratio, Uric Acid level in male rats

Parameters	CON	NMB	p-Value	% Change
Creatinine	0.75±0.03	0.73±0.04	0.711	2.67 % Decrease
Urea	34.42±1.38	42.07±0.69	0.001	22.23 % Increase
BUN	16.08±0.65	19.66±0.32	0.001	22.26 % Increase
BUN/Creatinine	21.65±1.04	27.66±1.67	0.01	27.76 % Increase
Urea/Creatinine	46.34±2.22	59.19±3.58	0.01	27.73 % 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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DISCUSSION

Proteins are important parts of all cells and tissues. The total protein test measures the total amount of two classes of proteins found in the fluid portion of blood: albumin and globulin. Albumin helps prevent fluid from leaking out of blood vessels and globulins are an important part of immune system [27, 28]. Drugs that can increase total protein measurements include anabolic steroids, androgens, corticosteroids, dextran, growth hormone, insulin, phenazopyridine, and progesterone [29].

Serum albumin test can help to determine if a patient has liver disease or kidney disease, or if the body is not absorbing enough protein. In the study, on the contrary to the findings regarding serum total protein content, the albumin content was decreased in NMB treated male rats and the decrease was statistically significant. Serum albumin level decreased due to liver dysfunction, malnutrition and mal-absorption, hypothyroidism, nephrotic syndrome due to kidney disease, protein losing-enteropathy, chronic illness, chronic inflammatory diseases, inflammation, insufficient anabolic hormones such as Growth Hormone, DHEA and testosterone [30]. The decrease of albumin in the NMB treated experimental population can be due to any of the factors mentioned above.

Globulins are the key building block of antibodies. Globulins include gamma globulins (antibodies), beta globulins, alpha-2 globulins, and alpha-1 globulins and a variety of enzymes and carrier or transport proteins. Since the gamma fraction usually makes up the largest portion of the globulins, antibody deficiency should always come to mind when the globulin level is low [29]. Chronic infections, liver disease (biliary cirrhosis), fatty necrotic liver, kidney dysfunction (Nephrosis), ulcerative colitis, rheumatoid arthritis, leukemia, multiple myelomas, increased amount of nonspecific protein, and autoimmune disorders such as collagen diseases can affect globulin level. The increase of globulin in the NMB treated experimental population can be due to any of the factors mentioned above. The liver can function adequately on 20% of liver tissue, thus early diagnosis by lab methods is difficult. A reversed A/G ratio may be a helpful indicator. Normally this ratio exceeds 1.0 but in disease conditions which selectively affect albumin levels, are associated with lesser ratios [29]. A low A/G ratio may reflect overproduction of globulins such as seen in multiple myeloma or autoimmune diseases or underproduction of albumin such as may occur with cirrhosis or selective loss of albumin from the circulation as may occur with kidney disease (nephrotic syndrome), liver dysfunction. The decrease of Albumin/Globulin ratio (A/G ratio) in the NMB treated experimental population can be due to any of the factors mentioned above.

BUN stands for blood urea nitrogen. The BUN test is often done to check kidney function. BUN Increases by 10-20 mg/dl/day if renal function absent. Serum creatinine is a better measure of renal function and BUN is reabsorbed at renal tubules [31-33]. Decrease of BUN level may be seen in severe liver disease, malnutrition, and sometimes when a person is overhydrated. A decrease of BUN level may indicate lower risk of kidney disease. BUN-to creatinine ratio is considered a reliable test that helps in detecting kidney problems. BUN and creatinine are two compounds found in the blood and the amount of these substances is directly governed by the functioning of the kidneys. The principle behind this ratio is the fact that both urea (BUN) and creatinine are freely filtered by the glomerulus, however urea reabsorbed by the tubules can be regulated (increased or decreased) whereas creatinine reabsorption remains the same (minimal reabsorption) [31-33]. Any dysfunction of the kidneys can increase or decrease the quantity of these compounds in the blood. In this study, NMB noticeably increase the BUN level and BUN to Creatinine ratio. So the drug may have nephrotoxic effect.

CONCLUSION

From the above data it can be concluded that NMB should not be administered chronically at a higher dose as it may cause liver or kidney disease. Further studies should be done by reducing the administered dose. Thus NMB is to be taken under medical supervision only at a dosage of 12–24 ml once or twice a day usually advised after food. If needed, it can be mixed with equal quantity of water.

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