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Original Research Article

Hematological Studies of an Ayurvedic Medicine "Vata Gajendra Singha" Used in Rheumatoid Arthritis After Chronic Administration to Male Sprague-Dawley Rats

Nilay Saha[1]*, Md. Mamun Sikder[2], Md. A. Uddin[1], Ferdousi Akter[1], Umma H. Asha[3], Tahmina A. Sony[1], Nuruzzaman Neon[1], Saquiba Yesmine[1], M Shahabuddin K. Choudhuri[1]

[1]*Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh;*
[2]*Department of Biochemistry, Kagawa University School of Medicine, 1750-1 Ikenobe, Miki, Kagawa 761-0793, Japan;*
[3]*Bangabandhu Sheikh Mujibur Rahman Science & Technology University, Gopalganj, Bangladesh.*

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ABSTRACT

Vata Gajendra Singha (VGS), an Ayurvedic preparation, is used in traditional medicine to treat rheumatoid arthritis in the rural population. The effect of chronic administration of Vata Gajendra Singha (VGS) on the hematological parameters was studied. The acute test of VGS recorded no death, even at the highest dose of 80 ml/ Kg body weight. During chronic testing, the animals were divided into three groups. The first two groups were given VGS preparation at a low dose of 50 mg/kg and a high dose of 400 mg/kg for 28 days, while the third group that served as the control received water for the same period. After 28 days of chronic administration of the VGS preparation, some hematological parameter changes were noted. There were notable changes in the percentage of neutrophils, lymphocytes, Mean Corpuscular Hemoglobin, and platelets attributed on the high and low dosage of Vata Gajendra Singha. These hematological parameters are some important factors related to a variety of disease conditions. Vata Gajendra Singha negatively affect some hematological parameters abnormally in treated rats and should not be administered chronically at a higher dose.

Keywords: Vata Gajendra Singha, Ayurvedic preparation, rheumatoid arthritis, hematological.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disorder that primarily affects joints resulting in warm, swollen, and painful joints. RA is a chronic disorder that affects about 24.5 million people in 2015 around the world.[1] This is between 0.5 and 1% of adults affected in developed countries making a major concern about this disease.[2] Middle age and women are affected 2.5 times as frequently as men.[2,3] It resulted in 38,000 deaths in 2013, 49,000 deaths and 28,000 deaths in 1990 due to RA globally.[2,4] The age at which the disease is most commonly affect women between 40 and 50 years of age, and for men somewhat later.[5] Ayurvedic medicines are manufactured from natural sources widely used in different diseases for their well content of medicinal properties though chronic studies found fluctuation in lipid profile and hormonal imbalance in the treated animals.[6,7] Vata Gajendra Singha (VGS) is an Ayurvedic preparation. It is used in traditional medicines to treat rheumatoid arthritis in the rural population. Vata Gajendra Singha (265 p.) is included in the Bangladesh National Formulary of Ayurvedic Medicine 1992.[8]

*Corresponding author. E mail: nilay.saha.ju@gmail.com
Tel: +1(307) 761-0491

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Bangladesh National Formulary of Ayurvedic Medicines is compiled by the National Unani and Ayurvedic Formulary Committee and published by the Bangladesh Board of Unani and Ayurvedic Systems of Medicine, 38, Bangabandhu Avenue, Dhaka-1000, under the authority vested in the Board vide section 13(j) of the Bangladesh Unani and Ayurvedic practitioners Ordinance, 1983 in collaboration with the World Health Organization.[9]

Permission to manufacture at an industrial scale is printed on page no. 533 (column 1: Product code 12.43). Directorate of Drug Administration has issued Notification DA/Admin/1-10/96/6212 dated 19th October 1996 has issued a license under Drug Act, 1940 and Rules there under and Drug (Control) Ordinance 1982 for local manufacture and sales in Bangladesh. (Published Bangladesh Gazette #24 Part VI dated Thursday, June 11th 1998.). At present, many Ayurvedic manufacturers are manufacturing and marketing the Classical Ayurvedic Medicinal Preparation.[10-14]

Materials and Methods

Drugs Chemicals, and Reagents: Vata Gajendra Singha (VGS) was collected from Sri Kundeswari Aushadhalaya Ltd., Chittagong in 2018 (Table 1). Ketamine injection was purchased from ACI. Pharmaceuticals Ltd., Bangladesh in 2018. All other reagents, assay kits, and chemicals used in this research were purchased from Human GmbH, Wiesbaden, Germany.

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 (JU), were used in the hematological experiment. These animals were apparently healthy and weighed around 60-70 g. The animals were kept in a well-ventilated, clean experimental animal house under steady environmental and sufficient nutritional conditions throughout the experiment. They were fed with rat chow made according to the formula developed by the Bangladesh Council of Scientific and Industrial Research (BCSIR). Water was provided ad libitum and the animals were retained at 12 hours day and 12 hours night cycle. The rats were fed with standard pellet diets (prepared according to the formula developed at BCSIR,

Dhaka, Bangladesh). The animals were handled in accordance with the ethical guidelines and the study protocol was approved by the Biosafety, Biosecurity and Ethics Committee of Jahangirnagar University (Ethics approval no: BBEC, JU/ M 2018 (5)2), Savar, Dhaka, Bangladesh.[38]

Experimental Design Acute pharmacological study: The acute oral pharmacological test was performed following the guidelines of the Organization for Economic Co-operation and Development (OECD) for testing chemicals with minor adjustments (OECD Guideline 425).[15] Sixteen male mice (30-40 g body weight) were separated into four groups of four animals each. Different doses (50 ml/Kg, 60 ml/Kg, 70 ml/Kg, and 80 ml/Kg) of the experimental drug Vata Gajendra Singha (VGS) were administered orally. The dose was divided into two sections and given within twelve hours. Then all the experimental animals were observed for mortality and clinical toxicity signs (general behavior, cardiovascular signs, respiratory pattern, reflexes, motor activities, 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 Vata Gajendra Singha (VGS) administration.

Chronic hematological studies: The rats were randomly divided into 3 groups of 8 animals each. Two groups were treated with VGS at a low dose of 50mg/kg and a high dose of 400mg/kg, and another was used as a control (Table 2). The control animals were administered with distilled water only as per the same volume as the drug-treated group for 28 days. After acclimatization, Ayurvedic medicinal preparation was administered to the rats by an intra-gastric syringe between 10 am to 12 am daily throughout the study period. All experiments on rats were carried out in absolute compliance with the ethical guidelines for the care and use of laboratory animals. The experimental animals were marked carefully on the tail, which helped identify a particular animal. By using identification marks, responses were noted separately for a particular period prior to and after the administration.[16]

Blood Samples Collection and Preparation of Serum: At the end of the 28 days treatment period, after 18 hours of fasting, rats from each group were anesthetized by administration (i.p) of ketamine (500 mg/Kg body weight).[17] Blood samples were accumulated from the post vena cava of rats into EDTA (Ethylene di-amine tetra acetic acid) sample tubes for hematological analysis and into plain sample tubes for serum production for biochemical analysis. Serum was obtained after allowing the blood coagulate for 30 minutes and centrifuged at 4000 g for 10 minutes using a benchtop centrifuge instrument (M.S.E. Minor, England). The supernatant serum samples were collected by dry Pasteur pipette and stored in the refrigerator for further analysis. All analyses were finished within 12 hours of sample collection.[18]

Determination of Hematological Profile Studies: Hematological profile studies involve the analysis of parameters such as Red Blood Cells (RBCs) and platelet levels determined by the Electrical Impedance method.[19] Hemoglobin (HGB) level was determined by the modified hemoglobin cyanide method.[20] The HCT was *calculated* from the RBC count and the MCV as follows:

HCT = (RBC x MCV)/10

MCV, MCH, and MCHC are calculated according to the formula as given by Wintrobe[21] and Diem and Clenter.[22]

MCV = [HCT (%) / RBC count (millions)] X 10

MCH = [Hb (g/dL) / RBC count (millions)] X 10

MCHC = [Hb (g/dL) / HCT (%)] X 100

Mathematically the RDW is calculated with the following formula:

RDW = (Standard deviation of MCV ÷ mean MCV) × 100.[23]

Two WBC values were provided by the CELL-DYN 3700 System:(1) The W.I.C. (WBC Impedance Count) and (2) The WOC (WBC Optical Count). Impedance resistance was used for the measurement of platelet indices in all blood samples (analyzers CELL DYNN 1700 and GENS), such as platelet count (PLT), mean platelet volume (MPV), and platelet distribution width (PDW)).[24] Erythrocyte Sedimentation Rate (ESR) were measured by Westergren Method.[25]

Statistical Analysis: Values are presented as mean±S.E.M (n=8). SPSS (Statistical Package for Social Science) for WINDOWS was applied for the analysis of data. One-way ANOVA followed by Dunnett's multiple comparisons was performed to analyze this dataset when compared against control. Dunnett t-tests treat one group as a control and compare all other groups against it. For Dunnett test, $^{X}p<0.05$, $^{Y}p<0.01$, $^{Z}p<0.001$.

Table 1: Name of the ingredients/herbs used in the preparation of "Vata Gajendra Singha (VGS.)"[39]

Sl no	Ingredient	Plant part	Botanical/Zoological or Calyx name	Family	Amount
1	Sutaka	Kajjali	Hydragentum		20 g.
2	Gandhaka		Purified Sulphur oxide		
3	Abhraka bhasma	Calyx	Purified Mica oxide $K(Mg,Fe)_3AlSi_3O_{10}$ $(Fe,OH)_2$		10 g.
4	Lauha bhasma	Calyx	Purified Iron oxide Fe_3O_4		10 g.
5	Tamra bhasma	Calyx	Purified Copper oxide (CuO)		10 g.
6	Naga bhasma	Calyx	Purified Plumbum oxide (PbO,Pb_3O_4)		10 g.
7	Tankana	Lavana	Borax ($Na_2B_4O_7$, $10H_20$)		10 g.
8	Saindhava	Lavana	Rock salt (Sodium chloride)		10 g.
9	Vatsanabha	Rhizome	*Aconitum ferox Wall.*	Ranunculaceae	10 g.
10	Lavanga	Flower	*Syzygium aromaticum Merr & L.M.Perry.*	Myrtaceae	10 g.
11	Hingu bhrsta	Exudate	*Ferula asafetida L.*	Apiaceae	10 g.
12	Jatiphala	Seed	*Myristica fragrans Houtt.*	Myristicaceae	10 g.
13	Tvak	Stem Bark	*Cinnamomum zeylanicum Blume*	Lauraceae	5 g.
14	Ela laghu	Seed	*Elettaria cardamomum Maton.*	Zingiberaceae	5 g.
15	Patra	Leaf	*Cinnamomum tamala Nees & Eberm.*	Lauraceae	5 g.
16	Amalaki	Fruit Pulp	*Emblica officinalis Gaertn.*	Euphorbiaceae	5 g.
17	Haritaki	Fruit Pulp	*Terminalia chebula Retz*	Combretaceae	5 g.
18	Bibhitaka	Fruit Pulp	*Terminalia bellerica Roxb.*	Combretaceae	5 g.
19	Jiraka	Seed	*Cuminum cyminum L.*	Apiaceae	5 g.
20	Kanya(ghrtakumari)	Exudate	*Aloe barbadensis Mill.*	Liliaceae	

Results and Discussion

Acute pharmacological study: (VGS) administered up to a high dose of 80 ml/Kg produced no mortality of the experimental animals. Thus the LD50(Median Lethal Dose) value was found to be greater than 80 ml/Kg body weight. The animals did not demonstrate any sign of restlessness, general irritation, respiratory distress, or convulsion. Since VGS has been in clinical use for rheumatoid arthritis treatment 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 supporting the test material's low or non-toxicity and immortality nature, the limit test at the highest starting dose level (80 ml/Kg body weight) was conducted. No mortality and toxicity signs were observed at 80 ml/Kg body weight. Therefore, it can be concluded that VGS when administered at a single dose, is non-toxic and can be used safely in oral formulations.

Chronic Hematological Study Studies

At a low dose, there was a decrease in the percentage of the Neutrophil count of the male rat, the decrease though not significant, yet it was prominent (p=0.316). On the contrary, at the higher dose, there was a statistically significant (p=0.045) increase in the percentage of the Neutrophil count of the male rat. At a low dose, there is a negligible increase in the percentage of Lymphocyte count of the male rat, which was statistically not at all significant (p=0.790). In contrast, on the contrary, at the higher dose, there is a statistically highly significant (p=0.010) decrease in the percentage of Lymphocyte count of the male rat. In both doses, there is a decrease in the Mean corpuscular volume and a red cell index of the male rat; the decrease is statistically very highly significant (p=0.004 and p=0.001, respectively). Both at the low dose and the high dose, there is a decrease respectively in the Mean corpuscular hemoglobin, a red cell index of the male rat; the decrease is statistically significant (p=0.023 and p=0.016, respectively). Both at the low dose and the high dose, there is an increase respectively in the mean platelet volume of the male rat; the increase was statistically significant (p=0.022 and p=0.050, respectively (Table 3).

Blood is a tissue that is very vulnerable to a wide range of drug or toxic insults, and the effects may be far-reaching and grave.[26] To a large extent, toxicity to the bone marrow can be assessed by peripheral blood measurements. Marked hematological changes at all dose levels would indicate that a drug would not be suitable for development. However, the sensitivity of hematological measurements is constantly increasing with the development of micro-automated methods. As a result, it should be recognized that subtle but significant treatment-related changes may come to light which has little clinical relevance. The analysis of blood parameters is strongly related to risk evaluation because when tests involve rodents, the hematological system has a higher prognostic value of any abnormal toxicity signs and symbols in humans[26,38]. We found noticeable hemolytic changes on some major hematological parameters after chronic administration of VGS.

Neutrophils are phagocytic granulocytes that constitute a vital component of the rapid "non-specific" immune defenses. Neutrophils have a short circulating life span after leaving the bone marrow and then go through apoptosis.[27] Antiapoptotic signals generated by growth factors and cytokines can affect neutrophil survival and increase neutrophil numbers.[28] In our study, we found that in high doses (400mg/kg) after chronic administration of the VGS, the percentage of neutrophils was significantly increased (<p=.05) and the percentage of lymphocytes was highly significantly decreased (<p=.01) in high dose (Table 3). The neutrophil increase can occur due to several mechanisms, such as a shift from marginating to circulating pool, increased release from bone marrow, increased production by the bone marrow, and decreased migration into tissue. Neutrophils too high, known as neutrophilia,[29] may cause acute bacterial infections, inflammation, and tissue death. Low lymphocyte concentration is associated with increased infection rates after surgery or trauma. Lymphocytes too low, known as lymphocytopenia[29] may cause autoimmune disorders such as rheumatoid arthritis and bone marrow damage.

The value of Mean Corpuscular Volume (MCV) highly significantly decreased (<p=.01) in low dose and very highly significantly decreased (<p=.001) in high dose to the VGS treated rats in our study (Table 3). Low levels of MCV are a sign that red blood cells are smaller in volume and are, therefore, not able to hold an adequate quantity of hemoglobin in them.

Table 2: Hematological profiles after chronic administration of VGS in low dose 50mg/kg and high dose 400mg/kg to the male rates.

Parameters	Control	VGS 50mg/kg	VGS 400mg/kg
WBC	4.950 ± 0.4536	5.440 ± 0.4411	4.240 ± 0.2400
Neutrophil	1.2738 ± 0.08850	1.3660 ± 0.10386	1.3440 ± 0.15523
Lymphocyte	3.1575 ± 0.57371	4.0200 ± 0.34028	2.8280 ± 0.11342
Monocyte	.0562 ± 0.00778	.0560 ± 0.00510	.0580 ± 0.00917
% Neutrophil	26.12 ± 1.141	23.20 ± 1.594	31.40 ± 2.015 ^{X}
% Lymphocyte	72.75 ± 1.082	73.80 ± 0.583	67.00 ± 1.897 ^{Y}
%Monocyte	1.12 ± 0.125	1.00 ± 0.000	1.40 ± 0.245
RBC.	6.5838 ± 0.15688	6.8260 ± 0.15661	7.0340 ± 0.14278
Hemoglobin	11.888 ± 0.2900	11.700 ± 0.2121	12.040 ± 0.3444
Hematocrit	36.9625 ± 0.84683	36.4200 ± 0.61188	37.2600 ± 0.82073
MCV	56.162 ± 0.5057	53.400 ± 0.5348 ^{Y}	53.000 ± 0.5413 ^{Z}
MCH.	18.050 ± 0.2104	17.160 ± 0.1965 ^{X}	17.100 ± 0.2490 ^{X}
MCHC.	32.162 ± 0.2044	32.160 ± 0.0678	32.280 ± 0.2332
RDW.	12.8125 ± 0.31984	12.5400 ± 0.29933	12.3600 ± 0.17493
Platelet	437.50 ± 23.12621	475.00 ± 27.29469	485.00 ± 10.48809
MPV.	4.0025 ± 0.06391	4.2840 ± 0.07827 ^{X}	4.2420 ± 0.06880 ^{X}
P.D.W.	15.9125 ± 0.10596	16.3000 ± 0.20976	16.2200 ± 0.08602
ESR.	1.38 ± 0.183	1.00 ± 0.000	1.00 ± 0.000

Values are presented as mean ± SEM (n=8). One way ANOVA followed by Dunnet's multiple comparisons was performed to analyze this dataset when compared against control. For Dunnet test, $^{X}p<0.05$, $^{Y}p<0.01$, $^{Z}p<0.001$

Table 3: Research outline

	Low dose = 50 mg/kg				High dose = 400 mg/kg			
Parameters	**Trend**	**Significance Level / p value**	**Percentage**	**Incr/Decr**	**Trend**	**Significance Level / p value**	**Percentage**	**Incr/Decr**
WBC	↑	0.647	9.90%	Incr+	↓↓↓	0.421	14.34%	Decr-
NEU(abs)	↑	0.794	7.24%	Incr+	↑	0.873	5.51%	Incr+
LYMP (abs)	↑↑↑	0.37	27.32%	Incr+	↓↓↓	0.851	10.44%	Decr-
MONO(abs)	≈↓	1	0.36%	Decr-	↑	0.983	3.20%	Incr+
NEU(%)	↓↓↓	0.316	11.18%	Decr-	↑↑↑	*0.045	20.21%	Incr+
LYMP (%)	↑	0.79	1.44%	Incr+	↓	**0.010	7.90%	Decr-
MONO(%)	↓↓↓	0.793	10.71%	Decr-	↑↑↑	0.361	25%	Incr+
RBC	↑	0.475	3.68%	Incr+	↑	0.111	6.84%	Incr+
HAEM	↓	0.872	1.58%	Decr-	↑	0.913	1.28%	Incr+
HCT	↓	0.861	1.47%	Decr-	≈↑	0.955	0.80%	Incr+
MCV	↓	**0.004	4.92%	Decr-	↓	***0.001	5.63%	Decr-
MCH.	↓	*0.023	4.93%	Decr-	↓	*0.016	5.26%	Decr-
MCHC.	≈↓	1	0.01%	Decr-	≈↑	0.884	0.37%	Incr+
RDW	↓	0.756	2.13%	Decr-	↓	0.483	3.53%	Decr-
Platelet	↑	0.427	8.57%	Incr+	↑↑↑	0.273	10.86%	Incr+
M.P.V.	↑	*0.022	7.03%	Incr+	↑	*0.05	5.98%	Incr+
PDW	↑	0.107	2.43%	Incr+	↑	0.22	1.93%	Incr+
ESR	↓↓↓	0.148	27.54%	Decr-	↓↓↓	0.148	27.54%	Decr-

Having a low MCV level means that might be a patient of thalassemia or prolonged suffering from iron deficiency anemia. MCH test helps diagnose the type of anemia.[29] There are several reasons that can contribute to a person having a low MCH level, such as blood loss, folic acid, and or Vitamin B12 deficiency. The value of Mean Corpuscular Hemoglobin (MCH) significantly decreased (<p=.05) in both doses in the VGS-treated rats (Table 3). Low MCV or MCH levels can be due to lead poisoning, microcytic anemia or hemoglobinopathy.[30] Microcytic anemia is usually caused by an iron deficiency. Hemoglobinopathies are genetic disorders such as sickle cell anemia that result in abnormally shaped hemoglobin, making it unable to efficiently carry oxygen through the blood.[31]

MPV is a useful prognostic marker in myriad medical conditions, including ischemic heart disease, infective endocarditis, and cerebral infarction.[32–34] MPV may act as a risk factor for recurring myocardial infarction independent of established risk factors, which is simple and economical laboratory aid along with traditional cardiac biomarkers for risk stratification of acute coronary syndrome patients admitted to the emergency.[35] In both doses, MPV increased significantly (<p=.05) after chronic administration of the VGS (Table 3). When there is the destruction of platelets, MPV is higher. This may be seen in inflammatory bowel disease,[36] myeloproliferative diseases, immune thrombocytopenic purpura (ITP), and Bernard-Soulier syndrome. It may also be related to pre-eclampsia, recovery from transient hypoplasia and potential surrogate marker of predicting liver cirrhosis.[37] An increase in MPV is observed in idiopathic thrombocytopenic purpura, hyperthyroidism, atherosclerosis, diabetes, and myeloproliferative disorders.

Conclusion

From the results, it can be concluded that Vata Gajendra Singha (VGS) should not be administered chronically at a higher dose. ; Thus, it necessitates further investigation to picture out the reason for this discrepancy in case of different parameters that represent the proper functioning of hematological profiles.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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