

Full Length Research Paper

Investigation of hemotoxicologic potential of an ayurvedic preparation “Kutajarista” used in Sprue syndrome after chronic administration to male Sprague-Dawley rats

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The ayurvedic medicinal system is the world's most primitive medical systems which were originated in India. For the treatment of Sprue syndrome, a very well-known ayurvedic preparation named Kutajarista (KTJ) is used in the south Asian region. This study aimed to detect the impact of chronic administration of KTJ on hematological parameters. The experimental animals were subdivided into two groups. A high dose (50 mL/kg) of body weight was given to the first group for 42 days and the second group which was considered as the control group was treated with normal water for the same period. There was a remarkable reduction [10.37%, $p=0.092$] in the total numbers of red blood cells (RBC). A prominent decrease [10.75%, $p=0.097$] is also observed in the hemoglobin content of the blood. There was a noticeable [11.05%, $p=0.077$] decrease in the hematocrit level of the blood and a very high [64.35%, $p=0.19$] increase in the number of white blood cell (WBC) count of the male Sprague-Dawley rat. A remarkably high increase was observed in the absolute count of Neutrophils (220.161%, $p=0.12$), Eosinophils (368.75%, $p=0.11$), Lymphocytes [11.29%, $p=0.06$], and Monocyte [142.781%, $p=0.13$] count. Also a prominent [\uparrow 14.15%, $p=0.42$] increase in the number of platelet counts was observed. Rat cutaneous tail bleeding time and whole blood clotting time shortening were also observed and the result is statistically prominent. As the result shows a significant change in different hematological parameter it can be summarized that KTJ have potential hemotoxicity at a higher dose. Further study is required at a lower dose.

Key words: Ayurvedic, Kutajarista, hemotoxicity, red blood cells (RBC), white blood cells (WBC).

INTRODUCTION

Ayurvedic system of medicine is the world's most ancient medical system that originated in India about 3000 years

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Table 1. Name of ingredients used in the formulation of ayurvedic preparation of Kutajarista.

Component	Scientific Name	Quantity	Quantity (%)
Kutajamula (Rt. Bk.)	<i>Holarrhena antidysenterica</i>	4.800 kg	7.610
Mrdvika (draksa) (Dr.Fr.)	<i>Vitis vinifera</i> L.	2.400 kg	3.805
Madhuka puspa (F1)	<i>Madhuca indica</i>	480 g	0.761
Kasmari (gambhari) (St. Bk.)	<i>Gmelina arborea</i> Roxb.	480 g	0.761
Water for decoction	-	49.152 L reduced to 12.288 L	77.929
Guda	<i>Medicinal Sugar</i>	4.800 kg	7.610
Dhataki (Fl.)	<i>Woodfordia fruticosa</i>	960 g	1.520

ago. Most of the primitive traditional methods of healing such as Tibetan, Chinese and Greek medicine were influenced by the ayurvedic medicine system. In the Indian sub-continent, nearly 80% of the population is currently using ayurvedic preparations and medicinal plants to meet their primary level of disease recovery. At the very beginning of the twentieth-century world health organization recommended larger-scale use of ayurvedic medicine in developing countries as an alternative medicinal system and the use of ayurvedic and herbal origin extracts is increasing day by day (Mohammed et al., 2020; Pehlivan and Sevindik, 2018; Sevindik et al., 2017; "WHO, 2002).

The Sprue syndrome is known in Ayurveda as Grahani is characterized by anemia, weight loss, diarrhea, frothy stools, sore mouth, and indigestion. Besides, mucus appears in the patient's stool. In the morning after rising from bed, the patients significantly discharge a large amount of stool (Bennett et al., 2014; Ramakrishna et al., 2006). Kutajarista (KTJ) is prepared as liquid ayurvedic medicinal form, and currently used at the dose of 12 to 24 mL once or twice daily for the treatment of sprue syndrome, mal-absorption syndrome, ulcerative colitis, diarrhea, irritable bowel syndrome, dysentery or blood dysentery fever, Croon's disease, etc. (Verma, 1991; Dash, 1984; Dastur, 1960; Nadkarni, 1976; Mishra, 2010). It acts as an anti-motility and anti-secretory agent thus helps in the treatment of diarrhea (Shamkuwar and Shahi, n.d.). 5 to 10% self-generated alcohol present in the liquid KTJ preparation provides a media to deliver various herbal active components to the body after ingestion (Government of India, 1990).

Bangladesh National Formulary of Ayurvedic Medicine included KTJ as herbal medicine in 1992 (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, 2011. The formulation of KTJ is given in Table 1 (Anonymous, 2003; Classical Ayurvedic Prescriptions for Common Diseases (Only for registered ayurvedic medical practitioners), 2010). 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.

Ayurvedic medicines have a good safety profile (Ernst, 2002). But recent studies have reported that heavy metal in the ayurvedic preparation (e.g. Lead, Arsenic) shows toxicity (Keen et al., 1994; Olujohungbe et al., 1994). The safety profile of most of the ayurvedic medicine is not yet completely investigated. Considering this issue, this research work has been launched to detect the level of hemotoxicity after chronic administration of the marketed ayurvedic medicinal preparation, KTJ.

MATERIALS AND METHODS

Drugs, chemicals, and reagents

For this hemotoxicological study, KTJ 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 purchased from Human GmbH, Wiesbaden, Germany.

Experimental animals

For acute toxicological study female Swiss Albino mice with body weight in the range of 35 to 40 g were used and the hemotoxicological experiment use 45 to 60 day old male Sprague-Dawley rats that were bred and maintained at the animal house of the Department of Pharmacy, Jahangirnagar University. These animals were weighed in the range of 150 to 170 g and were healthy. Plastic cages having dimensions of 30 × 20 × 13 cm and softwood shavings as the bed were used for housing of all animals. They were fed with rat chow prepared according to the formula developed at Bangladesh Council of Scientific and Industrial Research (BCSIR) and water was provided *ad libitum*. The rats were maintained at scheduled 12 h a day and 12 h night cycle. Ethical guide for the care and use of laboratory animals approved by the Ethical Review Committee, Faculty of Life Sciences, Department of Pharmacy, Jahangirnagar University was fully followed throughout the experiment on animals.

Design of the study

Acute toxicity study

Guidelines of the Organization for Economic Co-operation and Development (OECD) were followed to detect the acute oral toxicity level (Oecd/Ocde, 2008). Non-pregnant twenty four female mice with body weight in the range of 35 to 40 g were subdivided into six

groups (n=4). Different doses of experimental drug Kutajarista (KTJ) (50, 60, 70, 80, 90 and 100 mL/kg of body weight) were administered by intra-gastric syringe. The dose was divided into two equal fractions and administered within 12 h. The observation was done for mortality and clinical signs of toxicity (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) for all experimental animals at 1, 2, 3 and 4 h and thereafter once a day for the next three days following KTJ administration.

Chronic toxicity studies

The experiment of detection of chronic hemotoxicity level on rats was started with randomly divided sixteen rats into 2 groups (n=8). One group was treated with KTJ in the dose of 50 mL/kg of body weight for 42 days and another was used as a control and was administered with distilled water only as per the same volume as the drug-treated group for the same period (Gad, 1988). After acclimatization, ayurvedic medicinal preparation KTJ was administered to the rats by an intra-gastric syringe between the 10 and 12 am daily throughout the 42 days long study period. To identify a particular animal all the experimental animals were marked carefully on the tail. By using the identification mark, responses were noted separately for a particular period before and after the administration (Stevens and Gallo, 1989).

Blood samples collection

After completion of 42 days of long treatment with KTJ, the food supply stopped for 18 h. Then 24 h after the last drug administration, Ketamine (500 mg/kg i.p.) was injected to anesthetize the animals. Whole blood samples were collected from post vena cava and transferred to EDTA-added tubes immediately and marked properly. All analyses of blood were completed within 12 h of sample collection (Wolford et al., 1986).

Determination of hematologic parameter

Hematologic profile studies involved analysis of blood parameters such as Red Blood Cells (RBCs) and WIC (WBC Impedance Count) level determined by Electrical Impedance method, Hemoglobin (HGB) level determined by Modified hemiglobincyanide method, Platelet level was determined by Electrical Impedance method, WOC (WBC Optical Count) level was determined by Laser light scatter (Tatsumi et al., 1999; van Kampen and Zijlstra, 1961). Differential analysis was done by the CELL-DYN 3700 System. Erythrocyte Sedimentation Rate is determined by Wintergreen Method (Brigden, 1999). The modified procedure of Mohamed et al. (1969) was used for the determination of bleeding time (Dejana et al., 1979). Clotting time was determined with the method of Goldstein and Abramson (1996). MCV, MCH, and MCHC, HCT, and PCT are calculated according to the formula given by Diem and Konrad (1975) and Ketley (1993).

$$MCV = \frac{\text{HCT (\%)}}{\text{RBC count (millions)}} \times 10$$

$$MCH = \frac{\text{Hb (g/dL)}}{\text{RBC count (millions)}} \times 10$$

$$MCHC = \frac{\text{Hb (g/dL)}}{\text{HCT (\%)}} \times 100$$

$$\text{HCT} = \frac{\text{RBC} \times \text{MCV}}{10}$$

$$\text{PCT} = \frac{\text{PLT} \times \text{MPV}}{10,000}$$

$$\text{Differential count of Neutrophil (\%)} = \frac{\text{Number of Neutrophil}}{\text{Total number of Leucocytes}} \times 100$$

$$\text{Differential count of Eosinophil (\%)} = \frac{\text{Number of Eosinophil}}{\text{Total number of Leucocytes}} \times 100$$

$$\text{Differential count of Basophil (\%)} = \frac{\text{Number of Basophil}}{\text{Total number of Leucocytes}} \times 100$$

$$\text{Differential count of Lymphocytes (\%)} = \frac{\text{Number of Lymphocytes}}{\text{Total number of Leucocytes}} \times 100$$

$$\text{Differential count of Monocytes (\%)} = \frac{\text{Number of Monocytes}}{\text{Total number of Leucocytes}} \times 100$$

The platelet distribution width (PDW) is a measure of the heterogeneity of the platelet population. Red cell distribution width (RDW) is a measurement factor for the detection of the level of the heterogeneity of the RBC population.

Statistical analysis

The group data are expressed as Mean \pm Standard Error of the Mean (SEM). Unpaired "t" tests were done for statistical significance tests. Statistical Package for Social Science (SPSS) for WINDOWS (Ver. 22) was applied for the analysis of data. Differences between groups were considered significant at $p < 0.05, 0.01, \text{ and } 0.001$.

RESULTS

Acute toxicity study

No mortality was observed at the highest administered dose of 100 mL/kg of body weight. Thus the LD₅₀ value was found to be greater than the highest administered dose in the current study. No convulsion, respiratory distress, general irritation, and restlessness were noticed. Since KTJ is used for many years in ayurvedic medicinal system for the clinical purpose of treatment of diarrhea, dysentery, irritable bowel syndrome, or sprue syndrome, a limit test was performed in acute oral toxicity study. OECD test guideline 425 suggests that 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 100 mL/kg body weight was launched. Therefore, it can be summarized that KTJ when administered at a high dose is non-toxic oral

Table 2. Effect of KTJ on different blood cell count.

Blood cell type	Control (Mean±SEM)	KTJ (Mean±SEM)	P Values	%Change
RBC (million\µL)	7.150±0.0880	6.408±0.065	0.092	↓ 10.37
WBC (thousand\µL)	5.453±0.3984	8.962±1.906	0.19	↑64.35
Platelets (thousand\µL)	471.820±5.36	538.400±77.040	0.42	↑ 14.15
Neutrophil (thousand\µL)	1.24±0.069	3.97±1.6474	0.12	↑220.161
Eosinophil (thousand\µL)	0.012±0.0120	0.075±0.0292	0.11	↑368.75
Basophil (thousand\µL)	0	0	-	-
Lymphocyte (thousand\µL)	4.010±0.255	4.463±0.346	0.061	↑ 11.29
Monocyte (thousand\µL)	0.187±0.0079	0.454±0.089	0.13	↑142.781

formulations.

Chronic hematologic profile study

Effect on different blood cell count

Comparing with the control group a statistically noticeable decrease [10.37%; $p=0.092$] in the total number of red blood cell count of the male rat was observed after 42 days administration of KTJ. Again a very high increase [64.35%; $p=0.19$] observed in white blood cell (WBC) count that is statistically prominent. The absolute count of different WBC such as Neutrophil, Eosinophil, Lymphocyte, and Monocyte counts was also increased grossly. The platelet count of the male rat also showed a high [14.15%; $p=0.42$] increase. Different blood cell count data is presented in Table 2.

Effect KTJ on differential (WBC) count in male rats

Blood cell count result obtained by Electrical Impedance method on blood sample of 42 days KTJ administered rat shows an insignificant but noticeable [94.80%; $p=0.067$] increase in the percentage of Neutrophil count and an increase in the percentage of Eosinophil count [185.214%; $p=0.034$] and the result not significant. Lymphocyte count decreased significantly [32.28%; $p=0.027$] and Monocyte count increased [47.721%; $p=0.021$]. All the results are presented in Table 3.

Effect of KTJ on hemoglobin, hematocrit, MCV, MCH, MCHC, and RDW in male rats

After 42 days long administration of KTJ at a dose of 50 ml/kg on male rat the blood sample analysis reports recommend that the hemoglobin content of the blood decrease [10.75%; $p=0.097$] which is statistically not significant but prominent and hematocrit level of the blood decrease [11.05%; $p=0.077$] which is statistically noticeable. Different red cell index showed a statistically

insignificant change as mean corpuscular volume (MCV) increase [0.67%; $p=0.891$], mean corpuscular hemoglobin (MCH) decrease [2.45%; $p=0.411$], mean corpuscular hemoglobin concentration (MCHC) also decrease [3.56%; $p=0.501$], Red cell volume distribution width (RDW) increase [8.43%; $p=0.099$]. All the results are presented in Table 4.

Effect of KTJ on ESR, BT, CT, MPV, PCT and PDW in male rats

Some other blood parameter tests like erythrocyte sedimentation rate, bleeding time, clotting time, mean platelet volume, platecrit value, and platelet volume distribution width were done and result of the drug administered group compared with the control group rat to detect any potential change the level of different blood constituents. The test report suggests a statistically insignificant [10.04%; $p=0.60$] increase in erythrocyte sedimentation rate and a statistically prominent [18%; $p=0.15$] shortening of male Sprague-Dawley rat's cutaneous tail bleeding time. A little [6.81%; $p=0.10$] shortening of whole blood clotting time in male rats was noticed, the decrease though not significant was prominent. Statistically prominent result was observed in different platelet parameter as in the Mean Platelet Volume (MPV) increase [9.42%; $p=0.14$], Platecrit value (PCT) increase [22.91%; $p=0.30$]. A very negligible [2.02%; $p=0.85$] decrease in the platelet volume distribution width of the male rat was noticed which was statistically not significant. All the results are presented in Table 5.

DISCUSSION

To investigate the level of hemotoxicity of an administered drug or preparation it is essential to assess the hematological profile 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 (Olson et al., 2000).

Table 3. Effect KTJ on differential (WBC) count in male rats.

Parameter	Control (Mean+SEM)	KTJ (Mean+SEM)	P Values	%Change
Neutrophil (%)	22.739±0.109	44.298±3.459	0.067	↑ 94..80
Eosinophil (%)	0.293±0.0285	0.836±0.004	0.034	↑ 185.214
Basophil (%)	0	0	-	-
Lymphocyte (%)	73.530±0.352	49.800±4.824	0.027	↓ 32.280
Monocyte (%)	3.429±0.123	5.065±1.102	0.021	↑ 47.721

Table 4. Effect of KTJ on hemoglobin, hematocrit, MCV, MCH, MCHC, and RDW in male rats.

Parameter	Control (Mean+SEM)	KTJ (Mean+SEM)	P Values	%Change
Hemoglobin(g/dL)	13.020±0.397	11.62±0.294	0.097	↓10.75
Hematocrit (%)	42.980±0.428	38.320±1.326	0.077	↓11.05
MCV (fL)	60.140±0.3893	60.54±1.0599	0.891	↑0.67
MCH (pg)	17.520±0.261	17.09±0.387	0.411	↓ 2.45
MCHC (%)	30.010±0.582	28.94±1.10	0.501	↓ 3.56
RDW (%)	9.206±0.068	9.982±0.387	0.099	↑ 8.43

MCV=Mean corpuscular volume; MCH= Mean corpuscular hemoglobin; MCHC= Mean corpuscular hemoglobin concentration; RDW= Red cell distribution width.

Table 5. Effect of KTJ on ESR, BT, CT, MPV, PCT and PDW in male rats.

Parameter	Control (Mean+SEM)	KTJ (Mean+SEM)	P-Value	%Change
ESR (mm/h)	2.39±0.2204	2.63±0.2449	0.60	↑ 10.04
BT (s)	50.0±3.00	41.0±3.00	0.15	↓ 18.00
CT (s)	220.0±5.00	205.0±3.00	0.10	↓6.81
MPV (fl)	3.765±0.07	4.12±0.11	0.14	↑ 9.42
PCT (%)	0.179±0.003	0.22±0.030	0.30	↑22.91
PDW (%)	14.80±.2395	15.10±.1462	0.85	↑2.02

ESR= Erythrocyte sedimentation rate; BT= Bleeding time; CT= Clotting time; MPV= Mean platelet volume; PCT= Plateletcrit; PDW= Platelet distribution width.

Reviewing the overall study we found a noticeable change in the hematologic parameters that include the possibility of occurrence of an anemic condition, hypersensitivity, allergic symptoms, and leukemia.

Bone marrow parenchyma cell is responsible for the generation of new red blood cells which is also known as erythrocytes (Khan et al., 2013). The principal function of RBC is to carry and protect hemoglobin (Hb) which is responsible for oxygen transport to body tissue. The level of Hb concentration in the blood determines the level of tissue oxygenation. The number of red blood cells or hemoglobin concentration per unit of blood dropped below the normal range, then the condition is termed as anemia. In anemic body condition, cells get less oxygen than their demand (Longo et al., 2015). At any point in the life cycle of RBC (production, recycling, or regulating) anemia may occur in the body. The previous study

suggests that six to eight-week-long intake of some ayurvedic preparation reduce the level of RBC and hemoglobin level in the blood of human and animal (Gunturu et al., 2011; Nipa et al., 2016; Rahman et al., 2020). In the current study, the drug KTJ noticeably reduces the RBC count in the blood of Sprague-Dawley rats and that is why we can recommend that the drug has the potential to cause anemia when used for a longer time.

WBC serves in the body's defense mechanism by detecting and destroying the disease-causing agents that enter into the body. Five different types of cell lines (neutrophils, eosinophils, basophils, lymphocytes, and monocytes) comprises the WBC population with all having individual function in the body defense system. A high increase in WBC count is considered as a nonspecific marker of inflammation which is linked with

the immune system and correlate both acute and chronic infection and exposure to irritating or toxic agents (O'Byrne and Dalglish, 2001; Pai et al., 2004; Rader, 2000; Ridker et al., 2000; Ross, 1999). In the current study, we noticed a significant and high increase in the WBC count after chronic administration of KTJ that is matched with the previous study (Nipa et al., 2016; Rahman et al., 2020; Suresh Gupta et al., 2006). A very high increase was also noticed 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 or cardiovascular problem (Majd et al., 2014).

Platelets are not nucleate and are the smallest formed element in the blood (~1-4 μm , ~3-15 fl) with a life cycle of only 4 to 10 days. These are derived from the myeloid stem cell. In most species, counts range between 200,000 and 500,000 μL^{-1} (rats~800,000 μL^{-1} ; mice, >1,000,000 μL^{-1}) (Corey et al., 2014). The primary function of platelets is to maintain homeostasis. More specifically, they can form a plug at the sites of endothelial cell lining injury. Previous studies suggest that a change in the platelet count correlates various infections, inflammation, and myeloproliferative disorders (Sankar, 2017). The current study shows a prominent increase in platelet count that may indicate the presence of infections, inflammation after chronic administration of KTJ.

The main component of the red blood cell is hemoglobin which comprises approximately 33% of a total red blood cell. It is responsible for oxygen transport to tissue which is one of the main functions of blood. The concentration hemoglobin in the whole blood normally is about one-third of the hematocrit. Hemoglobin concentration test in the blood is done as it is a common indicator of iron deficiency. The hematocrit is measured as a percentage and helps to measure the ratio of blood volume occupied by RBCs (Tripathi et al., 2014). Both hemoglobin and hematocrit are considered as a late marker of iron deficiency anemia though it is non-specific (Wu et al., 2002). A correlation observed in the concentration of hemoglobin and hematocrit value, which is the lower value of hematocrit indicates a low number of circulating RBC which ultimately implies decreased oxygen-carrying ability by hemoglobin and produce symptoms of anemia.

A high hematocrit value may reflect an absolute increase in the number of RBCs or a decrease in plasma volume (Lokwani, 2013). In the current study, after chronic administration of the drug Kutajarista prominently decreases hemoglobin concentration and hematocrit level which is a clear indication of hemotoxicity.

Some morphological parameters as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are calculated to identify the types of anemia (Beutler, 1981). Decrease the value of MCV indicates the presence of iron-deficiency anemia (Lokwani, 2013). MCH values are

reduced in anemia patients, and the MCHC value is reduced in severe disease. The RDW is a marker of the variation in cell volume within the red cell population. Different sizes and shapes of the red cell can be identified by the RDW test and a high value of RDW rule out liver disease, hemolytic anemia, vitamin B12 deficiency, and folic acid deficiency. In the current study, we observed a very slight change in the MCV, MCH, MCHC, and RDW values.

Any kind of inflammation can be screened non-specifically by the erythrocyte sedimentation rate test. The increased presence of plasma fibrinogen, immunoglobulins, and other acute-phase reaction proteins causes the red blood cell to settle more rapidly under the disease state (Saha et al., 2018). Time taken for platelet plug formation and blood vessel constriction to occur is bleeding time. The clotting time will be prolonged if plasma factors such as prothrombin or fibrinogen are reduced. This study shows an increased erythrocyte sedimentation rate (ESR) value which indicates the presence of any type of inflammation. Bleeding time and clotting time decreased noticeably.

The size of the platelet of a given sample can be measured by mean platelet volume (MPV). An increase in MPV value implies the presence of larger sized platelet which clinically diagnosed as thrombocytopenia (Liu et al., 2012). Plateletcrit (PCT) value reflects the number and size of the platelets and also platelet distribution width (PDW) reflects the variation of platelet size. These markers identify the degree of anisocytosis or heterogeneity of platelet size (Chandrashekar, 2013). This study shows a significant change in the MPV and PCT values but a very negligible change in PWD.

Conclusion

From the experiment, it can be concluded that Kutajarista (KTJ) should not be administered chronically at a higher dose as it noticeably reduces red blood cell (RBC) count, hemoglobin, hematocrit, MCH, MCHC and a very high increase in numbers of white blood cell count and platelet count. Further studies should be done by reducing the administered dose. Thus KTJ is to be taken safely only at a dosage of 12 to 24 mL once or twice a day normally advised after food. If needed, it can be mixed with an equal quantity of water.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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