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# Safety / Toxicity studies of ayurvedic formulation-Navratna rasa

**G.S. Lavekar[1], B. Ravishankar[2], S. Venugopal Rao[1], S.N. Gaidhani[1], B.K. Ashok[2] and V.J. Shukla[2]**

[1]Central Council for Research in Ayurveda and Siddha , Janakpuri, New Delhi-110058,

[2]CCRAS Pharmacological Research Unit, (G.A.U.), Jamnagar, India.

## Abstract

**Navratna rasa, an ayurvedic proprietary medicine based on classical formulation has been in use since long for general debility, rickets and calcium deficiency in Ayurvedic practices. The drug was screened for its safety/toxicity studies in acute and chronic models. No mortality and behavioral changes were observed during the course of acute toxicity study. The chronic toxicity study reveals that, the test drug has no serious toxicity potential to most of the important organs in therapeutics doses.**

**Keywords:** Ayurvedic drug, toxicity, rats

## Introduction

Initially the Central Council for Research in Ayurveda and Siddha, Dept. of AYUSH, Ministry of Health and Family Welfare has conducted the safety/toxicity studies of nine ayurvedic preparations. Out of nine drug formulations, Navratna rasa was one of the drug formulations. This study is very much relevant in the background of the heavy metal content much above the limits in some of the marketed ISM drugs (Saper *et al.,* 2004).

The findings of studies will be helpful in clearing misconception among Physicians, Scientists and consumers as well.

The chemical analysis for heavy metal contents and evaluation of safety/toxicity profiles of this drug biologically through acute and chronic toxicity (depend on clinical regimen) studies at council's institutes was conducted.

Navratna rasa, one of the ayurvedic preparations widely used in the treatment of general debility, rickets and calcium deficiency was tested.

Since, there is no literature available with regards to its safety/toxicity and standardization the present studies were undertaken for its heavy metal content and safety/toxicity studies.

***Corresponding author**
E-mail: ccras_dir1@nic.in

## Materials and Methods

### Ingredients of Navratna rasa

| | | |
|---|---|---|
| 1. Hirak (Diamond) Bhasma | : | 14 mg |
| 2. Panna (Emarald)Bhasma | : | 16 mg |
| 3. Manikya (Ruby) Bhasma | : | 20 mg |
| 4. Pushparaj (Topaz) Bhasma | : | 22 mg |
| 5. Nilam (Saphire) Bhasma | : | 24 mg |
| 6. Vaidurya (Cat's eye) Bhasma | : | 28 mg |
| 7. Gomedmani (Zircon) Bhasma | : | 26 mg |
| 8. Mukta (Pearl) Bhasma | : | 30 mg |
| 9. Praval (Coral) Bhasma | : | 30 mg |
| 10. Vaikrant (Garnet) Bhasma | : | 160 mg |
| 11. Sulphide of Mercury | : | Q.S. |
| 12. Piper longum | : | 250 mg |
| 13. Dugdha sarkara | : | 250 mg |

### I. Estimation of heavy metals

To determine the metal concentration in the drug, sample was analyzed by Atomic Absorption Spectrophotometer (Kenawy *et al.,* 2000). The sample was weighed and taken in porcelein basin and ashed in muffle furnace at 525°C. The ash was extracted with 1:1 HCl solution and then volume was made up. Heavy metals of the extracted solution were estimated with Atomic Absorption Spectrophotometer. For estimation of Arsenic, Hydride Generator was used.

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### II. Acute toxicity studies

The animals were obtained from the animal house facility attached to Institute of Post Graduate Teaching, Research in Ayurveda (IPGT & RA), Gujarat Ayurved University (Jamnagar). They were maintained in ideal laboratory conditions with ambient temperature and humidity. The experiments were carried out in accordance with the guidelines of the Institutes Animal Ethical Committee (IAEC). Swiss albino mice of either sex with an average body weight of 20-32 g were used in the study. The animals were divided into four groups of eight animals each (4 male and 4 female). The drug Navaratna rasa was suspended in distilled water and was administered in the erapeutic effective doses of 19.5 (TED), 97.5 (Average 5 times TED) and 195 mg/kg, orally (Higher, 10 times TED), once daily and another group served as a control. The animals were then observed for any toxic symptoms or mortality up to 72 hours. Dose for experimentation was calculated by dose conversion table (Paget and Barnes, 1964) with reference to suggested human doses (i.e.150 mg)

### III. Chronic toxicity studies (90 days)

Charles Foster strain albino rats of either sex weighing 160-250 g in groups of 10 for each dose level of Navratna rasa were used for experiment. Chronic toxicity was evaluated after single daily administration of test drug at therapeutic effective dose 13.5 (TED), 67.5 (Average, 5 times TED) and 135 mg/kg (Higher, 10 times TED), p.o. for 90 days. Test drug was given as solution in distilled water by gavage with a control group receiving the vehicle (distilled water).All the rats were maintained on "Amrut" brand rat feed (Mfg. by Pranav Agro Industries Ltd.) given *ad libitum* with water. Toxicity was evaluated in terms of body weight and gross behavior, gross and histological appearance of vital organs (thymus, heart, liver, spleen, kidney, testis and uterus) biochemical changes (blood sugar, serum cholesterol, serum triglyceride, serum urea, serum creatinine, serum alkaline phosphatase, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), total protein, serum albumin, and serum globulin) and hematological parameters (WBC, RBC, lymphocyte percentage, MCV, monocyte percentage, hematocrit, granulocyte Percentage, MCH, MCHC, MRBC, haemoglobin. The qualitative analysis of urine, estimation of sodium, potassium in urine and pH of urine were done.

## Results and Discussion

### Estimation of Heavy Metals

Table 1 lists the metal contents of Navratna rasa (heavy metals viz. Lead, Mercury, Arsenic and Cadmium). Mercury and Arsenic were within permissible limits as laid down by WHO (2004).

### Acute toxicity studies

In none of the group no mortality or any gross changes were observed during the study period of 7 days.

### Chronic toxicity studies

No significant behavioral changes were observed in any of the group studied. These parameters remained unaffected in all groups during the study period. Normal body weight gain was observed in control as well as test drug administered groups. TED group showed no change in body weight gain in comparison to control group, while a marginal decrease in weight gain was observed in five times therapeutic dose groups. The body weight gain was apparently higher in ten times therapeutic dose group. However, none of the changes observed were found to be statistically significant (Table 2).

The test drug administration did not affect the weight of thymus and heart, in all the three treated

**Table 1: Heavy Metal estimation in ayurvedic formulation Navaratna rasa**

| | Pb (10ppm)* | Hg (1ppm)* | As (10ppm)* | Cd (0.3ppm)* |
|---|---|---|---|---|
| JAMA[1] | 600 µg/g | 104000 µg/g | 60 µg/g | --- |
| CCRAS[2] | 47.05 ppm | 0.05 ppm | 4.18 ppm | 0.48 ppm |

*Limits of heavy metals (WHO, 1964).
[1]Technique used for estimation x-ray fluorescence spectroscopy (Saper *et al.*, 2004); [2]Technique used for estimation Atomic Absorption Spectrophotometer.

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**Table 2: Effect of different dose level of Navratna rasa on body weight in albino rats**

| Groups | Dosage (mg/kg) | Body weight (g) | | Body weight change in (g) | Body weight change in (%) |
|---|---|---|---|---|---|
| | | Initial | At the end of study | | |
| Control | Distilled water | 194.66±08.44 | 246.66±15.84 | 52.00±18.19 | — |
| TED | 13.5 | 183.33±06.88 | 235.00±08.00 | 51.67±10.78 | 00.36 ↑ |
| TED ´ 5 | 67.5 | 206.00±14.44 | 249.00±18.90 | 43.67±08.11 | 16.01 ↓ |
| TED ´ 10 | 135 | 166.00±05.50 | 256.16±15.30 | 90.00±17.76 | 73.01 ↑ |

Data: Mean±SEM: ↑ – Increase; ↓ – Decrease

**Table 3: Effect of different dose level of Navratna rasa on organs weight (mg) of albino rats**

| Name of organ | Control (Distilled water) | Navratna rasa | | |
|---|---|---|---|---|
| | | TED 13.5 mg/kg | TED × 05 67.5 mg/kg | TED × 10 135 mg/kg |
| Thymus (mg) | 488.00±34.00 | 492.00±23.69 | 463.33±43.89 | 500.83±39.84 |
| Heart (mg) | 795.17±14.70 | 770.00±40.39 | 781.67±61.78 | 823.33±47.79 |
| Liver (mg) | 5775.00±540.12 | 5358.00±340.00 | 5335.00±568.35 | 5622.50±432.67 |
| Spleen (mg) | 506.67±53.01 | 454.17±25.31 | 480.83±38.13 | 528.33±31.48 |
| Kidney (mg) | 1578.33±151.07 | 1480.83±109.10 | 1516.66±111.40 | 1540.83±124.40 |
| Testis (mg) | 2398.33±130.13 | 2790.00±035.11 | 2106.66±162.71 | 2693.33±150.14 |
| Uterus (mg) | 525.00±44.81 | 856.66±143.33 | 440.00±40.00 | 680.00±120.00 |

Data: Mean ± SEM

groups in comparison to control group. A marginal but statistically non-significant decrease in weight of liver was observed in test drug administered groups in comparison to control group. (Table 3). The moderate increase observed in blood glucose level in test drug administered groups in comparison to control group and was found to be statistically non-significant. In both average and higher dose treated groups an apparent and statistically significant decrease in serum cholesterol level was observed in comparison to control group. A marginal increase in SGOT activity was observed in TED treated group and decrease was observed in average and higher dose treated groups in comparison to control group. However, the decrease of SGPT activity was observed in all the dose levels which were found to be statistically non-significant. At all the three dose levels studied, the test drug produced marginal decrease in serum globulin level, which was found to be statistically non-significant in comparison to control group. A marginal increase in serum triglyceride was observed in therapeutic and higher dose and decrease in average dose in comparison to control group (Table 4).

There was decrease in mean corpuscular volume observed at all the three dose level studied. However, only the decrease observed in TED group was found to be statistically significant in comparison to control group. A marginal and statistically non-significant increase in mean corpuscular hemoglobin concentration was observed at all the three dose levels studied in comparison to control group (Table 5). The test drug administration did not affect on urine pH to significant extent in comparison to control group. A moderate increase in sodium excretion was observed in TED treated group in comparison to control group. A moderate increase in potassium excretion was observed in TED and average dose treated groups in comparison to control group (Table 6).

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**Table 4: Effect of different dose level of Navratna rasa on biochemical parameters in albino rats.**

| Parameter studied | Control (Distilled water) | TED 13.5 mg/kg | TED × 05 67.5 mg/kg | TED × 10 135 mg/kg |
|---|---|---|---|---|
| Blood Glucose (mg/dl) | 42.00±02.20 | 48.00 ± 01.65 | 48.33 ± 02.95 | 44.83 ± 02.57 |
| Serum Cholesterol (mg/dl) | 089.50±04.09 | 129.66 ± 24.63 | 066.50 ± 05.41** | 067.50 ± 08.15* |
| Serum Triglycerides (mg/dl) | 089.00±04.95 | 095.83 ± 10.60 | 080.66 ± 07.65 | 106.60 ± 10.12 |
| Blood Urea (mg/dl) | 38.50±02.44 | 47.00 ± 02.55* | 44.00 ± 01.66 | 34.16 ± 01.92 |
| Serum Creatinine (mg/dl) | 00.76±00.03 | 00.80 ± 00.03 | 00.75 ± 00.02 | 00.75 ±00.04 |
| Serum Alkaline Phosphatase (IU/L) | 203.00±19.95 | 206.00 ± 13.20 | 263.66 ± 22.91 | 195.66 ± 12.99 |
| S.G.O.T (IU/L) | 332.00±52.69 | 356.00 ± 38.59 | 301.16 ± 48.90 | 298.83 ± 43.54 |
| S.G.P.T (IU/L) | 81.00±08.38 | 79.00 ± 08.98 | 68.33 ± 05.48 | 59.40 ± 05.55 |
| Serum Total Protein (g/dl) | 8.10±00.17 | 7.66 ± 00.18 | 7.55 ± 00.12 | 7.55 ± 00.22 |
| Serum Albumin level (g/dl) | 04.18±00.24 | 04.08 ± 00.07 | 04.13 ± 00.16 | 03.91 ± 00.06 |
| Serum Globulin (g/dl) | 03.91±00.14 | 03.58 ± 00.14 | 03.36 ± 00.25 | 03.63 ± 00.18 |

Mean±SEM; **P<0.01; *P<0.05

**Table 5: Effect of different dose level of Navratna rasa on hematological parameters in albino rats**

| Parameter studied | Control (Distilled water) | TED 13.5 mg/kg | TED × 05 67.5 mg/kg | TED × 10 135 mg/kg |
|---|---|---|---|---|
| WBC ($10e^3$/ μl) | 01.34±00.24 | 01.62±00.22 | 01.35±00.18 | 01.71±00.43 |
| RBC ($10e^6$/ μl) | 06.38±00.17 | 06.80±00.26 | 05.90±00.28 | 06.00±00.20 |
| Lymphocyte % | 92.08±00.58 | 91.33±00.99 | 94.71±00.37* | 91.51±00.53 |
| Monocyte % | 01.51±00.11 | 01.66±00.15 | 01.40±00.14 | 01.65±00.14 |
| Granulocyte % | 05.60±00.60 | 06.98±00.95 | 03.88±00.37* | 06.86±00.43 |
| Lymphocyte count ($10e^3$/ μl) | 01.23±00.21 | 01.48±00.21 | 01.26±00.18 | 01.56±00.40 |
| MCV (fl) | 84.52±03.09 | 75.81±00.76* | 77.38±01.18 | 76.82±01.33 |
| Haematocrit (%) | 49.45±01.30 | 51.50±01.75 | 47.16±01.47 | 45.83±01.07 |
| MCH (Pg) | 14.73±00.05 | 14.86±00.24 | 15.56±00.49 | 15.31±00.43 |
| MCHC | 19.01±00.27 | 19.61±00.27 | 20.15±00.58 | 20.01±00.35 |
| MRBC % | 01.08±00.07 | 00.80±00.05* | 00.90±00.05 | 00.91±00.05 |
| Haemoglobin (g) | 09.40±00.22 | 10.08±00.29 | 09.13±00.18 | 09.16±00.15 |

Mean±SEM; *P<0.05

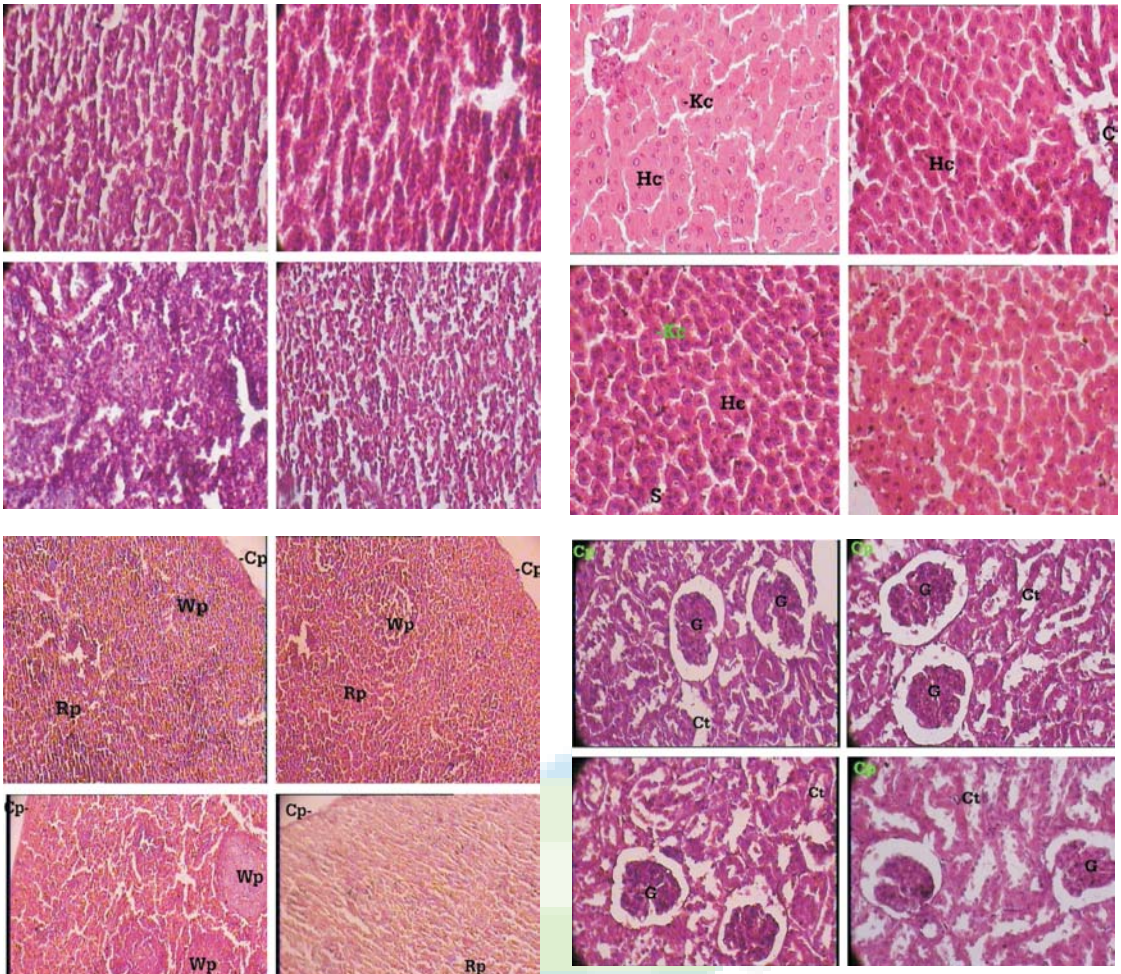
**Table 6: Effect of different dose level of Navratna rasa on urine pH sodium and potassium excretion in albino rats**

| Groups | Dosage (mg/kg) | Urine pH | Sodium Excretion(mEq/I) | Potassium Excretion(mEq/I) |
|---|---|---|---|---|
| Control | Distilled water | 10.50±00.00 | 27.47±03.38 | 165.76±50.00 |
| TED | 13.5 | 10.33±00.14 | 33.65±10.06 | 199.54±59.30 |
| TED × 05 | 67.5 | 10.33±00.14 | 28.43±03.52 | 170.54±33.97 |
| TED × 10 | 135 | 10.33±00.14 | 22.33±09.84 | 134.73±24.05 |

Mean±SEM

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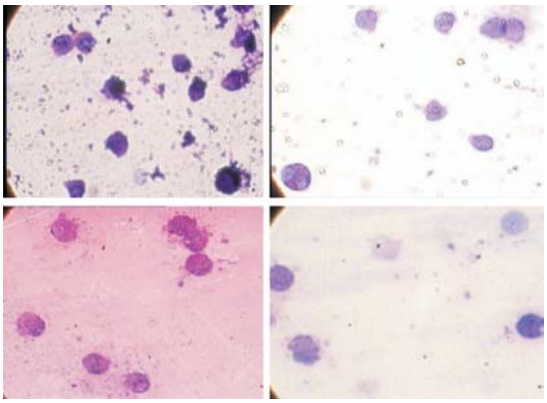



**Fig.1 (A)** Photomicrograph of representative sections of anterior part of Pituitary gland of albino rats from control group.**(B)** Photomicrograph of representative section of anterior part Pituitary gland of albino rats from TED group. **(C)** Photomicrograph of representative section of anterior part Pituitary gland of albino rats from TED×05 group.**(D)** Photomicrograph of representative sections of anterior part Pituitary gland of albino rats from TED×10 group. Magnification: 1×400 **Fig.2 (A)** Photomicrograph of representative sections of Liver of albino rats from control group **(B)** Photomicrograph of representative sections of Liver of albino rats from TED group **(C)** Photomicrograph of representative sections of Liver of albino rats from TED × 05 group **(D)** Photomicrograph of representative sections of Liver of albino rats from TED × 10 group. Hc-Hepatic Cell; Kc-Kupffer Cell; Cv-Central vein (Note: Normal cyto-architecture). Magnification: 1×400. **Fig.3 (A)** Photomicrograph of representative section of Spleen of albino rats from control group **(B)** Photomicrograph of representative sections of Spleen of albino rats from TED group (Note: Increased white pulp proportion) **(C)** Photomicrograph of representative sections of Spleen of albino rats from TED×05 group (Note: Increased white pulp proportion) **(D)** Photomicrograph of representative sections of Spleen of albino rats from TED×10 group. Cp-Capsule; Rp-Red pulp; Wp-White pulp. Maginfication: 1×100 **Fig.4 (A)** Photomicrograph of representative sections of Kidney of albino rats from control group **(B)** Photomicrograph of representative sections of Kidney of albino rats from TED group **(C)** Photomicrograph of representative sections of Kidney of albino rats from TED×05 group **(D)** Photomicrograph of representative sections of Kidney of albino rats from TED×10 group. Cp-Capsule; Ct-Convoluted tubule; G-Glomerulus. Maginfication: 1×400 **Fig.5 (A)** Photomicrograph of smear of bone marrow from control group (1x 1200 Magnification) **(B)** Photomicrograph of smear of bone marrow from TED group (1x 1200 Magnification) **(C)** Photomicrograph of smear of bone marrow from TED×05 group - (1x 1200 Magnification) **(D)** Photomicrograph of smear of bone marrow from TED×10 group (1x 1200 Magnification) (Note: Normal cyto architecture).

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Beside this the test drug did not produce any significant changes in the cytoarchitecture of any organ studied at various dose levels. There were no apparent gross lesions at necropsy and histological examination of vital organs revealing any pathological changes, except very rare incidence of cells with micronuclei in bone marrow smear. (Photomicrographs).

No change could be observed in any of the parameters studied. Hence it may be concluded that the test drug has no serious toxicity producing potential which may involve tissue loss or body weight loss. At TED dose level none of the biochemical parameter was found to be significant in comparison to control group. At average dose level two parameters were found to be affected- they were significant increase in lymphocyte percentage and significant decrease in granulocyte percentage. The observed effect may be indicative of the increased activity of lymphoid organs and decrease in myeloid activity. Since, it is not dose dependent and only moderate in magnitude it may not have any serious toxic implications.

Analysis of the histopathological scanning shows that the drug Navratna rasa is well tolerated at all the doses levels. Though some changes were observed which were not of serious nature. Hence, it may be concluded that the test drug has no serious toxicity producing potential in most of the organs studied.

It seems Saper *et al*. (2004) have failed to analyze the different forms by which the elements are bound but have projected only the quantum of elemental distribution. This is critical since these elements could be chelated in the formulation and will be safe to use. The results of the present study also confirms the nontoxic nature of the drug as the final product in bhasma/raskalpas are different from the raw materials since they would be transformed to therapeutic compounds by different processes like detoxification, titration, heating etc. Hence, it is unlikely that free elements would be present in these products.

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