

Toxicological Studies of the Ayurvedic Medicine “Naradiya Laksmivilasa Rasa” Used in Sinusitis

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Abstract

In this study, the toxicological effects along with possible side effects of the classical Ayurvedic formulation Naradiya Laksmivilasa Rasa (NMB), which is used as a traditional medicine in the treatment of sinusitis in the rural population, were evaluated. During this study, various experiments on body growth rate, organ-body weight ratio, and tissue hydration indices were performed to evaluate its efficacy and toxicity. To find out the toxicological characteristics of NMB, it was administered chronically to male Sprague-Dawley rats at a dose of 100 mg/kg. After 32 days of chronic administration of the NMB preparation, the following toxicological changes were noted. Throughout the experimental period, the NMB-treated animals showed negligible [1.04% loss ($p = 0.914$) to 3.18% gain ($p = 0.753$)] changes in body weight and no statistically significant increase or decrease was noted. The study involving comparison of the relative weight of the major organs of the rats revealed some significant results. There was a statistically highly significant increase in the relative percentage weight of the male rat kidney, along with an increase in the absolute weight of the male rat spleen and an increase in the relative percentage weight of the male rat spleen. In the tissue hydration index experiment, there was a statistically significant increase in the water content of the male rat liver. As NMB increases abnormally, the weight of several organs increases in the body of treated rats, so it should not be administered chronically at a higher dose.

Keywords: Naradiya Laksmivilasa Rasa; Toxicity; Absolute weight; Organ percentage weight; Organ water content

Introduction

Sinusitis, also known as rhinosinusitis, is inflammation of the paranasal sinuses. It can be due to infection, allergy, or autoimmune problems. Most cases are due to a viral infection and resolve over the course of 10 days. Sinusitis is a common condition with about 24-31 million cases occurring in the United States annually [1,2]. Chronic sinusitis affects approximately 12.5% of people [3].

Ayurvedic medicines have reputation as decent and effective remedies for a number of diseases [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 deliver health-care services at the primary health-care level [5]. According to WHO, an estimated 1.5 billion people of the world are now getting treatment with these medicines [6,7]. They also have a good safety profile [8].

Nardiya Laksmivilasa Rasa is an Ayurvedic medicine in tablet or powder form. It is used in the treatment of sinuses, chronic skin diseases, diabetes, and urinary tract disorders [9-13]. The dose of NMB is 250 mg once in the morning, before or after food, or as directed by an Ayurvedic doctor. Naradiya Laksmivilasa Rasa is incorporated in Bangladesh National Formulary (pages 308-309) of Ayurvedic Medicine 1992, which was approved by the Ministry of Health and Family Welfare, Government of Bangladesh, Memo No. Health-1/Unani-2/89/(Part-1) 116 dated 3-6-1991. Unani and Ayurvedic Formulary Committee compiled the Bangladesh National Formulary of Ayurvedic Medicine and this formulary was published by the Bangladesh Board of Unani and Ayurvedic Systems of Medicine under section 13(j) under the authorization of the Board of Bangladesh Unani and Ayurvedic Practitioners Ordinance, 1983. In June 1992, Bangladesh National

Formulary of Ayurvedic Medicine and WHO together published the first edition. In June 2011, the board exclusively revised and published the second edition by own finance [ISBN 978-984-33-3254-7]. In page 533 (column 1: Product code 12.31), permission is given for manufacturing Ayurvedic medicines at industrial scale. The Directorate of Drug Administration has issued Notification DA/Admin/1-10/96/6212 dated October 19, 1996, and given license to companies for local manufacture and sale in Bangladesh under Drug Act, 1940, and its associated rules and Drug (Control) Ordinance 1982 [Ay-58-A-061] (Published Bangladesh Gazette #24 Part VI dated Thursday, June 11, 1998) [9].

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 Limited, Bangladesh. All other reagents and chemicals used in this work were analytical grade.

Experimental animals

Six- to 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 60-70 g. The animals were sheltered 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 were maintained at 12 h day and 12 h night cycle. All experiments on the rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals approved by the Ethical Review Committee,

Ingredients	Botanical/English name/Calyx name	Amounts
1. Krsnabhra curna (bhasma)	Mica (calcined)	48 g
2. Ras (parada)	Mercury (purified)	24 g
3. Gandhaka	Sulfur (purified)	24 g
4. Candra (karpura)	<i>Cinnamomum camphora</i>	12 g
5. Jatikosa (Ar.)	<i>Myristica fragrans</i>	12 g
6. Jatiphala (Sd.)	<i>Myristica fragrans</i>	12 g
7. Vrddhadaraka bija (Sd.)	<i>Argyres speciosa</i>	12 g
8. Dhusturaka (dhattura) bija (Sd.)	<i>Datura alba</i>	12 g
9. Trailokya vijaya (bhanga) bija (Sd.)	<i>Cannabis sativa</i>	12 g
10. Vidari mula (Rt. Tr.)	<i>Pueraria tuberosa</i>	12 g
11. Narayani (satavari) (Rt.)	<i>Asparagus recemoso</i>	12 g
12. Nagabala (mula) (Rt.)	<i>Grewia populifolia</i>	12 g
13. Atibala (mula) (Rt.)	<i>Abutilon indicum</i>	12 g
14. Goksuraka bija (Fr.)	<i>Tribulus terrestris</i>	12 g
15. Nicula bija (Sd.)	<i>Barringtonia acutangula</i>	12 g
16. Parnapatra (tambula) Rasa (Lf)	<i>Piper betle</i>	Quantity sufficient

Table 1: Name of the ingredients used in the preparation of Naradiya Laksmivilasa Rasa (NMB)

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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 modifications (OECD Guideline 425) [14]. Sixteen male mice (30-40 g body weight) were divided into four groups of four animals each. Different doses (50, 60, 70, and 80 ml/kg) of experimental drug (NMB) were administered by a stomach tube. The dose was divided into two fractions and given within 12 h. Then all the experimental animals were observed for mortality and clinical signs of toxicity (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, and changes in skin and fur texture) at 1, 2, 3, and 4 h and thereafter once a day for the next three days following NMB administration.

Chronic toxicity studies

Prior to the experiment, the rats were randomly divided into two groups of eight 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 32 days. For all the pharmacological studies the drugs were administered via oral route at a dose of 100 ml/kg body weight [15]. After acclimatization, the Ayurvedic medicinal preparation was administered to the rats by an intragastric syringe between 10 am and 12 am daily throughout the study period. All experiments on the rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. The experimental animals were marked carefully on their tails, which helped to identify a particular animal. By using the identification mark, responses were noted separately for a particular period prior to and after the drug administration [16].

Growth analysis

Careful monitoring of body weights of the rats of both sexes was performed throughout the 32-day drug administration period. Body

weights were recorded at regular intervals (2-3 days) until the treatment period was completed. All rats were kept under close observation throughout the experimental period. An equal numbers of animals of the same species were also maintained as the control group and these were also kept under close observation. Statistical analysis of the initial and final growth rates was performed. The growth rate was expressed as percentage increment in the body weight. The growth rate of the treatment group was compared with that of the control group.

Body weight: organ weight ratio analysis

At the end of the 32-day treatment period, the animals were fasted for 18 h and also 24 h after the last administration. Ketamine (500 mg/kg i.p.) was administered for the purpose of anesthesia [17]. The rats of both NMB and control groups were sacrificed after the completion of the 32-day period and examined macroscopically for external lesions. Necropsy was performed to examine gross pathological lesions of various internal organs. Specific organs of interest were then detached and preserved in 13% formalin and sent for the evaluation of histological anomalies, if any. The tissues thus subjected to histopathological evaluation are heart, kidneys, lungs, liver, spleen, thymus, stomach, cecum, pancreas, adrenal glands, urinary bladder, and reproductive organs, which include testis, seminal vesicles, prostate gland, and epididymis in case of males and ovaries, fallopian tube, and uterus in case of females. Portions of heart, lungs, liver, and spleen were excised and preserved for histological examination. The remaining portions were dried for determination of water content.

$$\text{Relative weight of organ} = \frac{\text{AOW}}{\text{BW}} \times 100$$

AOW = absolute organ weight

BW = body weight

$$\text{Water content in tissue} = \frac{\text{OW}_1 - \text{OD}}{\text{OW}_1 - \text{OF}} \times 100$$

OW₁ = organ wet weight

OD = organ dry weight

OF = organ foil weight

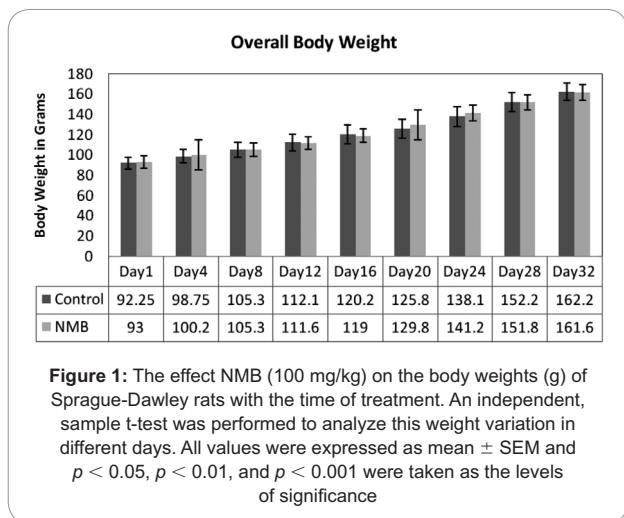
Statistical analysis

The data were analyzed using independent sample t-test with the help of SPSS (Statistical Package for Social Science) Statistics 16 package (SPSS Inc., Chicago, Ill.). All values were expressed as mean ± SEM (standard error mean) and $p < 0.05$, $p < 0.01$, and $p < 0.001$ were taken as the levels of significance.

Results

Acute toxicity study

The drug (NMB) administered up to a high dose of 80 ml/kg produced no mortality. Thus the LD₅₀ value was found to be greater than 80 ml/kg body weight. The animals did not manifest any signs of fever, chronic skin diseases, diabetes, urinary tract disorders, sinuses, nonhealing wounds, fistula, obesity, rheumatoid arthritis, ascites, headache, gynecological disorders, and diseases of ear, nose, throat, and eyes. Since NMB is in the clinical use for treating the aforementioned ailments for many years, a limit test was performed in an acute oral toxicity study. According to the OECD test guideline 425, when there is information in support of low or no toxicity and the immortality nature of the test material, then the limit test at the highest starting dose level (80 ml/kg body weight) is conducted. There were no mortality and



Parameters	Control	NMB	p value	%increase/decrease
Heart	0.4255 \pm 0.0184	0.4311 \pm 0.02955	0.874	\uparrow 1.32
Lung	0.8844 \pm 0.03365	0.9705 \pm 0.05132	0.182	\uparrow 9.74
Liver	5.2442 \pm 0.32213	5.9321 \pm 0.47373	0.25	\uparrow 13.12
Kidney	0.4931 \pm 0.01812	0.5692 \pm 0.03858	0.105	\uparrow 15.43
Spleen	0.4611 \pm 0.02856	0.8601 \pm 0.09827	0.002**	\uparrow 86.53
Testis	1.0184 \pm 0.03086	1.076 \pm 0.04623	0.317	\uparrow 5.66

\uparrow : increase, \downarrow : decrease; $p^* \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$

Table 2: The effect of Naradiya Mahalaksmivilasa Rasa (NMB) (100 mg/kg) on the absolute organ weights of male rats

Parameters	Control	NMB	p value	%increase/decrease
Heart	0.2632 \pm 0.00521	0.2651 \pm 0.00678	0.827	\uparrow 0.72
Lung	0.5498 \pm 0.02052	0.6021 \pm 0.02346	0.115	\uparrow 9.51
Liver	3.2368 \pm 0.12244	3.6354 \pm 0.14397	0.053	\uparrow 12.31
Kidney	0.3063 \pm 0.00928	0.3501 \pm 0.00858	0.004*	\uparrow 14.30
Spleen	0.2876 \pm 0.01912	0.5285 \pm 0.04612	0.001***	\uparrow 83.76
Thymus	0.1451 \pm 0.01363	0.1693 \pm 0.01327	0.226	\uparrow 16.68
Testis	0.6341 \pm 0.02297	0.6681 \pm 0.01583	0.242	\uparrow 5.36

\uparrow : increase, \downarrow : decrease; $p^* \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$

Table 3: The effect of Naradiya Mahalaksmivilasa Rasa (NMB) (100 mg/kg) on the relative organ weights of male rats

Parameters	Control	NMB	p value	%increase/decrease
Heart	75.916 \pm 1.35881	74.28 \pm 3.0234	0.629	\downarrow 2.16
Lung	79.9438 \pm 0.53312	78.8674 \pm 2.05619	0.626	\downarrow 1.35
Liver	72.6499 \pm 0.3112	74.7377 \pm 0.82288	0.042*	\uparrow 2.87
Kidney	77.8321 \pm 1.28889	62.2155 \pm 16.71138	0.367	\downarrow 20.06
Spleen	76.7662 \pm 0.47337	74.0995 \pm 2.13907	0.26	\downarrow 3.47
Testis	86.8774 \pm 0.18917	87.3404 \pm 0.31098	0.213	\uparrow 0.53

\uparrow : increase, \downarrow : decrease; $p^* \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$

Table 4: The effect of Naradiya Mahalaksmivilasa Rasa (NMB) (100 mg/kg) on various tissue hydration indices of male rats

toxicity signs observed at 80 ml/kg body weight. Therefore, it can be concluded that NMB when administered at single dose is nontoxic and can be used safely in oral formulations.

Chronic growth study

Effect of NMB on overall body weight

The total treatment period was of 32 days. All throughout the experimental period the NMB-treated animals were maintaining a decrease in body weight; in the body weight study, the NMB-administered animals were weighing 2.76-4.60% less than their control counterparts. All throughout the experimental period no statistically significant decrease was noted.

Effect of NMB on organ toxicity study

In case of absolute organ weight, there is a statistically highly significant ($p = 0.002$) increase in the absolute weight of the male rat spleen (86.53% increase). In case of relative organ weight of male rats there is a statistically highly significant ($p = 0.004$) increase in the relative percentage weight of the kidneys (14.30% increase). There is a statistically very highly significant ($p = 0.001$) increase in the relative percentage weight of the male rat spleen (83.76% increase).

Effect of NMB on tissue hydration index

In case of tissue hydration index, there is a statistically significant ($p = 0.042$) increase in the organ water content of the male rat liver (2.87% increase). No significant increase or decrease was noticed in case of water content of other organs of NMB-treated male rats.

Discussion

Effect of NMB on overall body growth

The administration of herbal preparations without any standard dosage along with insufficient scientific studies on their safety profile has raised concerns on their toxicity [18]. Change in body weight is a sign of impairment in the normal functioning of the body. All throughout the experimental period the NMB-treated animals were maintaining a decrease in body weight; in the body weight study, the NMB-administered animal were weighing 2.76-4.60% less than their control counterparts but the decrease was not statistically significant. Rapid body weight loss may be due to decreased feed and/or water consumption, disease, dental maladies, or specific toxic effects [19].

Effect of NMB on various organs: organ-body weight ratio

Relative organ weight (ROW) may serve as a sign of pathological and physiological status in man and animals. Toxic substances induce abnormal metabolic reactions that affect the primary organs (e.g., heart, liver, spleen, kidneys, and lungs) [20]. Change in organ weight is a symbol of impairment in the normal body functioning. Organ-body weight ratio may indicate organ swelling, atrophy, or hypertrophy [21].

Administration of xenobiotics may alter renal weight and as a consequence any renal weight changes in toxicity studies should be assessed with care. In this study we found the kidney weight significantly increases in the NMB-treated rats. When increases in renal weight are manifestations of toxicity, they are frequently associated with macroscopic appearances of swelling and pallor of the kidneys and evidence of significant damage on histological examination. When increases in renal weight occur in the absence of histopathological alterations, it is reasonable to assume that the changes are a manifestation of adaptive responses to increased physiological demands placed on

the renal tissue in the elimination of the xenobiotic. Some xenobiotics, notably angiotensin-converting enzyme (ACE) inhibitors, have been associated with a reduction in renal weight without evidence of renal cellular damage, presumably as a result of reduced renal demand [22].

In this study we found the spleen weight significantly increased in the NMB-treated rats. In rodents, evidence of increased red cell turnover in the spleen is indicated by increased splenic weight, changes in splenic pigmentation, presence of foam cells, and intense erythropoiesis. The red pulp may expand and develop marked hematopoiesis under a variety of circumstances. In rodents many of these stimuli are nonspecific and occur sporadically in long-term studies. Drugs and chemicals that affect blood cells may activate intense hematopoiesis in the spleen, the cytological nature of which varies with the type of cell affected. Thus, increased hematopoiesis as a result of increased red cell demand shows predominantly cells of the red cell series in the spleen whereas less specific processes such as infections tend to be associated with a more diverse cell population, including megakaryocytes. However, increased erythropoiesis may be difficult to distinguish from plasma cell hyperplasia that also occurs in the red pulp. The presence of pigment-laden macrophages as well as numerous erythropoietic cells in the red pulp implies that there is increased red cell turnover in the spleen.

Effect of NMB on tissue hydration index

Water comprises from 75% body weight in infants to 55% in elder people and it is essential for maintaining cellular homeostasis.

In our study we found that NMB causes significant increase in the percentage water content of liver. It can be suggested that this drug has positive impact on maintaining cellular hemostasis.

Conclusion

From the above experiment it can be concluded that NMB should not be administered chronically at a higher dose as it increases the weight of heart, lungs, liver, kidneys, spleen, and testes. Further studies should be done by reducing the administered dose.

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