



## A review on toxicological studies of selected anti-diabetic herbal formulations

S Ruby\*, Rkarthik, R Mohit Rahul, K Manojkumar, M Kumar

Department of pharmaceutical chemistry, Vinayaka Missions College of pharmacy, Salem, Tamil Nadu, India

### Abstract

The main aim is to review about the Siddha system of medicines, one of the indigenous systems of drugs, is being practiced in India. The following review states about the toxicological profile of a commonly used herbal formulation was studied, and also toxicological profile of anti-diabetic herbal formulations in this current review.

**Keywords:** toxicity studies, anti-diabetic herbal formulations

### Introduction

Siddha System of drugs homogenous of Indian network of herbal medicines, which is extremely potent and unique system in comparison with other traditional systems alive. Siddha Medicine is concentrating more on the health caring of Siddha Vaidya must be on the crown of all the normal arts of the herbal drug world due to its richness. Siddha medicine, one among the medical systems legally recognized and supported by the govt of India, is, however, little known outside Tamil Nadu and its practice limited mainly to the Tamil populations of India and overseas [1, 2, 3].

### Terminology

Diabetes mellitus is a metabolic sickness withinside the endocrine system. Diabetes is a continual sickness in metabolism of carbohydrate, proteins, and fats because of absolute or relative deficiency of insulin secretion with / without various diploma of insulin resistance. Also, it can be described as a ailment wherein the frame produces little insulin / ceases to provide insulin, or turns into modern resistance to its actions [4, 5].

### Siddha System of Diabetes Medicine

In siddha system of drugs is an insulin-dependent. Siddha system of drugs is claimed to alleviate the basis explanation for the diseases by maintaining the ratio of tridoshas; Vatham, Pitham and Kapham. These unique humors operate constantly between environment and individual and are required to take care of the integrity of a living system. Siddhars, fathers of Siddha system of drugs grouped the DM under Pitha disorders of "Mega" disease. Mega disease means excessive discharge of urological secretions and excretions from the body [6, 7].

### Anti-diabetic property of siddha medicines

Many of the siddha medicinal flora own the anti-diabetic property. Siddha medication has higher treatments for remedy and control of diabetes. Anti-diabetic capsules has principal range of medication like sirukurinjan (*Gymnemasylvestre*), Vendhayam (*Trigonella foenum graecum*), Avarai (*Cassia auriculata*), kondrai (*Cassia fistula*), Naval (*Eugenia jambolana*), Vilvam (*Aegle marmelos*) having antidiabetic properties [8, 9].

### Toxicological Studies

#### Definition

Toxicology may be a branch of science that deals with toxins and poisons and their effects and treatment. Toxicological screening is extremely important for the event of latest drugs and for the extension of the therapeutic potential of existing molecules [10].

#### Historical development of toxicological studies

##### Paracelsus (1493-1541):

who decided unique chemical substances answerable for the determined toxicity of flowers and animals. He proven the innocent and useful outcomes of pollutants and proved dose-reaction relationships for the outcomes of drugs. Paracelsus, who changed into a physician, alchemist, and astrologer, is broadly appeared as the pater of toxicology. The following declaration of his is frequently quoted: "All materials are poisons; there's none which isn't not a poison.

Mathieu Orfila (1787-1853),

Spanish physician, decided the connection among poisons and their organic houses and confirmed particular organ harm resulting from toxins. Orfila is called the daddy of present day toxicology. Toxicological screening strategies and toxicological studies on man or woman materials advanced withinside the mid-1900s, and environmental toxicological research advanced withinside the mid-20 th century [11, 12].

J.W TREVAN 1920,

Proposed the usage of the 50% deadly dose (LD) take a look at to decide the deadly dose of man or woman chemical compounds. After the advent of LD, a FDA scientist John Draize advanced a technique for trying out eye and pores and skin inflammation the use of rabbits, and this technique become broadly prevalent for trying out the consequences of chemical compounds and prescribed drugs on the attention and pores and skin. Later, the United States National Cancer Institute (NCI) advanced a take a look at to become aware of carcinogenic chemical compounds thru the day by day dosing of rats and mice for two years.

In the early 1960s,

The heaps of infants have been born with debilitating beginning defects resulting from thalidomide. After this, all of the regulatory businesses focused on figuring out the toxicity profiles of all pharmaceutical materials to be had for

ordinary affected person use and made obligatory the acceptance of toxicity profiles of new drug application (NDA).

In the late 1980s,

The Organization for Economic Co-operation and Development (OECD) and the International Conference on Harmonization (ICH) introduced out the pointers for toxicity trying out of pharmaceutical substances <sup>[13]</sup>.

### **Herbal formulation toxicological methods:**

#### **Selection of animals**

Two distinct animal species mice and rabbits had been decided on for gift research acute toxicity research had been achieved on mice and sub-persistent research had been completed on rabbits <sup>[14]</sup>.

#### **Acute toxicity testing (ld50)**

Acute oral toxicity (LD50) became completed via way of means of the technique of Lorke. Albino mice of both intercourse; weighing 20 - 25 gm, had been maintained below chronic environmental situations  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and had been furnished general weight-reduction plan and water advert libitum. Three agencies of mice every comprising of three animals became administered with 10, one hundred and a thousand mg/kg of natural method via way of means of mouth and tested for mortality inside 24 hours. Following the effects of mortality in every institution, some other set of three agencies of mice had been administered better doses of the check drug, to acquire the least and maximum poisonous price and LD50 became calculated via way of means of geometric imply of the values <sup>[15]</sup>.

#### **Sub-chronic toxicity**

This check became completed on 24 healthful white rabbits of both intercourse from 1200 to 1800 grams. All animals had been similarly separated into 3 agencies, one institution appeared as manipulate and different obtained 20 and 60 mg/kg doses of natural method for consecutive 60 days via oral intubation tube. Doses had been organized in DMSO but manipulate institution obtained DMSO orally identical to the quantity of person doses in line with their frame weight. Before management of drug, bodily fitness of those animals became found all through the conditioning duration below the laboratory surroundings for per week explicitly seeing lack of hair, diarrhea, edema, ulceration and absence of activity <sup>[16]</sup>.

#### **Sample collection**

Blood pattern of round 6 ml had been amassed from those animals through cardiac puncture on the finishing touch of dosing on 61st day to decide numerous biochemical and hematological parameters <sup>[17]</sup>.

#### **Assessment of toxicities**

##### **physical examination**

Gross toxicities had been perceived each one-week after giving natural components for 60 days explicitly noticing pores and skin ulceration, common weight variation, lack of hair, lack of appetite, lack of activity, hematuria, vomiting, diarrhea, edema, lacrimation, salivation, muscle tone, tremor and competitive behavior.

Autopsy turned into accomplished after random selection, on the finishing touch of dosage and pattern series for biochemical tests <sup>[18]</sup>.

### **Biochemical evaluation**

Blood samples had been amassed from fasted animals previous to necropsy. Approximately 7 ml of blood samples had been amassed through aerobic puncture. Serum had been right away separated through centrifugation for 10 min at 4000 rpm and turned into tested for the subsequent parameters inside three hours of pattern series on Humalyzer 3000 (GmbH Germany) at  $37^{\circ}\text{C}$  using reagents furnished through Human GmbH Germany <sup>[19]</sup>.

1. Cardiac parameters: CK-NAC, LDH, AST.
2. Lipid profile: LDL cholesterol and triglycerides.
3. Blood glucose level.
4. Calcium and phosphorus.

### **Hematological evaluation**

Blood samples were collected under 10% EDTA at 7.2 pH and hematological parameters i.e. RBC, WBC, PLT, Hematocrit, & hemoglobin were explored using Humacount hematology analyzer GmbH 17400, a totally automated cell counter with a built-in veterinary software module <sup>[20]</sup>.

### **Microscopic examination**

Representative blocks from extraordinary regions of heart, liver and kidney had been reduce from every pattern after keeping apart all fats from respective organs. The blocks had been processed via Gilford a hundred and Ones computerized tissue processor <sup>[21]</sup>. Tissue slices of three - four micron had been taken from the wax blocks through rotary microtome. The tissue slices had been hooked up on slides and dehydrated softly through urgent with clear out out paper. The hooked up slides had been positioned in general for drying on a warm plate ( $45^{\circ}\text{C}$ ) for ninety mins after which left in an incubator at  $37^{\circ}\text{C}$  in a single day to dry earlier than microscopic examinations <sup>[21]</sup>.

### **Statistical analysis**

All the values for biochemical checks have been said because the imply and trendy mistakess to the imply (S.E.M.) and have been analyzed with the aid of using the usage of one manner unstacked ANOVA and p values have been found Results have been taken into consideration sizeable if p fee become much less than 0.05 and rather sizeable if p fee become much less than 0.005 <sup>[22]</sup>.

### **Toxicological Studies of Anti-Diabetic Drugs**

#### **Example of anti-diabetic drugs in toxicity studies**

1. Atthippattaiyathi Kasayam in Swiss Albino Mice and Wistar Rats of Toxicity Studies.
2. Naaval Kottai Mathiri in Rat Model of Toxicity Studies.
3. Nellikai in Rat Model of Toxicity Studies.

#### **Toxicological study of atthippattaiyathi kasayam**

- Preparation of aqueous extract.
- Experimental animals.
- Acute toxicity studies.
- Sub-acute toxicity studies.
- Histopathological study.

#### **Preparation of aqueous extract**

The aqueous extract for acute look at organized via way of means of powdered 2.7 gm of Atthippattaiyathi kasayam formula in 200ml of water and boiled it for a few mins to lessen 10 ml, permitting the decoction stand for half-hour

and filtered it thru paper filter. The quantity of the filtered answer became improved to ten ml with distilled water in order that 1 ml of the answer became equal to 270 mg of beginning fabric. For sub-acute look at, 2.7 gm, 13.5 gm and 27 gm of Atthippattaiyathi kasayam powder became delivered to 200ml of water one after the other and boils it for a few mins to lessen 10 ml, permitting the decoction stand for half-hour and filtering it thru paper filter. The quantity of the filtered answer became improved to ten ml with distilled water in order that 1 ml of the answer became equal to 270 mg, 1350 mg and 2700 mg of beginning fabric respectively [24].

#### **Experimental animals:**

Acute and sub-acute toxicity research had been accomplished in Swiss albino mice and Wistar rats, respectively. All the animals had been saved below widespread environmental condition ( $27 \pm 2$  diploma C). Adult mice (6 weeks antique) of both intercourse weighing 20-25 gm had been housed in polypropylene cages, five animals in line with cage with unfastened get right of entry to water and widespread pellet diet (Sai Meera meals Pvt. Ltd, Bangalore). 6-eight weeks antique Wistar albino rats of both intercourse weighing 150-2 hundred gm had been housed, five in line with cage in polypropylene cages with unfastened get right of entry to meals and water. The standards of laboratory animal care had been observed [24].

#### **Acute toxicity study**

For acute toxicity research, 20 mice had been used for the look at. The mice had been divided into 2 corporations containing 10 animals. The animals had been fasted in a single day and the drug became administered orally. Group I obtained distilled water (car for formula) and served because the Manage. Group II obtained 270 mg/kg b.w of Atthippattaiyathi kasayam aqueous extract unmarried dose orally. The animals had been determined constantly for the primary 4hrs then once in a while as much as 24hrs after which every day up to fourteen days, put up remedy to look at for any poisonous signs and mortality [24].

#### **Sub-acute toxicity study**

The animals were fasted overnight and thus the drug was administered orally. group one gets the value of formulation at the dose of 270, 1350, 2700 mg/kgb. Food and water consumption was checked daily. weight was checked on the starting stage of week intermission all over the study [24].

#### **Histopathological study**

All the animals had been sacrificed on day 29 below ether anesthesia. Necropsy of all animals became accomplished and the weights of the organs which include liver, kidneys, brain, heart, and lungs had been recorded. Tissue samples of organs from manage and handled animals had been preserved in 10% formalin for coaching of sections the use of microtome. The organs blanketed liver, kidneys, heart, lungs and belly of the animals had been preserved and that they had been subjected to histopathological examination. The organ pieces (three-five micron) had been constant in 10% formalin for twenty-four hours and washed in walking water for twenty-four hours. Samples had been dehydrated in tissue processor after which wiped clean in benzene to dispose of absolute alcohol. Embedding became achieved via way of means of passing the cleared pattern thru 3 cups

containing molten paraffin at 50 diploma Celsius after which a cubical block of paraffin made via way of means of the L moulds it became observed via way of means of microtome and the slides had been stained with haematoxylin-eosin stain. Stained sections of every organ had been tested below mild microscope at high (40x) energy magnification. All the histopathological slides had been organized at Dept of Pathology, Vels University, pallavaram, Chennai [24].

#### **Toxicological study of naaval kottai mathiri**

- experimental animals.
- Acute toxicity Study.
- Sub-chronic toxicity study.
- Histopathological studies.
- Statistical analysis.

#### **Experimental animals**

Healthy out bred Wistar Albino Rats of both intercourse weighing approximately 120 – a hundred and sixty g had been acquired from the Animal residence in tamil nadu. The girl rats acquired had been nulliparous and non pregnant. The animals obtained RO water advert libitum and fed with Rodent pellet which become bought from Shri Venkateshwara Traders, Bengaluru. Before the induction of toxicity take a look at, all of the animals had been acclimatized for seven days. The take a look at protocol has were given approval from Institutional Animal Ethical Committee of National Institute of Siddha, Chennai [25].

#### **Acute toxicity Study**

This take a look at become done with the aid of using following the method with the beginning dose of 2000 mg/kg frame weight of check drug referred to in OECD 423 guideline; six girl rats had been randomly decided on and acclimatized previous to the take a look at. The rats had been fasted in a single day earlier than the administering of check drug. After the management of check drug, the rats had been disadvantaged of feed for sixteen hour and water become now no longer allowed for preliminary three hours. The take a look at become performed to start with with the beginning dose of 2000 mg/kg administering in 3 rats and determined for mortality. As there has been no mortality, 3 greater rats had been subjected to the take a look at with the equal dosage of check drug. The check drug NKM become administered via oral gavage suspended withinside the distilled water as unmarried dose. The rats had been determined for mortality, behavioural modifications and medical symptoms and symptoms of toxicity for 1/2 of an hour as soon as in first 4 hours after dosing and thereafter periodically up to fourteen days on equal time of every day [25].

#### **Sub-chronic toxicity study**

This take a look at become done with the aid of using following OECD pointers followed for the trying out of chemicals – 408. In the literature Kannusamiyam ennum Pathartha Guna Vilakkam, Mooligai vakuppu, the human supposed dosage for NKM become encouraged as pattani length 500 mg two times a day (one thousand mg/day). On the premise of frame floor place conversion in opposition to human dose, 1000mg/kg/day dosage of NKM become calculated for rat (Paget and Barnes, 1964). In the existing take a look at, 3 doses of NKM of ninety mg/kg/day (Low

dose), 450 mg/kg/day (Intermittent dose) and 900 mg/kg/day (High dose) had been decided on for management. Wistar Albino rats of each intercourse had been randomized into 4 agencies of ten animals each (10 males, 10 females). Group I obtained a vehicle (distilled water) and served as manage institution. Group II, III and IV served as low, intermittent and excessive doses of NKM respectively. All the check materials had been administered as soon as day by day through oral path via gastric gavage for ninety days. All the rats had been determined day by day for mortality, morbidity and strange medical symptoms and symptoms on every day for the equal time. The frame weight change, water and meals intake of every rat become monitored as soon as a week. At the cease of ninety days treatment, stay rats had been fasted over night time and at the ninety one st day beneath mild chloroform anaesthesia, blood had been drawn the usage of capillary tube from the unfashionable orbital sinus and delivered right into a tube with potassium EDTA and a tube without anticoagulant. The blood samples without anticoagulant had been used for estimating biochemical parameters which includes Glucose, Cholesterol, Triglyceride (TG), Protein, Urea, Creatinine, Bilirubin, Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) and Alkaline Phosphatase (ALP) the usage of Erba gadget Pack kits in Fully Automated Biochemistry analyzer (Transasia EM 360). Sodium, Potassium and Chloride content material had been predicted with the aid of using the usage of electrolyte analyser from Roche®. After withdrawal of blood, all of the rats had been sacrificed for gross necropsy and histopathological take a look at. Organs inclusive of brain, lungs, heart, liver, kidney, spleen, testis and ovaries had been studied for gross necropsy and weighed for calculating relative organ weight.

#### Histopathological studies

liver, kidney, lungs, stomach, heart, spleen, brain, and femoro tibial joints had been done for manage and excessive dose institution. The tissues of gathered organs had been constant in 10% Neutral buffered formalin for twenty-four h. The tissues had been trimmed, embedded in molten paraffin wax and sectioned (4-five microns thickness) the usage of rotary microtome. The sections had been floated in warm water and located withinside the glass slide. Established in DPX and tested beneath mild microscope [25].

#### Statistical analysis

All information had been expressed as mean  $\pm$  wellknown deviation (SD). The check agencies had been as compared with manage institution for trying out importance and executed with the aid of using One-manner Analysis of Variance (ANOVA) accompanied with the aid of using Dunnett Multiple Comparisons Test the usage of GRAPH PAD INSTAT model three software program programs [25].

#### Toxicological study of *Phyllanthusemblica linn.* (Nellikai)

- Plant materials.
- Experimental animals.
- Evaluation of chronic toxicity.
- Hematological and blood chemical analyses.
- Necropsy.
- Statistical analysis.

#### Plant material

The end result of *P. emblica* had been accumulated from the wooded area of Nan province, Thailand. The plant cloth turned into recognized and the voucher specimen (PBM 01402) turned into stored at Faculty of Pharmacy, Mahidol University, Bangkok, Thailand [26].

#### Experimental animals

Seven weeks old, male and girl Sprague-Dawley rats, weighing inside two hundred-220 g had been acquired from the National Laboratory Animal Center, Nakorn Pathom, Thailand. Animals had been randomly assigned to govern and remedy companies (five rats/intercourse organization). They had been housed below fashionable environmental situations of temperature at 24 + 1 °C below a 12 h dark-mild cycle, and had been allowed unfastened get admission to to ingesting water and fashionable pellet diet. Rats had been stored in experimental facility for 1 week to permit them to be acclimated previous to dosing. The Animal Ethics Committee of Faculty of Medicine, Thammasat University, Pathum Thani, Thailand, authorised all experimental protocols (No. 0001/2007). Administration: Animals had been disadvantaged of meals besides water 16-18 hour previous to dosing on day 0. According to the World Health Organization (WHO) tenet (WHO 2000) and the Organization of Economic Cooperation and Development (OECD) tenet for trying out of chemical substances 420 (OECD 2001), the extract on the dose of five, 000 mg/kg turned into given orally to check organization of rats, at the same time as the manage organization obtained water withinside the equal quantity with the aid of using gavage the usage of a ball-tipped chrome steel feeding needle. Jaijoy *et al.* 50 Observation of toxicity symptoms and symptoms: Body weight, symptoms and symptoms of toxicity (trendy behavior, breathing pattern, cardiovascular symptoms and symptoms, motor activities, reflexes, and alternate in pores and skin and fur) and mortality had been determined after the management on the first, second, fourth and 6th hour and as soon as every day for subsequent 14 days. On the fifteenth day, all rats had been stored fasted overnight, after which anesthetized with thiopental sodium (50 mg/kg). Rats had been sacrificed for necropsy examination. The inner organs had been excised and weighed.

The gross pathological observations of the tissues had been finished with the aid of using histopathological examination [26].

#### Evaluation of chronic toxicity

##### Experimental animals

Seven weeks old, male and girl Sprague-Dawley rats had been randomly divided into 5 companies of 10 rats each. At the onset of dosing, girl weighed inside 160-a hundred and eighty g and male weighed inside two hundred-220 g. Administration: According to the WHO tenet (WHO 2000) and the OECD 452 (OECD 1981), the extract turned into administered orally at concentrations of 300, six hundred and 1, two hundred mg/kg to the 3 next remedy companies for consecutive 270 days, at the same time as the manage organization obtained distilled water. In order to evaluate reversibility and/or accumulation effects, the extract on the dose of 1, two hundred mg/kg turned into given as soon as every day to the satellite tv for pc organization of rats for 270 days, and stored for different 28 days submit remedy [26].

### Observation of toxicity signs

General behavior, breathing pattern, cardiovascular symptoms and symptoms, motor activities, reflexes, alternate in pores and skin and fur, mortality and the frame weight modifications had been monitored every day. The time of onset, intensity, and length of those symptoms and symptoms, if any, turned into recorded [26].

### Hematological and blood chemical analyses

At the cease of the observe, all animals had been stored fasted for 16-18 h after which anesthetized with intraperitoneal injection of thiopental sodium at a dose of fifty mg/kg at the 271st and 298th day (satellite tv for pc companies). Blood samples for hematological and blood chemical analyses had been taken from not unusual place carotid artery. Heparinized blood samples had been taken for figuring out whole blood count (white blood mobileular count, differential white blood mobileular count, platelet count, purple blood mobileular count, hematocrit, and hemoglobin) with the aid of using Sysmex SF-3000 completely automatic hematology analyzer. The serum from non-heparinized blood turned into cautiously accumulated for blood chemistry and enzyme evaluation (glucose, blood urea nitrogen (BUN), creatinine, overall protein, albumin, overall and direct bilirubins. These stages had been routinely decided the usage of the COBAS INTEGRA analyzer.

### Necropsy

All rats had been sacrificed after the blood collection. The positions, shapes, sizes and hues of inner organs had been evaluated. Heart, lungs, thymus, livers, pancreas, spleen, kidneys, adrenals, small intestine, belly and duodenum, muscle with sciatic nerve, thoracic spines, brain, eyes, intercourse organs, uterus and epididymis had been eliminated from all rats to visually stumble on gross lesions, and weighed to decide relative organs' weights. All tissues had been preserved in 10% impartial buffered formaldehyde answer for histopathological examination. The tissues had been embedded in paraffin, after which sectioned, stained with haematoxylin and eosin and had been tested microscopically.

### Statistical analysis

Results had been expressed as mean + fashionable blunders of mean (S.E.M.). Statistical importance turned into decided with the aid of using one-manner evaluation of variance (ANOVA) and submit hoc least-full-size difference (LSD) test. The facts acquired from acute toxicity observe had been analyzed the usage of Student's t-test. P values much less than 0.05 had been taken into consideration full-size [26].

### Conclusion

The Siddha System of drugs is an integrated a part of Indian System of medicines. The medicinal plant as drugs, many siddha formulations containing more than one as ingredient are used in traditional medicine to treat diabetes. Inspection on acute toxicity, sub-chronictoxicity, evaluation of herbal medicines does not reveal any significant toxicity; therefore it may indeed be concluded as safe formulation.

### Reference

1. Wu Lei, Wanyue Chen, Zhang Wang. "Traditional Indian medicine in China: the status quo of recognition,

- development and research." *Journal of Ethnopharmacology*, 2021, 114317.
2. Wangkheirakpam, Sujata. "Traditional and folk medicine as a target for drug discovery." *Natural Products and Drug Discovery*. Elsevier, 2018, 29-56.
  3. Biswas, Jayanta Kumar *et al.* "The flop side of using heavy metal (oids) s in the traditional medicine: toxic insults and injury to human health." *Biomedical applications of metals*. Springer, Cham, 2018, 257-276.
  4. Patel DK, Kumar R, Laloo D, Hemalatha S. Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pacific Journal of Tropical Biomedicine*, 2012;2(5):411-20.
  5. Zhao. Ruichen Drug delivery system in the treatment of diabetes mellitus. *Frontiers in Bioengineering and Biotechnology*, 2020;29:8:880.
  6. Sathasivampillai SV, Rajamanoharan PR, Heinrich M. Siddha Medicine in Eastern Sri Lanka today—Continuity and change in the treatment of diabetes. *Frontiers in pharmacology*, 2018;10(9):1022.
  7. Merish S, Tamizhamuthu M, Walter MT, Priya T. Evaluation of in-vitro Anti Diabetic Activity of a Siddha Herbo-Mineral Drug. *Current Traditional Medicine*, 2015;1(2):84-90.
  8. Susila R, Gladys RJ, Arunadevi R, Jothi J, Arunachalam C, Balagurusamy K. A review on anti-diabetic herbs of Siddha system with special reference to it's organoleptic quality (taste) as per tridosam concept. *Int J Herb Med*, 2017;5(5):97-101.
  9. Dhivyalakshmi K, Jothi SA, Anbu N, Sivaraman d. screening of anti-diabetic potential of the siddha formulation sarabendira siddha maruthuva sudar chooranam streptozotocin induced type ii diabetes in wistar rats. *Int J Trans Res Ind Med*, 2019;1(3):36-42.
  10. Ekman L, Hansson E, Havu N, Carlsson E, Lundberg C. Toxicological studies on omeprazole. *Scandinavian journal of gastroenterology. Supplement*, 1985;108:53-69.
  11. Nahar K, Murphy K, Paterson S. Toxicological Relevance of Pregabalin in Heroin Users: A Two-Year Post-Mortem Population Study. *Journal of Analytical Toxicology*, 2021, 11.
  12. Floriano JM, Rosa ED, do Amaral QD, Zuravski L, Chaves PE, Machado MM *et al.* Is tartrazine really safe? In silico and ex vivo toxicological studies in human leukocytes: a question of dose. *Toxicology research*, 2018;7(6):1128-34.
  13. Parasuraman S. Toxicological screening. *Journal of pharmacology & pharma cotherapeutics*, 2011;2(2):74.
  14. Khan RA, Aslam M, Ahmed S. Evaluation of toxicological profile of a polyherbal formulation. *Pharmacology & Pharmacy*, 2016;7(1):56-63.
  15. Karmaus A, Fitzpatrick J, Allen D, Patlewicz G, Kleinstreuer N, Casey W. Variability of LD50 values from rat oral acute toxicity studies: implications for alternative model development. *Society of Toxicology, San Antonio, TX*, 2018;11:3:11-5.
  16. Van Der Zande M, Vandebriel RJ, Groot MJ, Kramer E, Rivera ZE, Rasmussen K *et al.* Sub-chronic toxicity study in rats orally exposed to nanostructured silica. *Particle and fibre toxicology*, 2014;11(1):1-9.
  17. Feroz Z, Khan RA, Afroz S. Adverse effects of antiepileptic, antihypertensive, antidiabetic and

- antiarrhythmic drugs on hematological and hepatic parameters. *Lat. Am. J. Pharm*,2011;30(2):229-36.
18. Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL *et al*. Chimeric antigen receptor T-cell therapy—assessment and management of toxicities. *Nature reviews Clinical oncology*,2018;15(1):47-62.
  19. Khan RA, Aslam M, Ahmed S. Evaluation of toxicological profile of a polyherbal formulation. *Pharmacology & Pharmacy*,2016;7(1):56-63.
  20. Hilderink JM, Klinkenberg LJ, Aakre KM, de Wit NC, Henskens YM, van der Linden N *et al*. Within-day biological variation and hour-to-hour reference change values for hematological parameters. *Clinical Chemistry and Laboratory Medicine (CCLM)*,2017;55(7):1013-24.
  21. Ahmed S, Khan RA, Feroz Z. Assessment of sub-chronic, hematological and histopathological toxicities of a herbal combination. *Pakistan journal of pharmaceutical sciences*, 2015, 28(6).
  22. Motulsky HJ. Prism 4 statistics guide—statistical analyses for laboratory and clinical researchers. GraphPad Software Inc., San Diego, Cam, 2015, 122-6.
  23. Kokil RG, Rewatkar VP, Verma A, Thareja S, Naik RS. Pharmacology and chemistry of diabetes mellitus and antidiabetic drugs: a critical review. *Current Medicinal Chemistry*,2010;17(35):4405-23.
  24. Sofia HN, Kumari HV, Walter TM, Kumar SS. Acute and sub acute toxicity study of an anti Diabetic Siddha Polyherbal Preparation, Atthippattaiyathi Kasayam. *Journal of Pharmacy Research*,2014;8(7):915-9.
  25. Latha Rani M. *An open Clinical Study on Madhumegam (Diabetes Mellitus) with the Evaluation of Siddha Drug Naval Kottai Chooranam* (Doctoral dissertation, Government Siddha Medical College, Chennai), 2016.
  26. Charoentearaboon JN, gamkitidechakul C, Soonthornchareonnon N, Jaijoy K, Sireeratawong S. Antioxidant activities of the standardized water extract from fruit of *Phyllanthus emblica* Linn. *Sonklanakarin Journal of Science and Technology*,2010;32(6):599.