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Original Research Article

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

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ABSTRACT

Background: Krishna Chaturmukha (KMC) is an ayurvedic formulation predominantly used as a traditional medicine in the rural population to get relief from various neurological disorders.
Methods: The acute pharmacological test of KMC recorded no death or any signs of effectivity even at the highest dose of 4000 mg/kg body weight. The effect of chronic administration of KMC on hematological parameters was determined by administrating chronically to the male Sprague-Dawley rats at a dose of 100 mg per kg body weight for 40 days.
Results: There was a (10.12 %) decrease in the number of white blood cell count of the male rats, which, although not statistically significant yet it was prominent (p=0.248). Similarly, a negligible (0.12 %) decrease in the red blood cell count was observed, which was not statistically significant at all (p=0.965). Conversely, the platelets count showed a (4.99 %) increase in the experimental animals, while this increase was not significant, yet it was prominent (p=0.296). Furthermore, a (1.89 %) decline was observed in the platelet volume distribution width of the experiment rats, which, although not significant, yet it was noticeable (p=0.076).
Conclusions: From this study, it is evident that there is no significant change in the hematological parameters in the body of the treated rats, therefore it is safe to use in usual dose. Further research and analysis with biochemical parameters should be done to reconfirm the safety of this ayurvedic medicine.

Keywords: Ayurvedic formulation, Krishna Chaturmukha, Hematological, Neurological disorders

INTRODUCTION

Ayurveda is an ancient Indian medical system, based on natural products derivative from plants, animals, metals, and minerals, used to treat various physical and mental conditions. Ayurveda is based on a "natural" and holistic approach to physical and mental health. Ayurvedic medicine is the traditional medicine which has the reputation as safe and effective remedies for various of diseases such as neurological diseases, digestive complications, skin diseases, head pain, cough, and lung diseases.[1,2] These types of traditional medicines are widely accepted among the general population, particularly in rural areas, due to the good safety perception and inexpensive price of these medicines.[3]

Understanding the importance of ayurvedic medicines, the World Health Organization (WHO) has already recognized and recommended an extended use of herbal (Unani and Ayurvedic) medicines, predominantly in the developing countries, to ensure the maximum health care services at the primary healthcare level as an alternative

system of usual mediciness.[4] According to WHO, approximately 1.5 billion people from all over the world are now being treated with these traditional medicines.[5,6]

Krishna Chaturmukha is included in the Bangladesh National Formulary of Ayurvedic Medicine 1992.[7] It is used in the treatment of asthma, anemia, diabetes, digestion power, diabetes, asthma, abdominal colic, anorexia, hiccups, dyspepsia, epilepsy, gout, herpes, abscess, psychotic disorders and hemorrhoids.

It consists of Shuddha Parada (Herbal purified Mercury), Shuddha Gandhaka (Herbal purified Sulphur), Loha Bhasma (Bhasma prepared from Iron), Abhraka Bhasma (Purified and processed Mica), Kumari (Aloe vera juice extract), Errand (Castor Ricinus communis leaves) (Table 1).

Fine power of above ingredient is ground with juice extract or decoction, made into paste and pills are prepared.[8] The present study was undertaken to explore the effect of Krishna Chaturmukha (KMC) in terms of hematological profile of rat after chronic administration of the drug to male Sprague-Dawley rats.

METHODS

Drugs, chemicals and reagents

For the hematological study, Krishna Chaturmukha (KMC) was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong, Bangladesh. Ketamine injection was purchased from ACI Pharmaceuticals Limited, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Human GmbH, Wiesbaden, Germany.

Experimental animal

Eight-week-old male Sprague-Dawley rats bred and maintained at the animal house of the Department of Pharmacy, Jahangirnagar University, were used in the haematological experiment. Experimental animals were apparently healthy, active and weighed between 60 g to 70 g.

The house was well-ventilated clean experimental animal house where the experimental animals were housed in 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.

Experimental design

Acute toxicity studies

The guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals with minor modification (OECD Guideline 425) was followed for the acute oral toxicity test [10]. Sixteen healthy male rats, body weight between 50g and 70g, were divided into four groups consisting of four rats in each group and different doses (1000 mg/kg, 2000 mg/kg, 3000 mg/kg, and 4000 mg/kg) of experimental drug, Krishna Chaturmukha (KMC) 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 toxicity signs such as general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture etc. at 1, 2, 3 and 4 hours and thereafter once a day for the next three days following Krishna Chaturmukha (KMC) administration.

Chronic toxicological studies

Sixteen male mice (60-70 g body weight) were randomly divided into two groups of eight animals each in both control and experiment group. One group was administered with KMC in a dose of 100 mg/kg and another was used as the control group.

The animals from control group were administered with distilled water as placebo as par the same volume as the drug treated group for the same duration, 40 days. After acclimatization, the 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.[11]

Blood samples collection and preparation of serum

At the end of the 40 days treatment period, after 18 hours fasting, rats from each group were anaesthetized by administration (i.p) of ketamine (500 mg/kg body weight).[12]

Blood samples were collected from 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 generation for biochemical analysis.

Serum was obtained after allowing blood to coagulate for 30 minutes and centrifuged at 4000 g for 10 minutes using bench top centrifuge (MSE Minor, England). The supernatant serum samples were collected using dry Pasteur pipette and stored in the refrigerator for further analysis. All analyses were completed within 12 hours of sample collection.[13]

Determination of hematological profile studies

Hematological profile studies were involved analysis of parameters such as Red Blood Cells (RBCs) and platelet level determined by Electrical Impedance method.[14] Hemoglobin (HGB) level determined by modified hemoglobin cyanide method.[15] The HCT is 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 and Diem and Clenter.[16,17]

MCV =HCT (%) / RBC count (millions) ×10
MCH =Hb (g/dl) /RBC count (millions)×10
MCHC =Hb (g/dl)/ HCT (%) ×100

Mathematically the RDW is calculated with the following formula, RDW = (Standard deviation of MCV ÷mean MCV) × 100.[18] Two WBC values are provided by the CELL-DYN 3700 System.[18] The WIC (WBC Impedance Count). 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).[20]

Erythrocyte Sedimentation Rate (ESR) is measured by Westergren Method. For determination of bleeding time, modified procedure of Mohamed et al (1969) was used.[21,22]

Statistical analysis

The data were analyzed using independent sample t-test with the help of SPSS (Statistical Package for Social Science) Statistics 11.5 package (SPSS Inc., Chicago Ill). All values are expressed as mean±SEM (Standard Error Mean) and $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ was taken as the level of significant.

RESULTS

Acute toxicity study

No mortality was observed when the rats were administered with the highest dose of KMC (4000 mg/Kg body weight) (Table 2). So, it could be predicted that LD50 (median lethal dose) value was larger than 4000 mg/kg body weight. Moreover, the animals were out of any signs of restlessness, respiratory distress, general discomfort, or convulsions. Because this ancient ayurvedic medicine has been used by generations for centuries to treat various diseases, a limit test was performed in the acute oral toxicity test. According to the OECD guideline (425), the limit test at the highest beginning dose level (4000 mg/kg body weight) was performed because there is evidence of low or non-toxicity and immortality of the test agent. Therefore, this study shows that acute administration or single dose of KMC in usual dose (100mg/kg body weight) doesn't possess toxic effects and safe to use in oral formulation.

Effect of chronic administration of KMC on Hematological profiles of male rats (Table 3)

There was a (10.12 %) decrease in the number of white blood cell count of the male rats, which, although not statistically significant yet it was prominent (p=0.248). Similarly, a negligible (0.12 %) decrease in the red blood cell count was observed, which was not statistically significant at all (p=0.965). Conversely, the platelets count showed a (4.99 %) increase in the experimental animals, while this increase was not significant, yet it was prominent (p=0.296). A statistically insignificant (p=0.582) decrease (5.85 %) in the absolute count of neutrophils was found in the rats. Similarly, there was a statistically insignificant (p=0.633) decrease (10.14%) in the absolute count of monocytes of the male rat.

However, there was a negligible (1.15 %) decrease in the absolute count of lymphocytes of the male rat, which was not statistically significant at all (p=0.919). On the other hand, there was a statistically insignificant (P=0.501) increase (2.02%) was observed in the percentage of lymphocyte count of the male rats. There was a negligible (6.52 %) decrease in the percentage of Monocyte count of the male rats, which was not statistically significant at all (p=0.738).

In case of the Hemoglobin content of the blood of the experiment animals, a statistically insignificant (p=0.594) increase (1.82 %) was discovered. In a similar way, there was a negligible (0.64 %) increase in the Hematocrit level of the blood of the male rats, which was not statistically significant at all (p=0.787). Regarding the cell index of a red blood cell of the experiment animals, a negligible (0.28%) increase in the mean corpuscular volume was observed which was not statistically significant at all (p=0.803) and a negligible (0.11 %) increase in the Mean corpuscular hemoglobin was found, which was not also statistically significant at all (p=0.925). Oppositely, there was a statistically insignificant (p=0.513) decrease (0.70 %) in the Mean corpuscular hemoglobin concentration, a

red cell index of the male rat. In addition, a (1.76%) decrease was found in the red cell volume distribution width, which, though was not significant yet it was prominent (p=0.152). There is a negligible (3.20 %) increase in erythrocyte sedimentation rate in blood from the male rat, which was statistically not at all significant (p=0.887). A (9.77 %) shortening of male rats' cutaneous tail bleeding time was observed, which, though not significant yet it was prominent (p=0.346). There was a (1.07 %) shortening of whole blood clotting time in male rats, the decrease though not significant yet it was prominent (p=0.450). A (0.85 %) decrease was recorded in the mean platelet volume of the male rat, which was statistically insignificant (p=0.695). Furthermore, a (1.89 %) decline was observed in the platelet volume distribution width of the experiment rats, which, although not significant, yet it was noticeable (p=0.076).

Table 1: Name of the ingredients used in the preparation of KMC.

S. no	Name of the ingredients	Scientific name	Quantity used
1	Rasa (prada) suddha	Mercury	1 part
2	Gandhakasuddha	Sulphur	1 part
3	Loha- bhasma	Iron	1 part
4	Abhra (abhraka) bhasma	Mica	1 part
5	Kanya (kumara) swarasa	*Aloe barbadensis*	¼ part, formardana
6	Erandapatra	*Ricinus communis*	Q.S. for avestana

Table 2: Acute toxicity study of KMC in rats when administered orally.

	1000 mg/kg		2000 mg/kg		3000 mg/kg		4000 mg/kg	
	A	D	A	D	A	D	A	D
KMC	4	0	4	0	4	0	4	0
Mortality	0		0		0		0	

*Here, n = 4; A = Alive; D = Death

Table 3: Hematological profiles after chronic administration of Khrisna Chaturmukha (KMC) in dose 100 mg/kg to the male rates for 40 days.

Parameters	Control (Mean±SEM)	KCM (Mean±SEM)	p values	%Change
WBC	5.1500±0.32459	4.6286±0.27318	0.248	↓10.12
RBC	6.6950±0.13217	6.6867±0.11837	0.965	↓0.12
Platelet	545.62±21.327	572.86±10.793	0.296	↑4.99
Neutrophil	1.1137±.09409	1.0486±0.06010	0.582	↓ 5.85
Lymphocyte	3.9612±.26331	3.9157±.35991	0.919	↓1.15
Monocyte	0.0700±.01225	0.0629±.00680	0.633	↓10.14
Neutrophil (%)	21.62±1.438	20.14±1.335	0.468	↓6.85
Lymphocyte (%)	76.88±1.575	78.43±1.587	0.501	↓2.02
Monocyte (%)	1.38±0.183	1.29±0.184	0.738	↓6.52
RBC	6.6950±0.13217	6.6867±.11837	0.965	↓0.12
Haemoglobin	11.6875±0.28249	11.9000±0.26095	0.594	↑1.82
HCT	37.1125±0.61859	37.35±.54757	0.787	↑0.64
MCV	55.513±0.4692	55.671±0.3932	0.803	↑0.29
MCH	17.463±0.1700	17.483±0.1014	0.925	↑0.12
MCHC	31.488±0.2748	31.267±0.0803	0.513	↓0.70
RDW	12.375±0.1161	12.157±0.0751	0.152	↓1.76
Platelet	545.62±21.327	585.00 a± 13.093	0.153	↑7.48
ESR	1.25±0.164	1.29±0.184	0.887	↑3.20
Bleeding time	35.62±2.745	32.14±2.143	0.346	↓9.77
Clotting time	376.88±4.426	372.86±2.143	0.45	↓1.07
MPV	4.3512±0.06148	4.3143±0.06924	0.695	↓0.85
PDW	16.1625±0.10680	15.8571±0.11722	0.076	↓1.89

↑: increase, ↓: decrease

DISCUSSION

Hematological study is a part of the most preclinical and clinical safety studies and has become routine in monitoring a variety of novel and conventional therapies in humans and animals.[23,24] As with spontaneous disease, iatrogenic blood dyscrasias may be primary but are frequently secondary to other tissue toxicity. Blood and hematopoietic tissue rank with liver and kidney as target organs worthy of scrutiny in preclinical and clinical safety evaluations. Factors contributing to this include the high mitotic rate of hematopoietic tissue, the exposure of blood cells to agents administered systemically, and the consequences of blood cell damage and bone marrow impairment.[25]

Blood is a vital sample to determine the presence and level of drugs and other toxicants in the body. Blood is often used to investigate drug ingestion, poisoning, and driving impairment. It can also help determine the cause of death. Blood is a highly vulnerable and affected tissue by any therapeutic agents or toxic substances. Blood cells are exposed to any agent absorbed or injected into the bloodstream, even those rapidly metabolized and excreted. In clinical setting, blood manifestations are assayed for the purpose of diagnosis, treatment or prevention of diseases. Therefore, haematological alterations have higher predictive value for human toxicity when the data are interpreted from animal studies. Significant alterations in hematological profiles at all dose levels of a therapeutic agent would indicate that this drug would not be a suitable option for further use or development. From the study some prominent and noticeable hemolytic changes in some major hematological parameters were found.

White blood cells are the part of body's immune system and responsible for detecting and destroying diseases and foreign invaders which was decreased highly to the treated rats, though it was not statistically significant yet prominent. Platelets or thrombocytes are small blood cells stops bleeding from any injuries by forming plug (clot) and contribute to inflammation control. An elevation in the platelets count was found from this study in the rats those were treated with the medicine. High platelets count in the blood can lead to a condition called Thrombocytosis. Though this condition remain silent and diagnostic process is challenging can be led to thrombosis, a life-threatening condition. Conversely, thrombocytopenia is a condition, where subject has less count and remains exposed to the risk of bleeding.[26]

There are currently no established regulations or safety assessments regarding Ayurvedic medicines, which may lead to inconsistencies in the components utilized and their concentrations. Consequently, several Ayurvedic drugs have been reported to exhibit harmful effects post-administration.[27] It is essential that Ayurvedic preparations and formulations are subject to strict regulation by relevant authorities. The government should consider implementing restrictions to prevent direct access to or over-the-counter purchases of Ayurvedic medicines.

The findings of this study indicate that there is no significant alteration in the hematological parameters of the treated rats, suggesting that the usual dosage is safe for use. However, further investigation involving biochemical parameters is necessary, particularly with higher dosages, to reaffirm the safety profile of this Ayurvedic medicine.

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