A Subchronic 90-day Oral Toxicity Study of Sivanar Vembu Khuzhi Thailam in Rats

Ganesh Shenoy[1], Smita Shenoy[2]*, Sathish Pai B[3], Nitesh Kumar[4], Arul Amuthan[1], Manjunath Shetty[1], Mohandas Rao[5], Dr Kanthilatha Pai[6], Bharath Rao K[7]

1. Department of Pharmacology, Melaka Manipal Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India-576104.
2. Department of Pharmacology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India-576104.
3. Department of Dermatology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India-576104.
4. Department of Pharmacology and Toxicology, NIPER, Hajipur.
5. Department of Anatomy, Melaka Manipal Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India-576104.
6. Department of Pathology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India-576104
7. Department of Oral Biology, Melaka Manipal Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India-576104.

Abstract

The purpose of this study was to test the toxicity of a Siddha drug, Sivanar Vembu Khuzhi Thailam (SVKT), in Sprague Dawley rats. The chronic toxicity study was conducted in accordance with (OECD) test guideline 408. In this study, SVKT was administered orally every day to groups 1 to 3 of rats at doses of 40, 130, and 400 mg/kg body weight for a period of 90 days. An additional satellite group (group 4) received 400 mg/kg body weight of SVKT for 90 days and did not receive the drug for another 28 days. The experiment ended on day 118 for the group 4 and day 90 for the other groups. The animals' body weight was measured once a month. Hematological and biochemical analyses were performed at the conclusion of the experiment. Histopathological examination of vital organs of rats was performed for gross findings. Organ weights were also recorded.

There was no significant difference ($p > 0.05$) observed in the relative organs weight, body weight. However, histopathological findings seen in test groups were also seen in control. The increase in total WBC and differential counts were within normal physiological range.

Experimental article (J Int Dent Med Res 2022; 15(1): 88-93)

Keywords: Siddha, Sivanar Vembu Khuzhi Thailam, chronic toxicity study.

Received date: 11 December 2021 **Accept date:** 14 January 2022

Introduction

Medicinal plants are a storehouse of various bioactive chemicals with different medicinal qualities. According to a survey conducted by the World Health Organization, traditional herbal formulations are popular among 80 percent of the population in underdeveloped countries to treat ailments[1]. Many natural elements found in plants, such as polyphenols, flavonoids, and tannins, are protective and curative in various ways[2]. Siddha is a traditional medical system that dates back thousands of years. This system of medicine is used to address acute and chronic medical problems and is primarily practiced in the southern part of India[3]. Standardization of herbal formulations is vital in determining the quality of the drug based on the concentration of its active principle and assuring its safety profile, of which botanicals are an essential part of this type of traditional medicine[4]. Before beginning a human trial, preclinical toxicity research is essential to define a safe dose and find the medicine's negative effects[5].

Psoriasis mainly affects the skin and is a chronic autoimmune disease. Pathogenesis of this chronic inflammatory diseases involves mainly IL-23/Th17 axis and several molecules have been marketed which target this axis[67]. The disease affects 1 to 3 percent of the world's

Corresponding author:
Professor Dr. Smita Shenoy
Head, Department of Pharmacology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India-576104
E-mail : smita.shenoy@manipal.edu

population and is characterized by itching, erythema, and scaling[8]. Long-term treatment is essential because of the chronicity and recurrent nature of psoriasis. The severity of the condition, the presence of related comorbidities, and access to health care are all factors in determining how it is treated. Patients are classified as mild, moderate, or severe depending on the clinical severity of the lesion, the percentage of body surface area affected, and their overall quality of life. Psoriasis is treated using phototherapy, glucocorticoids, anticancer drugs, immunosuppressants, and biologicals. Yet, no treatment has been demonstrated to be effective[9]. Furthermore, long-term use of these medications has its own set of side effects. Siddha medicine, which originated in India and Sri Lanka, is an old and well-documented among Indian traditional remedies, based on careful observation and experimentation[10]. Siddha classifies skin illnesses into the following categories: Vida kadikal, Amman, Kuttam, Karappan, Punkal, and miscellaneous, with safe herbal and herbo mineral treatments for all of them. Under karappan diseases, psoriasis is synonymous with kutta karappan, mandai karappan, and chori kiranthi (kiranthi diseases)[11]. Although Siddha is widely used to treat psoriasis, there are no published studies on its clinical efficacy and safety. Siddha medicine, which originated in India and Sri Lanka and is based on meticulous observation and experimentation, is one of the well-documented and oldest Indian traditional treatments[3]. *Sivanar vembu kuzhi thailam* (SVKT)[12] is a polyherbal formulation described in traditional literature of siddha for Karappan (chronic weeping eczema in children), Megam (veneral diseases), Kushtam (leprosy), Katti (boils), Seelaipun (bed sores), Vandu kadi (insect bites), Visham (toxaemic states), Naatpatta viranam (chronic ulcers), and Kalanchaka padai (psoriasis). *Indigofera aspalathoides, Celastruss paniculatus, and Corallocarpus epigaeus* are the plants that make SVKT [13].

Materials and methods

Supplies and chemicals

SVKT was procured from SKM Siddha and ayurveda (GMP certified) Company (India) Ltd, Saminathapuram (Post). Modakkurichi, Erode District- 638 104, Tamilnadu. All other chemicals and clinical diagnostic kits were purchased from Agappe Diagnostics Limited, India.

Animal and experimental design

In this study, adult female Sprague Dawley rats aged 12–13 weeks were used. Under typical laboratory settings of light (12 h light/dark), humidity, and temperature, the animals were fed a standard feed and had free access to water.

Institutional animal ethics committee, Kasturba Medical College, Manipal (IAEC/KMC/78/2017 dated 28.10.2017) approval was obtained before the commencement of experiment.

A 90-day oral toxicity study was conducted at the central animal research facility, Manipal to assess the safety of SVKT. The body weight was documented before the commencement of experiment. The dosage levels that were targeted were 40, 130, and 400 mg/kg/day. The human dose of SVKT was converted to animal equivalent dose (AED) of 40 mg/kg/day using surface area ratios. The second dose chosen was approximately three times that of the AED, and the third dose was approximately ten times that of the AED.

Thirty rats were grouped into five of six animals each in this experiment. SVKT was dosed orally to four groups (groups 2 – 5) rats for 90 days continuously.

The control animals (group 1) were only given 10 mL/kg body weight of distilled water. Groups 2 to 4 received SVKT at the dose of 40, 130 and 400 mg/kg body weight respectively. The satellite group (group 5) animals were given the SVKT 400 mg/kg body weight orally for 90 days and then didn't get any therapy for another 28 days. This experiment ended on day 118 for the satellite group and day 90 for the other groups.

Throughout the study, all of the experimental animals were monitored for mortality daily. During the experimental period, visual observations for behavioral patterns (salivation, fur, lethargy, and sleep), changes in physical appearance, injury, pain, and signs of illness were made once daily. The animals' body weight was measured once a month.

At the end of the 90-day treatment period in groups 1–4 and 118-day treatment period in group 5, rats were anesthetized with pentobarbital (150 mg/kg) and blood was drawn

by retro-orbital puncture in EDTA vacutainers (for hematological analysis) and serum vacutainers (for biochemical analysis). Total white blood cell count, differential count, hemoglobin, mean corpuscular Hb conc, mean corpuscular Hb, total red blood cells, mean corpuscular volume, packed cell volume, red blood cells distribution width, and platelet count were all measured with a fully automated hematology analyzer (AGD Erma Inc., Japan).

A fully automatic analyzer (Star 21 Plus Semiautomatic Biochemistry Analyzer, India) was used to assess serum biochemical parameters such as urea, creatinine, uric acid, total protein, albumin, globulin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and serum electrolytes (sodium, potassium, and chloride). All rats were subjected to a gross necropsy at the end of the study. Heart, liver, spleen, kidneys, lungs, skin, and brain organs were dissected, trimmed, and weighed. Their relative weights were calculated. These organs were preserved in a 10% formalin solution and histologically examined.

Results

Clinical signs:

Daily oral administration of SVKT (all three doses) for 90 days caused no visible toxicity symptoms. There were no deaths or obvious clinical manifestations in any of the groups during the experiment. Physical examination of the treated rats revealed no evidence of toxicity in their mucus membranes, eyes, fur, or behavioral alterations such as salivation, tremors, sleep, diarrhea or coma.

Discussion

Dietary intake and metabolic process efficiency determine an individual's nutritional status. The bodyweight may determine an individual's health status; an increase in body weight is an indication of the normal health status of animals. Increase in the body weight of animals among all the study groups indicates nullification of the adverse impact of the drug on weight gain. The drug did not affect the relative weight of the heart, liver, spleen, kidney, lung, and brain at any dose level in rats compared to the control group. As a result, an increase in body weight from drug administration indicates that the drug is safe for mass balance and overall body functions, which is supported by an insignificant change in relative organ weights[14, 15]. During this oral toxicity study, there was no fatality or clinical signs/symptoms of broad systemic toxicity during this oral toxicity study[16]. The integrity and functionality of organs and organ systems are assessed using biochemical markers. They are also significant in determining the overall body health, assessment of risk, and pathological conditions[17]. Based on the biochemical findings of the current investigation, no significant changes in several indicators of kidney and liver function were detected. Clinical biochemistry values and hematological data are used to determine a drug's toxicity. An increase in these enzyme levels indicates that the liver parenchymal cells have been damaged[5]. The liver, being the primary organ for metabolising and detoxifying medications and poisons in the body, is vulnerable to chemical assault[18]. Serum ALT and AST are the most sensitive markers of liver damage because they are found in the cytoplasm and are released into the circulation following hepatocellular damage[19]. But SVKT administration did not show significant increase in AST, ALT, ALP, total protein levels, albumin, globulin, direct bilirubin, and serum electrolytes such as sodium, potassium, and chloride, indicating no damage to the parenchymal cells when correlated with the histological examination. However, there was a significant increase in the total bilirubin levels in the SVKT group (400 mg/kg bw) and satellite group. The increased serum total bilirubin may be due to the destruction of aged red blood cells, which results in increased bilirubin production, bile duct blockage, direct bilirubin buildup, and escape from the liver into the blood, impairing the liver's ability to transform bilirubin to the bile pigment-bilirubin glucuronide[20–22].

Toxic chemicals frequently target the hem atopoietic system, particularly the bone marrow, producing red blood cells. The hematopoietic sys tem is a primary tissue in which mature cells with a short life span are replaced by young cells. It is also one of the most sensitive organs in animals to test toxicity. In the current study, the majority of the parameters such as RBC, Hb, MCV, MCH, MCHc, RDWc, PLT Count, and PCV in the SVKT treated group was found to be comparable to control, with values within the normal physiological range, indicative of a lack of toxic

potential to the hematopoietic system[22–25]. The count of white blood cells and their indices are important in immune function. The immune system's ability to create antigenic specificity and the phenomena of immunological memory are two distinct features. Leukocytes (white blood cells) give immunity to the body against antigen invasion. Compared to control, SVKT 400 mg/kg BW and satellite groups, WBC, and differential leukocyte count levels were greater, showing an immune system boost, which might be related to the test drug's immunopotentiation action[26–28]. The elevated levels were within normal physiological range[29,30].

Some histological changes were seen only in the brain (pyknotic neurons), kidney (edematous cortex in some areas), and lungs (aggregation of inflammatory cells) of experimental animals as well as the satellite group. However, the histological changes observed in the experimental groups in the brain, kidney, and lungs were also observed in the normal group. They thus cannot be attributed to the administration of the SVKT, and these changes could be considered incidental. Histologically, all of the remaining organs were normal.

Conclusions

Our data indicate that subchronic exposure to *Sivanar vembu kuzhi thailam* at the dose of 40 and 130 mg/kg body weight did not significantly change any of the parameters analyzed. Hence this indicates that the traditionally recommended dose in human beings is safe.

Ethical considerations

The study was conducted after obtaining clearance from Institutional animal ethics committee, Kasturba Medical College, Manipal, Manipal Academy of Higher Education. Administrative and research advisory committee approvals were obtained before animal experiment was begun.

Declaration of Interest

The authors report no conflict of interest.

PARAMETERS	GROUPS				
	CONTROL	SVKT 40 mg/kg	SVKT 130 mg/kg	SVKT 400 mg/kg	SVKT satellite
TBIL (mg/dL)	0.02 ± 0.007	0.04 ± 0.023	0.03 ± 0.011	0.12 ± 0.051*	0.07 ± 0.022*, $
AST (IU/L)	160.7 ± 44.7	136.7 ± 13.7	133.6 ± 15.1	136.4 ± 20.8	135.6 ± 13.8
ALT (IU/L)	89.6 ± 9.5	80.2 ± 2.5	88.2 ± 10	90.1 ± 11.5	86.4 ± 3.5
ALP (IU/L)	204.3 ± 25.3	255.1 ± 51.0	249.5 ± 60.9	250.0 ± 30.6	250.6 ± 18.8
UREA (mg/dL)	11.1 ± 0.5	11.4 ± 0.6	11.8 ± 0.5	11.7 ± 0.5	11.5 ± 0.4
CREAT (mg/dL)	0.7 ± 0.04	0.6 ± 0.09	0.7 ± 0.11	0.8 ± 0.04	0.8 ± 0.02

Note: *p≤0.05 versus Control, $ p≤0.05 versus SVKT 400 mg/kg
One-way ANOVA followed by post hoc TUKEY test was performed. Values are represented as mean ±SD
TBIL: Total bilirubin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; CREAT: Creatinine

Table 1. Effect of SVKT on biochemical parameters in Rats.

PARAMETERS	GROUPS				
	CONTROL	SVKT 40 mg/kg	SVKT 130 mg/kg	SVKT 400 mg/kg	SVKT satellite
WBC (10^3/µl)	4.8 ± 0.9	5.9 ± 2.1	3.8 ± 1.8	7.7 ± 0.8$^{*,\#}$	7.3 ± 0.9$^{*,\#}$
NEUTRO (%)	24.6 ± 0.6	25.7 ± 0.9	25.6 ± 0.4	27.9 ± 0.5$^{*,¥,\#}$	27 ± 1.3*
LYMPHO (%)	65.8 ± 1.7	66.1 ± 1.2	66.2 ± 0.9	68.7 ± 1.1$^{*,¥,\#}$	66.6 ± 1.6
MONO (%)	6.1 ± 0.7	5.8 ± 0.4	5.7 ± 0.6	1.8 ± 0.5$^{*,¥,\#}$	1.9 ± 0.2$^{*,¥,\#}$
RBC (10^6/µl)	8 ± 0.7	7.8 ± 0.5	7.4 ± 0.2	7.6 ± 0.3	8.5 ± 0.3$^{\#,\$}$
Hb (g/dl)	14 ± 1	13.7 ± 0.2	13.1 ± 0.2	13.7 ± 0.3	14.2 ± 0.7
PLT (10^3/µl)	406.8 ± 152.5	429.6 ± 106.4	430.5 ± 78.9	355.3 ± 68.8	393.2 ± 95.4

*p≤0.05 versus Control, $^{¥}$p≤0.05 versus SVKT 40 mg/kg, $^{\#}$ p≤0.05 versus SVKT 130 mg/kg, $^{\$}$ p≤0.05 versus SVKT 400 mg/kg
One-way ANOVA followed by post hoc TUKEY test was performed. Values are represented as mean ±SD
WBC: Total white blood cells; NEUTRO: Neutrophils; LYMPHO: Lymphocytes; MONO: Monocytes; RBC: Total red blood cells; Hb: Hemoglobin; PLT: Platelet count

Table 2. Effect of SVKT on Haematology in Rats.

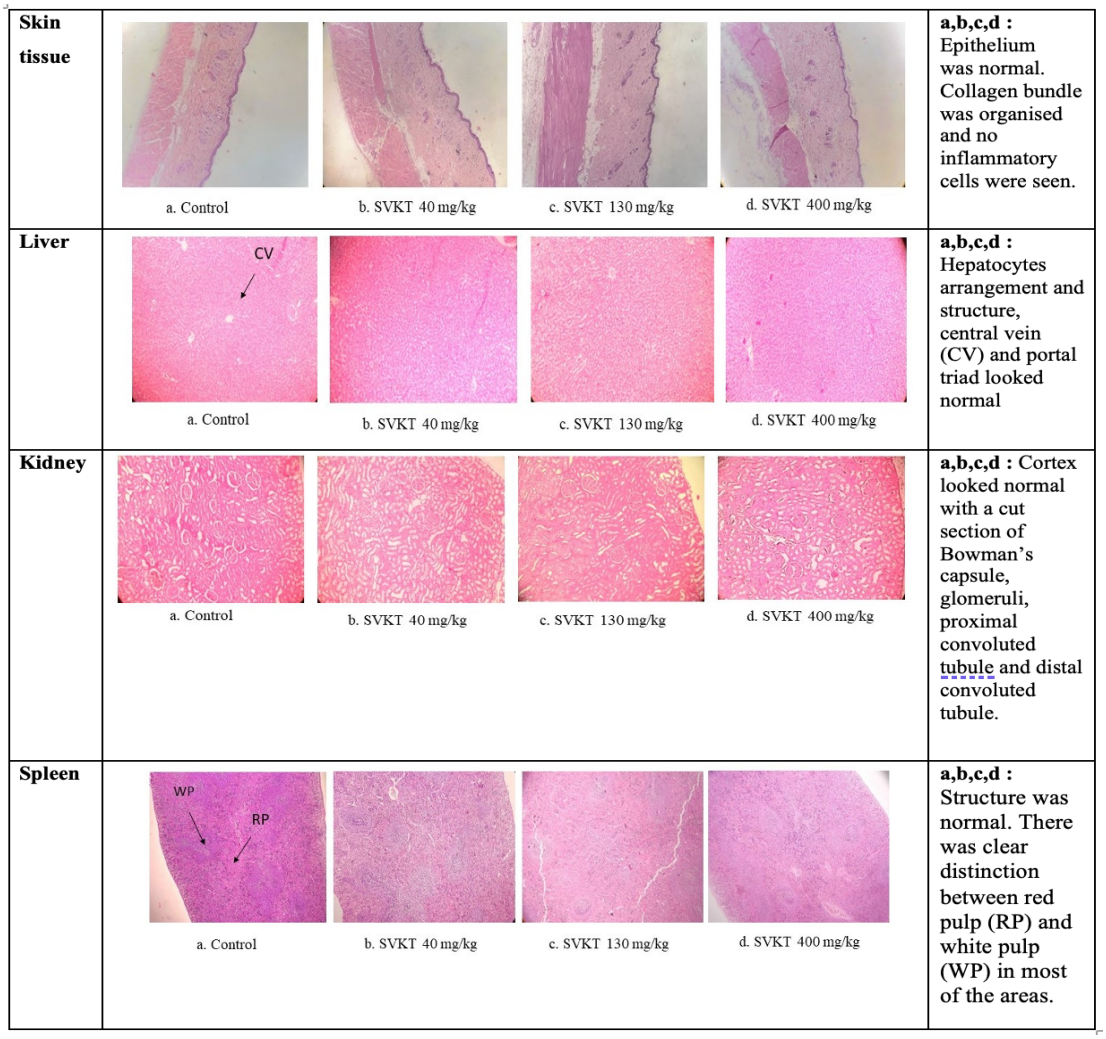

Figure 1. Qualitative assessment of Haematoxylin and Eosin (H & E) stained organ tissues.

References

1. World Health Organization. The Health of Indigenous Peoples. Fact Sheet 326. 2007;1:1-2.
2. Suwannakul, S., Chaibenjawong, P., & Suwannakul S. Antioxidant Anti-Cancer and Antimicrobial Activities of Ethanol Pandanus amaryllifolius Roxb . leaf extract (In Vitro)-A potential medical application. *J Int Dent Med Res*. 2018;11(2):383-389.
3. Thas JJ. Siddha Medicine—background and principles and the application for skin diseases. *Clin Dermatol*. 2008;26(1):62-78. doi:10.1016/j.clindermatol.2007.11.010
4. Sachan AK, Vishnoi G, Kumar R. Need of standardization of herbal medicines in Modern era. *Int J Phytomedicine*. 2016;8(3):300. doi:10.5138/09750185.1847
5. Dubey SK BA. Acute and sub acute toxicity studies on ethanolic fraction of Thuja occidentalis Linn. *Res J Pharm Technol*. 2008;1(3):245-248. doi:10.5958/0974-360X
6. Sulistio E, Masulili SLC, Lessang R AE. The influence of smoking on IL-17 cytokine in chronic periodontitis patients. *J Int Dent Med Res*. 2019;12(1):199-202.
7. Mas, N., Purwaningsih, S., Purwaningsih, N.M., Heah, K.G., Zhu, H., Rosdy, M.N., & Omar E. DEF6 Expression and Regulation in Cancer, Chronic Inflammatory Diseases and Autoimmune Diseases: A Review. *J Int Dent Med Res*. 2020;13(1):364-371.
8. Parisi R, Symmons DPM, Griffiths CEM, Ashcroft DM. Global Epidemiology of Psoriasis: A Systematic Review of Incidence and Prevalence. *J Invest Dermatol*. 2013;133(2):377-385. doi:10.1038/jid.2012.339
9. Mrowietz U, Kragballe K, Reich K, et al. Definition of treatment goals for moderate to severe psoriasis: a European consensus. *Arch Dermatol Res*. 2011;303(1):1-10. doi:10.1007/s00403-010-1080-1
10. Dubois Declercq S, Pouliot R. Promising New Treatments for Psoriasis. *Sci World J*. 2013;2013:1-9. doi:10.1155/2013/980419
11. Thambyayah M, Amuthan A. Infantile seborrheic dermatitis: A pediatric Siddha medicine treatise. *Clin Dermatol*. 2015;33(3):355-361. doi:10.1016/j.clindermatol.2014.12.013
12. SKM Siddha and ayurveda company I. *Therapeutic Index Siddha(Hospital Pharmacopoeia),*. 1st ed.; 2010.
13. Bogar. *Bogar Vaidhyam*.
14. MISHRA B, JOHN E, JOY K, R B, R A. TOXICITY PROFILE OF CELASTRUS PANICULATUS SEEDS: A PRECLINICAL STUDY. *Asian J Pharm Clin Res*. Published online May 8, 2020:115-118. doi:10.22159/ajpcr.2020.v13i7.37803
15. Sharma M, Patgiri B, Nariya M, Jamadagni S, Bedarkar P. Chronic toxicity study of Sameera Pannaga Rasa in Charle's foster albino rats. *AYU (An Int Q J Res Ayurveda)*. 2020;41(1):36. doi:10.4103/ayu.AYU_49_20
16. Shaikh M, Ahmed SS, Urooj M, Viquar U, Kazmi MH, Husain GM. 90-Days Repeated Dose Oral Toxicity Study on Majoon-e-Nisyan. *Int J Pharmacol Phytochem Ethnomedicine*. 2020;15:22-33. doi:10.18052/www.scipress.com/IJPPE.15.22
17. Lawal B, Shittu OK, Oibiokpa FI, Mohammed H, Umar SI, Haruna GM. Antimicrobial evaluation, acute and sub-acute toxicity studies of Allium sativum. *J Acute Dis*. 2016;5(4):296-301. doi:10.1016/j.joad.2016.05.002
18. I Arundina, Tantiana, I Diyatri, MDC Surboyo RA. Acute toxicity test of liquid smoke of rice hull (oryza sativa) on mice (mus musculus). *J Int Dent Med Res*. 2020;13(1):91-96.
19. Lokesha SB, kini RD, Kumar NA, Rashmi KS VN. A Study on Hepatoprotective Role of Virgin Coconut Oil on Liver Toxicity Induced by Cadmium Chloride in Rats. *J Int Dent Med Res*. 2021;14(3):1185-1189.
20. Dominique TK. Toxic Effect Study of Methanol Extract of Terminalia Glaucesens Leaves Following Single or Short-Term Repetitive Oral Administration in Swiss Mice. *Am J Biosci*. 2013;1(4):85. doi:10.11648/j.ajbio.20130104.16
21. Awotunde OS, Adewoye SO, P.S. D, Hawumba J. Subacute toxicity study of aqueous root extract of Terminalia schimperiana in male Wistar rats. *Toxicol Reports*. 2019;6:825-832. doi:10.1016/j.toxrep.2019.07.006
22. Hilaly J El, Israili ZH, Lyoussi B. Acute and chronic toxicological studies of Ajuga iva in experimental animals. *J Ethnopharmacol*. 2004;91(1):43-50. doi:10.1016/j.jep.2003.11.009
23. Testa NG, Dexter TM. Long-term hematopoietic damage: concepts, approaches, and results relevant to the study of environmental toxins. *Environ Health Perspect*. 1989;82:51-56. doi:10.1289/ehp.898251
24. Chitra B, Ramaswamy RS, Suba V. Toxicity Evaluation of Pūrṇa Cantirotaya Centūram, a Siddha Medicine in Wistar Rats. *Int Sch Res Not*. 2015;2015:1-10. doi:10.1155/2015/473296
25. Adeneye AA, Ajagbonna OP, Adeleke TI, Bello SO. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of Musanga cecropioides in rats. *J Ethnopharmacol*. 2006;105(3):374-379. doi:10.1016/j.jep.2005.11.027
26. Mythilypriya R, Shanthi P, Sachdanandam P. Oral Acute and Subacute Toxicity Studies with Kalpaamruthaa, a Modified Indigenous Preparation, on Rats. *J Heal Sci*. 2007;53(4):351-358. doi:10.1248/jhs.53.351
27. Hoffbrand AV MP. *Essential Haematology*. 6th ed. Willey-Blackwell Publisher; 2008.
28. WM A, DW N. Hematological Markers of In Vivo Toxicity. *J Hematol Thromboembolic Dis*. 2016;04(02). doi:10.4172/2329-8790.1000236
29. Lillie LE, Temple NJ, Florence LZ. Reference values for young normal Sprague-Dawley rats: weight gain, hematology and clinical chemistry. Hum Exp Toxicol. 1996;15(8):612-616. doi:10.1177/096032719601500802
30. Delwatta SL, Gunatilake M, Baumans V, et al. Reference values for selected hematological, biochemical and physiological parameters of Sprague-Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka. Anim Model Exp Med. 2018;1(4):250-254. doi:10.1002/ame2.12041