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Investigation of hematologic parameter of an Ayurvedic preparation "Amalaki Rasayan" used in jaundice after chronic administration to sprague-dawley rats

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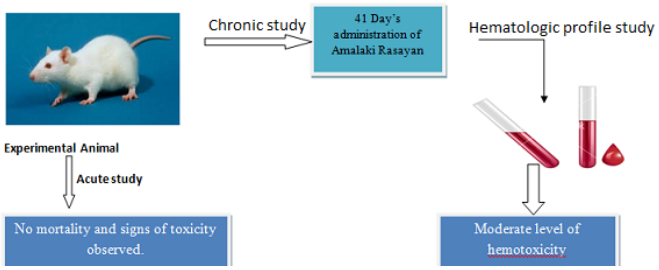
ABSTRACT



Amalaki Rasayan (MLK) is one of the prominent herbal formulations described in Ayurvedic classics which is used in treatment of jaundice. The effect of Amalaki Rasayan after chronic administration on hematological parameter was determined in this experiment. After 41 days administration of Amalaki Rasayan there was statistically prominent change in various blood cell count, differential wbc count and very slight change in other hematologic parameter. As the Result shows very moderate change in different hematological parameter it can be concluded that KTJ have less potential for hemotoxicity at high dose.

Keywords: Ayurvedic, Amalaki Rasayan, hemotoxicity, RBC, WBC

INTRODUCTION

The use of Ayurvedic medicine is one of the oldest approaches of treatment which was originated in South Asian region several thousand years ago. The modernized and modified practice of Ayurvedic traditions are a type of complementary or alternative medicine.[1] In recent years most of the population in Indian sub-continent is reported to use Ayurvedic medicine or medicinal plant to help meet their primary health care requirements.

Amalaki Rasayan (MLK) is one of the eminent herbal formulations described in Ayurvedic classics like Charak Samhita and Ashtang Hridaya.[2-3] It is widely used in view of the claim that it enhances life expectancy, fertility, body strength, intellect and reduces age-related debilities.[4] Recent study suggested that (MLK) have anti- secretory, anti-acid, and anti-ulcer activity.[5] It is also reported that dietary MLK suppressed neurodegeneration in fly models of poly Q-disorders or Alzheimer's disease without any severe side-effects.[6] Recent investigation suggested that Amalaki Rasayan even substantially inhibit induced apoptosis but not developmental one.[7] Amalaki Rasayan along with milk is very effective in treating ageing ailments.[8] It is used to relieve jaundice, heart disease, anemia, gout, hyperacidity non ulcer dyspepsia etc. It

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can also use as rejuvenator. MLK is prepared from fruits of Amla (*Phyllanthus emblica*, or *Emblica officinalis*) with some other ingredients through a specified process.[9]

Amlaki Rasayan (MLK), a classical Ayurvedic preparation which is included (page 70) in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (Approved by the Government of Bangladesh vide Ministry of Health and Family Welfare Memo No. Health-1/Unani-2/89/(Part-1) 116 dated 3-6-1991). Bangladesh National Formulary of Ayurvedic Medicine was 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.[10 -16]

By using Ayurvedic medicine expensive and extensive procedures of clinical investigations can be avoided in many cases. But Investigations of hemotoxicological studies are most often neglected for Ayurvedic medicines. Keeping in mind the present scenario, Amlaki Rasayan (MLK) an Ayurvedic preparation has been accomplished to explore a spectrum of its hematological parameters utilizing experimental animals. The objective was to have a better understanding of the possible hemogram profile of the drug under study and, to some degree, to justify about the safety of use of this drug is under the stated circumstances.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents:

For the toxicological study, Amlaki Rasayan was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was purchased from ACI Pharmaceuticals Limited, Bangladesh. All other reagents and chemicals used in this work were purchased from Human GmbH, Wiesbaden, Germany.

Experimental Animals:

The toxicological experiment use six to eight-week old male Sprague-Dawley rats that bred and maintained at the animal house of the Department of Pharmacy, Jahangirnagar University. These animals were apparently healthy and weighed 50-70 g. All of the rats were kept in plastic cages having dimensions of 30 x 20 x 13 cm and soft wood shavings were employed as bedding in the cages. 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.

Design of the Study:

Acute toxicity study:

The acute oral toxicity test was done according to the guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals and drug with minor modifications (OECD Guideline 425).[17] Sixteen female mice (non-pregnant, 30-35 g body weight) were divided into four groups of four animals each. Different doses of experimental drug (MLK) (50 ml/kg, 60 ml/kg, 70 ml/kg and 80 ml/kg) 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 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 hours and thereafter once a day for the next three days following MLK administration.

Chronic toxicity studies:

Before starting the experiment, Sprague-Dawley rats were randomly divided into 2 groups, each with 8 animals. One group was treated with MLK and another was used as control group. The control animals were administered with distilled water only as per the same volume as the drug treated group for 41 days. For all the pharmacological studies the drugs were administered per oral route at a dose of 40 ml/Kg body weight.[18] 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 tail 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.[19]

Blood Samples Collection:

At the end of the 41-day treatment period, the animals were fasted for 18 hours and also twenty-four hours after the last administration Ketamine (500 mg/kg i.p.) was administered for the purpose of anesthesia. Whole blood samples were collected from post vena cava and transferred to EDTA-added tubes immediately. All analyses were completed within 12 h of sample collection.[20]

Determination of hematologic parameter:

Hematologic profile studies involve analysis of parameters such as Red Blood Cells (RBCs) and WIC (WBC Impedance Count) level was determined by Electrical Impedance method,[21] Hemoglobin (HGB) level was determined by Modified hemiglobincyanide method,[22] Platelet level was determined by Electrical Impedance method,[21] WOC (WBC Optical Count) level determined by Laser light scatter.[23] Differential Analysis was done by the CELL-DYN 3700 System. Erythrocyte Sedimentation Rate is determined by Wintergreen Method.[24] For the determination of the bleeding time, modified procedure of Mohamed et al. (1969) was used.[25] Clotting time was determined with the method of Goldstein K.H et al.[26]. MCV, MCH and MCHC, HCT, PCT was calculated according to the formula as given by Wintrobe [27] and Diem and Clenter. [28]

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

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

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

HCT = (RBC x MCV)/10

PCT = (PLT x MPV)/10,000

Differential count of Neutrophil (%) = Number of Neutrophil\ Total number of Leucocytes

Differential count of Eosinophil (%) = Number of Eosinophil\ Total number of Leucocytes

Differential count of Basophil (%) = Number of Basophil\ Total number of Leucocytes

Differential count of Lymphocytes (%) = Number of Lymphocytes\ Total number of Leucocytes

Differential count of Monocytes (%) = Number of Monocytes\ Total number of Leucocytes

The platelet distribution width (PDW) is a measure of the heterogeneity of the PLT population. Red cell distribution width (RDW) is a measure of the heterogeneity of the RBC population.

Statistical Analysis

The group data are expressed as Mean ± SEM (Standard Error of the Mean). Unpaired "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 11) was applied for the analysis of data. Differences between groups were considered significant at $p < 0.05$, 0.01 and 0.001.

RESULTS

Acute toxicity study:

The drug (MLK) 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 sign of respiratory distress, restlessness, general irritation or convulsion. Since MLK is used for the clinical purpose of treatment of jaundice, heart disease, anemia, gout, hyperacidity non ulcer dyspepsia 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 (80 ml/kg body weight) was conducted. There were no mortality and signs of toxicity observed at 80 ml/kg body weight. Therefore, it can be concluded that MLK when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic Hematologic profile study:

In this experiment the Total Count (TC), Differential Count (DC), various erythrocyte parameters, platelet parameters, ESR and blood bleeding time and clotting times were determined. The results of the hemooxicological studies are given below.

Effect on different blood cell count.

There is a [6.66 %] decrease in the total numbers in the red blood cells of the male rat, the decrease though not significant yet it was prominent (p=0.31).There is a prominent (p=0.13, 25.99 %) increase in the number of white blood cell count and prominent (p=0.12, 14.51 %) increase in the number of platelet count of the male rat. There is an [28.11 %] increase in the absolute count of Neutrophils of the male rat, the increase though not significant yet it was prominent (p=0.22).There is a statistically insignificant (p=0.78, 33.33 %) decrease in the absolute count of Eosinophils of the male rat. There is also a statically prominent increase in both the absolute count of Lymphocytes (p=0.25, 24.83 %) and the absolute count of Monocytes (p=0.16, 39.78 %). of the male rat. All the results described in table 1.

Table 1: Effect of Amlaki Rasayana on different blood cell count in Male Rats.

Cell type	Control (Mean+SEM)	MLK (Mean+SEM)	P Values	%Change
RBC (million\µl)	7.120±.0880	6.646±.4342	0.31	↓ 6.66
WBC (thousand\µl)	5.302±.3984	6.680±.7140	0.13	↑↑↑ 25.99
Platelets (thousand\µl)	461.800±7.2484	528.800±34.6083	0.12	↑↑↑ 14.51
Neutrophil (thousand\µl)	1.060±.0772	1.358±.2139	0.22	↑↑↑ 28.11
Eosinophil (thousand\µl)	.012±.0120	.008±.0080	0.78	↓↓↓ 33.33
Basophil (thousand\µl)	0	0	---	----
Lymphocyte (thousand\µl)	4.004±.3255	4.998±.7416	0.25	↑↑↑ 24.83
Monocyte (thousand\µl)	.186±.0081	.260±.0434	0.16	↑↑↑ 39.78

Effect MLK on Differential (WBC) count in Male Rats:

There is a statistically insignificant (p=0.59, 9.14 %) decrease in the percentage of Neutrophil count of the male rat. There is a very high and statistically noticeable (p=0.09, 83.83 %] decrease in the percentage of Eosinophil count of the male rat and there a prominent (p=0.44, 88.94 %) increase in the percentage of Basophil count. There is a negligible and statistically insignificant (p=0.72, 2.20 %) decrease in the percentage of Lymphocyte count of the male rat. There is a very high [332.92 %] increase in the percentage of Monocyte count of the male rat, the increase though not significant yet it was prominent (p=0.37).All the results described in table 2.

Table 2: Effect of MLK on Differential (WBC) count in Male Rats

Parameter	Control	MLK	P Values	%Change
Neutrophil (%)	20.780±.2709	18.880±3.3080	9.14 %	↓ 9.14
Eosinophil (%)	.0878±.03359	.0142±.00700	83.83 %	↓↓↓ 83.83
Basophil (%)	2.080±.0520	3.930±.2.1685	88.94 %	↑↑↑ 88.94
Lymphocyte (%)	76.520±.4152	74.840±4.4991	2.20 %	↓ 2.20
Monocyte (%)	.5444±.15854	2.3564±1.80166	332.84 %	↑↑↑ 332.84

Effect of MLK on Hemoglobin, Hematocrit, MCV, MCH, MCHC and RDW in Male Rats:

There is a negligible decrease in both the Hemoglobin content of the blood (p=0.43, 0.06 %) and the Hematocrit level of the blood (p=0.21, 7.49 %) of the male rat. There is a statistically insignificant (p=0.53, 0.73 %) decrease in the Mean Corpuscular Volume, Mean Corpuscular Hemoglobin (p=0.36, 2.28 %) and Mean Corpuscular Hemoglobin Concentration (p=0.55, 1.45 %) which are red cell index of the male rat. There is an [8.97 %] increase in the red cell volume distribution width, a red cell index of the male rat, the increase though not significant yet it was prominent (p=0.13). All the results described in table 3.

Table 3: Effect of MLK on Hemoglobin, Hematocrit, MCV, MCH, MCHC and RDW in Male Rats

Parameter	Control	MLK	P Values	%Change
Hemoglobin(g\dl)	12.060±.4770	11.360±.6961	0.43	≈ Decr 0.06
Hematocrit (%)	42.980±.4994	39.760±2.3576	0.21	↓ 7.49
MCV (fl)	60.360±.3893	59.920±.5598	0.53	≈ Decr 0.73
MCH (pg)	17.520±.3261	17.120±.2517	0.36	↓ 2.28
MCHC (%)	29.040±.6021	28.620±.2817	0.55	↓ 1.45
RDW (%)	9.806±.0979	10.686±.5236	0.13	↑ 8.97

Effect of MLK on ESR, BT, CT, MPV, PCT and PDW in Male Rats:

There was no change noticed in Erythrocyte sedimentation rate in blood from the male rat.There is a statistically significant (p=0.03) shortening **[**25.00 % decrease] of male rat cutaneous tail bleeding time. There is a prominent (p=0.48, 2.74 %) shortening of whole blood clotting time in male rats. There is a prominent (p=0.11, 4.14 %) increase in the mean platelet volume of the male rat. There is a statistically significant (p=0.04**,** 19.29 %**)** increase in the Platecrit value of the blood of the male rat. There is a statistically insignificant (p=0.66, 0.832 %) decrease in the platelet volume distribution width of the male rat. All the results described in table 4.

Table 4: Effect of MLK on ESR, BT, CT, MPV, PCT and PDW in Male Rats

Parameter	Control	MLK	P Value	%Change
ESR (mm\hr)	2.400±.2449	2.400±0.2449	No change	= No change
BT (sec)	48.000±3.0000	36.000±3.6742	0.03	↓↓↓ 25.00
CT (sec)	219.000±6.0000	213.000±5.6124	0.48	↓ 2.74
MPV (fl)	3.816±.0645	3.974±0.0618	0.11	↑ 4.14
PCT (%)	0.1762±.00491	0.2102±0.01376	0.04	↑↑↑ 19.30
PDW (%)	14.420±.2395	14.300±0.1183	0.66	↓0.832

DISCUSSION

Investigation of the hematological profile is essential for the determination of toxicity of drug under experimentation, on the blood constituents of an animal. The evolution of risk is done by the analysis of hematological parameters as any abnormal toxicity sign in humans can be well predicted, when tests involve rodents.[29]In the overall study we found noticeable change in the hematologic parameter that include the possibility of occurrence of anemic condition and leukemic potential.

The main function of RBC's is to produce, carry, and protect hemoglobin (Hb) for oxygen transport. None but red blood cells are capable of carrying oxygen to cells.[30]Production of erythrocytes (RBC's) occurs extra-vascularly in the bone marrow parenchyma.[31] Blood hemoglobin concentration helps the determination of the degree of tissue oxygenation. Decrease in the numbers of red blood cells or hemoglobin level below the normal and the body cells get less oxygen than its demand is Anemia.[32] Anemia may occur at any point in the production, recycling or regulating of RBCs in the body. In the current study, the drug Amlaki Rasayan slightly reduces the RBC count and that is why we can say, the drug has less potential to cause anemia. White blood cells are responsible for detecting and destroying diseases that come into our body. Blood leukocytes (WBC's) comprises of five different types of cell lines (neutrophils, monocytes, eosinophils, basophils, and lymphocytes).In the current study we noticed slight increase in the number of WBC. There is also noticed a high increase in the differential count of different WBCs. A very high increase in the white blood cell count is the sign of hypersensitivity, inflammation and leukemia.[33-34] Platelets are derived from the myeloid stem cell. Platelets are anucleate and are the smallest formed element in blood (~1-4 µm, ~3-15 fl) with a life span of about 4-10 days. In most species, counts range between 200,000-500,000/µL (rats~800,000/µL; mice, >1,000,000/µL).[35] The primary function of platelets is to maintain homeostasis. More specifically, they can form a plug at the sites of endothelial cell lining injury. Our current study shows a prominent increase in platelets count that may produce myeloproliferative disorders, infections, inflammation and cancers.[36]

Hemoglobin is the amount of oxygen carrying protein present within the red blood cells. As red cells are approximately 33% hemoglobin, the concentration hemoglobin in the whole blood

normally is about one third of the HCT. Hemoglobin is most commonly used indicator of iron deficiency. Although, Hemoglobin and Hematocrit both are late markers of iron deficiency anemia and they are not specific for iron deficiency anemia.[37] The Hematocrit is measured as percentage and helps to measure the ratio of blood volume occupied by RBC's.[35] There is a linear relationship between the concentration of hemoglobin and hematocrit in normal conditions. A low value of hematocrit means a low number of circulating red blood cells that means a decrease in the oxygen carrying capacity which is an indicator of anemia. A high hematocrit value may reflect an absolute increase in the number of RBCs or a decrease in plasma volume.[38]In this study, the drug Amlaki Rasayan very slightly decreases hemoglobin concentration and hematocrit level. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), all are morphological measures and are helps to classify types of anemias. They are calculated values which calculated from the RBC count, Hct value, and Hb concentration. The mean corpuscular volume (MCV) is an indicator of iron-deficiency anemia and is usually decreased if iron-deficiency anemia is present .[39] MCH (the mean corpuscular hemoglobin) values are normally reduced in anemia patients, and the mean corpuscular hemoglobin concentration (MCHC) is reduced in severe disease. The RDW is a marker of the variation in cell volume within the red cell population. RDW test measures the different sizes and shapes of the red cell. A high value of MCV and RDW rule out liver disease, hemolytic anemia, Vitamin B12 deficiency, folic acid deficiency. Our study shows very slight change in the MCV, MCH, MCHC and RDW values.

The ESR is a simple and a non-specific screening test that indirectly measures the presence of any kind of inflammation in the body. It reflects the tendency of red blood cells to settle more rapidly in the face of some disease states, which is due to the increases in plasma immunoglobulins, fibrinogen and other acute-phase reaction proteins. Bleeding time test measurement of time taken for platelet plug formation and blood vessel constriction to occur. Clot is not allowed to form, so that the arrest of bleeding depends exclusively on blood vessel constriction and platelet action. The time taken for blood to clot mainly reflects the time required for the generation of thrombin in this manner. If the plasma concentration of prothrombin in plasma or of some of the other factors is low or if the factor is absent, or functionally inactive, the clotting time will be prolonged. This study shows no change in ESR value which indicates the presence of no inflammation. Bleeding time and clotting time is decreased noticeably.

Mean platelet volume (MPV) measure platelets size within a given sample, so the presence of some large platelets may not be an abnormal result. Large platelets have more clinical relevance in animals that are thrombocytopenic.[33] Plateletcrit (PCT) is the percent volume of the blood, occupied by platelets cell. It reflects the number and size of the platelets. Platelet distribution width (PDW) is a marker of variation in platelet size which can be a sign of active platelet release. This marker identifies the heterogeneity of platelet size (degree of anisocytosis). This study shows a very slight change in the MPV and PCT value but very negligible change in PWD.

CONCLUSION

From the above experiment it can be concluded that Amlaki Rasayan (MLK) is mildly Hemotoxic at a higher dose as it slightly reduce Red Blood Cell (RBC) count, Hemoglobin, Hematocrit, MCH, MCHC and moderately increase the number of white blood cell count and platelet count. This study may help the Ayurvedic prescriber to prescribe the MLK safely for the treatment of Jaundic. Thus it can be taken safely at a normal dosage of 12–24 ml once or twice a day as normally advised after food. Further studies can be done by increasing the administered dose to find out the level of chronic toxicity.

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