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Original Research Article

Preclinical hematological profile studies of an ayurvedic medicine Rohitakarista after chronic administration to male Sprague-Dawley rats

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ABSTRACT

Background: Rohitakarista (RHT) is a classical Ayurvedic formulation, traditionally used for the treatment of splenomegaly, particularly among rural populations.
Methods: The acute toxicity study of RHT in male Sprague-Dawley rats showed no mortality even at 80 ml/kg body weight. For chronic toxicological evaluation, rats were divided into two groups (n=10 per group). One group received RHT suspension orally at 40 ml/kg body weight daily for 41 consecutive days, while the control group received water. At the end of the treatment period, blood samples were collected to evaluate 25 different hematological parameters.
Results: RHT-treated rats showed a significant increase in absolute neutrophil count (153.96%, p=0.05) and neutrophil percentage (84.60%, p=0.04), alongside a significant decrease in lymphocyte percentage (24.62%, p=0.04). Red blood cell count (13.34% decrease, p=0.03), hemoglobin level (12.94% decrease, p=0.04), and hematocrit (14.29% decrease, p=0.01) were also significantly reduced. Non-significant but noticeable changes included increased WBC count (23.80%), eosinophils (216.67%), monocyte percentage (84.06%), and platelet count (17.71%). Other parameters such as MCV, MCH, MCHC, RDW, ESR, bleeding time, clotting time, and platelet indices showed minor and nonsignificant variations.
Conclusions: Chronic RHT administration led to significant hematological changes, particularly in neutrophil and erythrocyte indices, suggesting potential immunological and anemic risks. Further biochemical and histopathological studies are recommended to better understand its overall safety profile.

Keywords: Ayurvedic formulation, Hematology, Rohitakarista, Splenomegaly, Toxicology

INTRODUCTION

Ayurvedic medicine is a traditional Indian medical system widely used for various health conditions due to its perceived efficacy, safety, and holistic approach to healing. Rohitakarista (RHT) is a classical Ayurvedic polyherbal formulation recognized in several Ayurvedic pharmacopeias. It is traditionally indicated for spleen and liver disorders and is described in the Bhaisajyaratnavali, under the chapter Plihayakṛdroga Adhikara (verses 84-

85½).[1] It contains *Tecomella undulata* (Rohitaka stem bark) as the principal ingredient, along with other medicinal plants such as *Woodfordia fruticosa* (Dhataki), *Piper longum* (Pippali), *Plumbago zeylanica* (Citraka), *Zingiber officinale* (Sunthi), *Terminalia chebula* (Haritaki), *Terminalia bellirica* (Bibhitaka), *Emblica officinalis* (Amalaki) etc. (Table 1), each contributing unique therapeutic properties.[2]

Table 1: List of ingredients for the preparation of Rohitakarista (RHT)* suspension.

Ingredient name	Botanical name	Part used	Quantity (per batch)
Rohitaka	*Tecomella undulata*	Stem bark	4.8 kg
Dhataki	*Woodfordia fruticosa*	Flower	768 gm
Pippali	*Piper longum*	Fruit	48 gm
Pippali mula	*Piper longum*	Root	48 gm
Cavya	*Piper chaba*	Stem	48 gm
Citraka	*Plumbago zeylanica*	Root	48 gm
Sunthi	*Zingiber officinale*	Rhizome	48 gm
Tvak	*Cinnamomum zeylanicum*	Stem bark	48 gm
Ela	*Elettaria cardamomum*	Seed	48 gm
Patra	*Cinnamomum tamala*	Leaf	48 gm
Haritaki	*Terminalia chebula*	Pericarp	48 gm
Bibhitaka	*Terminalia bellirica*	Pericarp	48 gm
Amalaki	*Emblica officinalis*	Pericarp	48 gm
Jaggery (Guda)	—	Sweetener	9.6 kg
Water for decoction	—	Solvent	49.2 l (reduced to 12.3 l)

*Rohitakarista is approved for industrial-scale manufacture in Bangladesh, as listed in the National Ayurvedic Formulary (1992) by the Ministry of Health.

Traditionally, RHT has been used to manage conditions such as splenomegaly, liver disorders, abdominal diseases, jaundice, and skin ailments. It is also believed to enhance lymphocyte production and reduce toxins arising from infections.[2] Recent preclinical studies have demonstrated that chronic administration of RHT can significantly alter hematological parameters in animal models, highlighting potential impacts on immune function.[3,4] Moreover, toxicological evaluations have reported no mortality even at high doses, yet chronic use has indicated certain biochemical changes, particularly in renal function markers and lipid profiles, underscoring the importance of comprehensive safety evaluations for long-term clinical use.[5,6] Additionally, studies have reported alterations in thyroid and steroid hormone profiles following prolonged administration of RHT in animal models, necessitating further detailed investigations into endocrine safety.[7,8] Significant effects on hepatic enzymes and renal function parameters have also been observed.[9]

Given the wide therapeutic applications and increasing use of RHT, rigorous preclinical evaluations are imperative to ascertain its safety profile and therapeutic index. This study aimed to investigate the chronic toxicological impacts of RHT on various hematological parameters in male Sprague-Dawley rats, thereby contributing critical safety data essential for its clinical validation and broader acceptance.

METHODS

Drugs, chemicals and reagents

For this research, Rohitakarista (RHT) was obtained from Sri Kundeswari Aushadhalaya Limited, Chittagong, Bangladesh. Ketamine injection was procured from ACI Pharmaceuticals Limited, Bangladesh. All other reagents, assay kits, and chemicals used in this study were purchased from Human GmbH, Wiesbaden, Germany.

Experimental animal

Eight-week-old, healthy male albino rats (Rattus norvegicus, Sprague-Dawley strain) weighing 50-70 gm were bred and maintained in the animal house of the department of pharmacy, Jahangirnagar University for the hemotoxicological study.

All rats were housed in plastic cages (30 × 20 × 13 cm) with softwood shavings as bedding. They were maintained under a natural day night cycle in a well-ventilated and hygienic animal facility. The animals were fed ad libitum with a specially prepared ‘rat chow’ formulated according to the standard developed by the Bangladesh Council of Scientific and Industrial Research (BCSIR). Clean drinking water was also provided ad libitum.[10]

All experimental procedures were conducted in strict accordance with the ethical guidelines for the care and use of laboratory animals, and were approved by the ethical review committee, faculty of life sciences, department of pharmacy, Jahangirnagar University.

Experimental design

Acute toxicity studies

The acute oral toxicity test was conducted following the guidelines of the Organization for Economic Co-operation and Development (OECD) for the testing of chemicals with minor modifications, as described in OECD Guideline 425.[11] Additionally, methodological principles

described by Walum et al were followed for dose-ranging and observational parameters, as previously applied in our earlier study on *Citrus macroptera* extract.[12] Sixteen healthy male rats, weighing between 50 gm and 70 gm, were randomly divided into four groups, with four rats in each group. Different doses of the experimental drug RHT (50 ml/kg, 60 ml/kg, 70 ml/kg, and 80 ml/kg) were administered orally using a stomach tube. Each dose was divided into two fractions and administered within a 12-hour interval.

Following administration, the animals were closely observed for mortality and clinical signs of toxicity, including changes in general behavior, respiratory patterns, cardiovascular signs, motor activity, reflexes, and alterations in skin and fur texture. Observations were recorded at 1-, 2-, 3-, and 4-hours post-administration, and then once daily for the subsequent three days.

Chronic toxicological studies

Prior to the experiment, rats were randomly divided into two groups consisting of 10 animals each. One group received RHT, while the other served as the control. The control group was administered distilled water in the same volume as the RHT-treated group for a duration of 41 days. All administrations were performed via the oral route at a dose of 40 ml/kg body weight.

Following an acclimatization period, the Ayurvedic formulation was administered using an intra-gastric syringe once daily between 10:00 am and 12:00 pm throughout the study period. All animal experiments were conducted in strict accordance with the ethical guidelines for the care and use of laboratory animals. To ensure accurate tracking and data collection, each animal was marked on the tail for identification purposes. Observations were recorded individually for each animal at specified time intervals, both before and after administration.[13]

Blood samples collection and preparation of serum

At the end of the 41-day treatment period, following an 18-hour fasting phase, rats from each group were anesthetized by intraperitoneal (i.p.) administration of ketamine at a dose of 500 mg/kg body weight.[14]

Blood samples were collected from the post vena cava of each rat into EDTA (ethylene diamine tetra acetic acid) tubes for hematological analysis and into plain tubes for serum preparation for biochemical analysis. To obtain serum, the blood was allowed to clot for 30 minutes and then centrifuged at 4000 x g (relative centrifugal force, where g is the Earth's gravitational force of 9.8 m/s^2) for 10 minutes using a bench top centrifuge (MSE Minor, England). The supernatant serum was carefully collected using a dry Pasteur pipette and stored in a refrigerator until further analysis. All analyses were completed within 12 hours of sample collection.[15]

Determination of hematological profile studies

Hematological profile analysis included the evaluation of parameters such as red blood cell (RBC) count and platelet count, both determined using the electrical impedance method.[16] Hemoglobin (Hb) concentration was measured using the modified hemoglobin cyanide method.[17] Hematocrit (HCT) was calculated from the RBC count and mean corpuscular volume (MCV) using the following formula:

$$HCT = (RBC \times MCV) \div 10$$

The MCV, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using formulas described by Wintrobe and by Diem and Clenter:[18,19]

$$MCV = HCT\ (\%) \div RBC\ count\ (millions) \times 10$$

$$MCH = Hb\ (g/dl) \div RBC\ count\ (millions) \times 10$$

$$MCHC = Hb\ (g/dl) \div HCT\ (\%) \times 100$$

Red cell distribution width (RDW) was calculated using the formula:

$$RDW = (SD\ of\ MCV \div Mean\ MCV) \times 100.20$$

Total white blood cell (WBC) counts were obtained using the CELL-DYN 3700 system, which provides two values: WBC impedance count (WIC) and WBC optical count (WOC).

Platelet indices, including platelet count (PLT), mean platelet volume (MPV), and platelet distribution width (PDW), were determined by impedance resistance using automated analyzers (CELL-DYN 1700 and GENS).[21] The erythrocyte sedimentation rate (ESR) was measured using the Westergren method.[22]

Statistical analysis

The group data were expressed as Mean±SEM (standard error of the mean). Statistical analysis was performed using the independent samples t-test in IBM SPSS Statistics version 29.0.2 for Mac. Differences between groups were considered statistically significant at $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***).

RESULTS

Acute toxicity studies

No mortality was observed in rats administered the highest tested dose of RHT (80 ml/kg body weight) (Table 2). Therefore, it can be inferred that the median lethal dose (LD_{50}) of RHT is greater than 80 ml/kg body weight. Additionally, none of the animals exhibited signs of

restlessness, respiratory distress, general discomfort, or convulsions.

Given the long-standing traditional use of this Ayurvedic formulation for various ailments, a limit test was conducted in accordance with OECD Guideline 425. The limit test was performed at the maximum starting dose level (80 ml/kg body weight), supported by existing evidence indicating low or negligible toxicity and the absence of mortality. These results suggest that acute oral administration of RHT, even at doses higher than the usual therapeutic level (40 ml/kg body weight), does not induce observable toxic effects. Therefore, RHT may be considered safe for use in oral formulations under acute exposure conditions.

Table 2: Acute toxicity study of Rohitakarista (RHT) in male rats when administered orally.

Dose	50 ml/kg		60 ml/kg		70 ml/kg		80 ml/kg	
Status	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
RHT	4	0	4	0	4	0	4	0
Mortality	0		0		0		0	

Table 3: Hematological profiles after chronic administration of Rohitakarista (RHT) in dose 40 ml/kg to the male Sprague-Dawley rats for 41 days.

Parameters	Control (Mean±SEM)	RHT (Mean±SEM)	p value	% Change
White Blood Cells (WBC)	5.302±0.3984	6.564±0.6430	0.13	↑ 23.80
Neutrophil (Abs)	1.060±0.0772	2.692±0.6218	0.05*	↑ 153.96
Eosinophil (Abs)	0.012±0.0120	0.038±0.0159	0.22	↑ 216.67
Basophil (Abs)	0.000±0.0000	0.000±0.0000	-	-
Lymphocyte (Abs)	4.004±0.3255	3.576±0.1683	0.27	↓ 10.69
Monocyte (Abs)	0.186±0.0081	0.216±0.0616	0.65	↑ 16.13
Neutrophil (%)	20.780±0.2709	38.360±5.9206	0.04*	↑ 84.60
Eosinophil (%)	0.0878±0.0336	0.0152±0.0082	0.09	↓ 82.69
Basophil (%)	2.080±0.0520	2.954±0.6237	0.23	↑ 42.02
Lymphocyte (%)	76.520±0.4152	57.680±6.5741	0.04*	↓ 24.62
Monocyte (%)	0.5444±0.1585	1.0020±0.4321	0.36	↑ 84.06
Red Blood Cells (RBC)	7.120±0.0880	6.170±0.2993	0.03*	↓ 13.34
Hemoglobin (Hb)	12.060±0.4770	10.500±0.4254	0.04*	↓ 12.94
Haematocrit (HCT)	42.980±0.4994	36.840±1.5131	0.01**	↓ 14.29
MCV	60.360±0.3893	59.800±0.5753	0.44	↓ 0.93
MCH	17.520±0.3261	17.360±0.3414	0.74	↓ 0.91
MCHC	29.040±0.6021	29.060±0.3295	0.97	↑ 0.07
RDW	9.806±0.0979	10.822±0.6472	0.15	↑ 10.36
ESR	2.400±0.2449	2.200±0.2000	0.54	↓ 8.33
Bleeding time (BT)	48.000±3.0000	48.000±5.6124	1.00	-
Clotting time (CT)	219.000±6.0000	213.000±5.6124	0.48	↓ 2.74
Platelets	461.800±7.2484	543.600±37.4147	0.09	↑ 17.71
MPV	3.816±0.0645	3.784±0.0276	0.66	↓ 0.84
Platecrit (PCT)	0.1762±0.0049	0.2056±0.0137	0.07	↑ 16.69
PDW	14.420±0.2395	14.160±0.0748	0.33	↓ 1.80

↑: increase, ↓: decrease, p≤0.05 (*), p≤0.01 (**), and p≤0.001 (***).
Abs: absolute value, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell volume distribution width, ESR: erythrocyte sedimentation rate, MPV: mean platelet volume, PDW: platelet volume distribution width.

Hematological profiles after chronic administration of RHT in male rats (Table 3)

Chronic administration of Rohitakarista (RHT) at a dose of 40 ml/kg for 41-days resulted in several alterations in hematological parameters compared to the control group.

A significant increase was observed in absolute neutrophil count (↑ 153.96%, p=0.05) and neutrophil percentage (↑ 84.60%, p=0.04), indicating a shift in leukocyte distribution. A corresponding significant decrease in lymphocyte percentage (↓ 24.62%, p=0.04) was also noted, although the absolute lymphocyte count showed a non-

significant reduction (↓ 10.69%, p=0.27). Total white blood cell counts increased (↑ 23.80%, p=0.13), while changes in eosinophils, basophils, and monocytes, both absolute and percentage values, were not statistically significant.

Red blood cell parameters showed a significant decrease in RBC count (↓ 13.34%, p=0.03), hemoglobin level (↓ 12.94%, p=0.04), and hematocrit (↓ 14.29%, p=0.01). However, no significant differences were observed in MCV, MCH, MCHC, or RDW.

The erythrocyte sedimentation rate (ESR) was slightly reduced (↓ 8.33%, p=0.54), though not statistically significant. bleeding time (BT) remained unchanged, while clotting time (CT) showed a minimal, non-significant decrease (↓ 2.74%, p=0.48).

Platelet count increased by 17.71% (p=0.09), while platelet indices such as MPV, PCT, and PDW showed minor non-significant changes.

Overall, the data suggest that chronic RHT administration may affect leukocyte distribution and erythropoiesis, particularly by increasing neutrophils and reducing RBC-related parameters, without causing major disturbances in platelet function or coagulation time.

DISCUSSION

This study assessed the hematological effects of chronic oral administration of Rohitakarista (RHT) in male Sprague-Dawley rats over 41 days. The results revealed significant changes, indicating impacts on innate immunity and erythropoiesis. A rise in absolute neutrophil count and percentage suggests enhanced granulopoiesis, likely due to immunomodulatory compounds such as flavonoids and alkaloids in RHT. Similar trends were noted with immunostimulatory herbs like *Tinospora cordifolia* and *Withania somnifera*.[23,24] The reduced lymphocyte percentage, without significant change in absolute count, may reflect leukocyte redistribution, consistent with immune modulation.[25]

Significant reductions in RBC count, hemoglobin, and hematocrit indicate suppressed erythropoiesis or reduced red cell lifespan. As MCV, MCH, and MCHC remained unchanged, the anemia was likely normocytic and normochromic. Similar findings were reported with long-term exposure to *Boswellia sacra*.[26]

Platelet count increased moderately, but clotting time, bleeding time, and indices like MPV, PCT, and PDW remained unchanged, suggesting no adverse impact on platelet function.[27]

Non-significant increases in WBC count, eosinophils, and monocytes align with RHT's traditional role as an immune modulator. Stable ESR and coagulation values further indicate a lack of systemic inflammation or hematological toxicity.

CONCLUSION

In conclusion, chronic RHT administration altered leukocyte balance and red cell parameters but did not impair coagulation or induce hematotoxicity. Further studies are needed to explore long-term and dose-dependent effects.

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