IJPSM

Shivkanya Mhaske *et al*, International Journal of Pharmaceutical Sciences & Medicine (IJPSM), Vol.8 Issue. 10, October- 2023, pg. 107-116

ISSN: 2519-9889
Impact Factor: 5.9

A Review on Long Term Chronic Toxicity Study

Shivkanya Mhaske*[1]; Shivprasad Mhaske[*2]; Amrapali Pagare[*3]; Nikam Harshda[*4]
Department of Pharmacology, Rashtriya College of Pharmacy, Hatnoor Kannad, 431103

DOI: 10.47760/ijpsm.2023.v08i10.008

Abstract:
Toxicity study of a new drug compound is an essential step in the process of new drug development. Long term toxicity testing is done on various biological systems. Dose specific toxic effect on vital organs of an investigational products is essential. After the acute toxicity testing, sub chronic toxicity can be proceeded by studying the assessment of an investigational product on morphological, behavioral, biochemical, haematological and histopathological parameters on experimental animals.
Keywords: Sub chronic toxicological assessment, Biochemical, Hematological and Histopathological changes.

Introduction:

The term toxicology is overlapping with biology, chemistry, pharmacology and medicine that involves the study of adverse effect of chemical substance on living organism, the practice of diagnosing and treating and exposure to toxins and toxicant. The relationship between dose and its effects on the exposed organism is of high significance in toxicology. Toxicological screening is the main aim for the development of new drugs and for studying the extension of the therapeutic potential of existing molecules."Toxicity" is the degree to which a chemical substance (a toxin or poison) or a particular mixture of substances can damage an organism. The term toxicity refers to the effect on whole organism such as animal bacterium, or plant and also effect on substructure of the organism, such as a cell (cytotoxicity) or organ such as liver (hepatotoxicity). [(1)]

According to US Food and Drug Administration (FDA) toxicity study is essential to screen new molecules for pharmacological activity and toxicity potential in animals. The toxic effects of chemicals, food substances, and pharmaceuticals havegreat significance. Toxicity tests are used to examine specific end points and specific adverse events such as cardiotoxicity, skin eye irritation, and cancer. Toxicity testing also helpful for clinical studies to calculate the No Observed Adverse Effect Level (NOAEL) dose and pharmaceuticals on the eye and skin. In 1980s, the International Conference on Harmonization (ICH) and the Organization for Economic Co- operation and Development (OECD) brought the guidelines for toxicity testing of pharmaceutical substances.

ISSN: 2519-9889
Impact Factor: 5.9

Sources of Toxic Substances

Toxicants are classified based on their mode of action, chemical nature, or class (use class and exposure class).The use class classifies drug as therapeutic drugs, agriculture chemical, food additives, pesticides, drugs as abuse, plant toxins, and cosmetics. Exposure class classifies toxicant such as occurring in food, water, air, or soil. [(2)]

History of toxicity studies

The toxicity studies began with Paracelsus (1493-1541), who determined that specific chemicals being responsible for observed toxicity of animals and plants. Paracelsus, who was an alchemist, physician, and astrologer, is regarded as the father of toxicology. The following statement of his is often postulated as "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and remedy." Mathieu or fila (1787-1853) determined the relationship between poisons and their biological properties and demonstrated specific organ damage caused by toxins. Orfilais referred as the father of modern toxicology. Toxicological research on individual substances and toxicological screening methods were developed in the mid – 1900s, wheras the environmental toxicological studies were developed in the mid-20th century. The animal use in toxicity studies began in 1920. J.W. Trevan proposed the use of 50 % lethal dose (LD_{50}) test to determine the lethal dose of individual chemicals. John Draize developed a method for testing skin and eye irritation using rabbits, this method was accepted for testing the effect of chemicals. [(2)]

The basic principle of toxicology:

Toxicity assessment goal is to identify adverse effect(s) of a substance. [(3)]The route of exposure (oral, inhalation, or dermal) and dose(duration and concentration of exposure) of the toxicant influence the adverse effect. Dose of the test substances are explored and tested in both acute and chronic models.[(4)]Different sets of experiments are conducted to determine and examine other forms of toxicity. Some of the factors that influence chemical toxicityinclude [(5)]i. Dosage- both large single exposure (acute) and continuous small exposure (chronic) are studied. ii. Route of exposure –ingestion, inhalation, or skin absorption. Iii. Other factors such as – species, age, sex, health, environment and individual characteristics.

Types of Toxicology:

1) Descriptive toxicology – The science of toxicity testing to provide information for safety evaluation and regulatory requirement. It examines the organ toxicities of test agent and also identify possible acute and chronic toxicities. It includes genotoxicity, developmental, carcinogenicity and reproductive toxicity.

2) Mechanistic Toxicology (Research Toxicology) - Identification and understanding cellular, biochemical and molecular basis by which the chemical exerts toxic effects.

3) Regulatory Toxicology (Applied toxicology) - Determination of risk based on descriptive and mechanistic studies, and developing safety regulations.

4) Clinical Toxicology – Diagnosis and treatment of poisoning; evaluation of method of detection and intoxication, mechanism of action in humans and animals. Integrates toxicology, clinical medicine, clinical biochemistry/ pharmacology.

ISSN: 2519-9889
Impact Factor: 5.9

5) Environmental Toxicology – It is the branch of toxicology in which study of presence of different toxicants including their metabolites and degradation products in the environment and their effects on humans and animals. (6)

Sub chronic toxicological assessment:

Principle of Chronic toxicity studies (OECD 452):

Chronic toxicity studies are carried out in rodent species. The three main routes of administration used in chronic toxicity studies are oral, dermal, and inhalation. In chronic toxicity test chemical is administered daily in graduated dose to different groups of experimental animals, for 12 months and longer or shorter duration may also be chosen. The duration is chosen to be sufficiently long to allow any effect of cumulative toxicity to become manifest, without the confounding effects of geriatric changes. Chronic toxicity study design also include one or more interim kills, eg. 3 and 6 months and additional groups of animal may be included. Study included satellite group and sentinel group of animals. Satellite group included to monitor the reversibility of any toxicological changes induced by the chemical under investigation; these will be restricted for highest dose level of the study plus control. Additional group of sentinel animals (5 animal per sex) may also be included for monitoring of disease status if necessary during study. Interim kills or inclusion of satellite or sentinel group are planned, number of animals included in the study design should be increased by the number of animals schedule to be killed before the completion of the study. During the period of administration animals observed closely for signs of toxicity. Animals which die or killed during test are necropsied.(7)

There are four type of toxic entities; chemical, biological, physical and radiation.

Chemical toxicant includes inorganic substances; lead, mercury, chlorine gas, etc. and organic compounds. Biological toxicity of pathogens is difficult to measure because "threshold dose" may be single organism. Physical toxicants are substances that interfere with biological processes. It includes; coal dust, asbestos fibers or silicon dioxide. Water can act as physical toxicant, if taken in high doses, because the vital ions concentration decreases. Radiation toxicity occurs on exposure of harmful radiations such as x-rays, gamma rays etc.

Toxicity is measured by its effects on the target (organism, organ, tissue, or cell). Preclinical toxicity testing is done on different biological systems. It reveals species, organ, dose specific toxic effect of investigational product. Toxicity testing helps to calculate no observed adverse effect level (NOAEL) dose and which is a useful data in clinical studies. (8)

Acute toxicity – It involves harmful effects of an organism through a single or short-term exposure.

Sub chronic toxicity – It is ability of toxic substance to cause effects for more than one year but less than the life time of the exposed organism.

Chronic Toxicity – It is the ability of a substance or mixture of mixture of substances to cause harmful effects over an extended period, usually upon repeated or continuous exposure, sometimes lasting for the entire life of the exposed organism. (9)

Sub chronic and chronic toxicity mechanism: A Dose can be toxic when given repeatedly or a lower dose with repeated administration can produce significant toxicity. Mechanism of toxicity is not the same for all chemicals, when given in single high dose. High doses of alcohol lead to primary effect on CNS, while the lower repetitive doses which are result in liver injury. Some examples include

ISSN: 2519-9889
Impact Factor: 5.9

Anticholinesterase inhibition – Organophosphate pesticide mostly have little mammalian toxicity, until these pesticides are metabolically activated primarily in the liver. Organophosphate primary action is the inhibition of acetylcholinesterase (AChE) in peripheral nervous system (PNS) and brain. AChE is the enzyme that terminates stimulation of ACh. High level exposure of neuronal stimulation results in overstimulation of the cholinergic nervous system. This overstimulation results in host of symptoms including respiratory arrest. When Atropine is administered, it blocks the effect of acetylcholine and administration of pralidoxime chloride, reactivates the inhibited AChE.

Metabolic Activation – Chloroform, carbon tetrachloride, nitroso amines, acetylaminofluorene are metabolically activated to free radicals. High level of exposure results in "cellular injury or cellular death." The specific interaction and cellular target remain unknown. Some organ system has capability to activate these chemicals like liver, kidney, and lungs, these are the main target of injury. Particular cells within an organ may have greater or lesser capacity to activate or detoxicate the intermediate. Metabolism is one of the reasons for understanding pharmacokinetics which describes types of transformation, distribution and elimination of these intermediates.

Cancer mechanism –Cancer development is multi –stage process. Alterations in DNA (somatic mutation) in critical genes can cause cancerous lesions or increases susceptibility. Exposure to synthetic chemicals (like benzidine used as dye) natural chemicals (in cooked foods like fish) or physical agents (like UV light from the sun or industrial activity) all are supporters to somatic gene mutations. However natural and synthetic substances (anti– oxidants) and DNA repair processes maintain and protect homeostasis. Genetics is important factor in cancer. Different genetic disease syndrome such as in pigmentosum, xeroderma, there is lack of normal DNA repair, therefore increase susceptibility to skin cancer from exposure to ultraviolet light from the sun.[(10)]

Recent Studies:

Recently several studies were performed for assessment of sub chronic toxicity study. Akindele et.al.2015, performed 90 Days toxicological assessment of hydroethanolic leaf extract of *Ipomoea asarifolia* (Convolvulaceae) in rats.[(11)] In this study the author concluded that the hydroethanolic leaf extract of *Ipomoea asarifolia* is relatively safe when administered orally for long periods with potential renal in-vivo antioxidant activity hence the extract may cause reversible male sterility, anemia and hypernatremia.The haematological, biochemical, sperm, antioxidant indices analysis, histopathological and statistical analysis were done. A significant increase in the weight of liver and spleen, and MDA levels of the testeswere observed at the dose of *I. asarifolia*extract 40 mg/kg in the reversibility phase. In haematological analysis showed significant decrease in the red blood cell level and hemoglobin.Biochemical assays showed significant decrease in HDL level at highest dose administered in reversibility but not in the main study phase. Low level of HDL have increase the risk for atherosclerotic disease while high level of HDL shown to have a protective value against cardiovascular disease such myocardial infarction and ischaemic stroke. A significant decrease in the sperm motility and count was noted as compared with control group. This study concluded that hydroethanolic leaf extract of *Ipomoea asarifolia* is relatively safe for oral administration.

ISSN: 2519-9889
Impact Factor: 5.9

Amoateng et.al. 2016, performed long-term continuous administration of a hydro-ethanolic extract of *Synedrella nodiflora*Gaertn to assess the biochemical, haematological, and histopathological changes in male Sprague Dawley rat.[12]*Synedrella nodiflora* extract passes low toxicity on biochemical and haematological parameter and small histopathological changes in the vital organs from the rats. In this study haematological parameters were measured to determine the effect of extract on blood and blood forming tissues.SNE-100 mg/kg caused decrease in the white blood cell count and neutrophil counts. Effect of the extract on mammalian renal system at low dose of SNE-100 mg/kg showed significant increase in levels of serum potassium. Increase level of potassium indicateshyperkalemia. The study concluded that hydro-ethanolic extract of *Synedrella nodiflora* (L) Gaertn has low toxicity profile for 90-day continuous treatment with oral administration in male Sprague-Dawley rats. Low dose of 100mg/kg may cause leucopenia, hyperbilirubinemia and hyperkaliemia, and high dose of extract 1000mg/kg may result in cardiotoxicity.

Kabbaoui et.al 2017, performed acute and sub-chronic toxicity studies of the aqueous extract from leaves of *Cistus landaniferus* L. in mice and rats.[13] Study was conducted to assess toxicological profile by performing acute oral toxicity in mice for 14 days and sub-chronic toxicity in rats for 90 days treatment. In acute toxicity test, study showed at higher dose- 5000mg/kg induce toxic effect and mortality in animals. Mortality rate was high in female group. The result concluded that female mice are more sensitive to CL extract than male mice. In this study absolute and relative weight of liver was significantly increased in female rats at doses of 700mg/kg. At the end of sub-chronic study urinalysis performed showed that male rats treated with 1000 mg/kg group had increased urine proteins within normal limits. The hematological analysis showed decrease in MCV (mean corpuscular volume) level of male rats which were treated with dose of 500 mg/kg but this effect was not observed in the female groups. In this study when compared to the control group, the rats treated with different doses of *Cistus landaniferus* extract over 90 days showed significant decrease in blood glucose level at the end of treatment period. The study concluded that *Cistus landaniferus* extract at dose up to 1000mg/kg for a period of 90 days to be generally safe in rats.

Motiat B. et.al. 2016, performed sub-acute and chronic toxicity profile of *Markhamia tomentosa* ethanolic leaf extract in rats.[14] This study concluded that *Markhamia tomentosa* extract showed a non-toxic effect in kidney and liver function parameters in rats. The extract is safe when administered orally. When plant extract was compared to the control group it caused non-significant increase in the percent body weight. The highest body weight increase was recorded at doses of 40 and 200 mg/kg in 90 days treatment period. The 90 days treatment period showed a significant increase in weight of liver and testes with highest increase at dose of 40mg/kg. All serum biochemical parameters were measured after 90 days, only non-dose dependent increase of plasma TG at dose of 200 mg/kg and total protein at dose of 200mg/kg and 1000mg/kg were observed in treated rats of 28 days treatment period. Serum levels of AST, and ALT in treated rats showed a significant increase in a non-dose dependent manner compared with control rats. The hematology results showed significant increase in WBC, RBC, PLT, and MCV compared with control rats. No significant changes were observed with organ weights of the heart, kidney, lungs, and spleen. The study concluded that sub-acute and chronic oral toxicological profile of

ISSN: 2519-9889
Impact Factor: 5.9

Maekhamia tomentosa leaf extract did not show toxic effect and the results obtained from this study indicated the extract to be generally safe for oral administration.

Jian L. et.al. 2016, performed evaluation of toxicity studies of flavonoid fraction of *Linthocarpus polystachyus* Rehd in rodents.[15] In this sub-chronic toxicity study *Linthocarpus polystachyus*was administered a daily dose of 70, 140, 560 mg/kg/day for 24 weeks. Rats treated with each of these doses did not show any significant changes, toxic reaction or death. Body weight of treated groups were a little higher than the control group at 10 week and 22 weeks. The WBC level was significantly increased in 560 mg/kg/day as compared to control group. PLT was slightly increased at dose 140 mg/kg/day as compared to controls. The biochemical results showeda significant increase in the levels of AST and ALP in 560mg/kg/day dose level, which may indicate liver toxicity. The data showed that *Linthocarpus polystachyus* may have slight toxicity for the liver at 560 mg/kg/day after 26-week treatment period. In conclusion, the sub-chronic toxicity test indicated that *Linthocarpus polystachyus* given orally to rats did not show chronic toxic reaction.

Yuanbin Z. et.al. 2016, performed sub chronic toxicity study of ethanol root extract of baked *Aconitum flavum* in rats.[16] Sub chronic studies assessed undesirable effects of repeated exposure of plant extract over a portion of the average life span of experimental animals. In this study sub chronic toxicity of *Baked Aconitum* extract was evaluated in experimental animal rat at doses between 0.76-3.03g/kg/day for 90 days. Lungs, spleen, kidney and heart, where not adversely affected and showed no clinical signs of toxicity throughout the treatment. *Baked Aconitum* Because there was no reduction in relative organ weights of the treated animals at any of the tested doses. Study concluded that the *Baked Aconitum*extract has no toxicity in the analyzed organs. Haematological study conducted for all *Baked Aconitum* extract and control group and the results show significant increase in Hb, RBC, MCV, MCH and MCHC at any dose. The Significant effect of the extract showed that the *Baked Aconitum* extract affects the morphology, erythropoiesis, or osmotic fragility of the RBC. The serum hematology and biochemistry analyses were also performed. The hepatic function parameters like total protein and albumin significantly decreased at all doses. Level of serum CL, Total Protein, Glucoseand Triglycerides were decreased significantly but urea increased in treatment group at dose 3.03 and 1.52g/kg in female rats. In this study morphological and histopathological changes were not observed in any of the vital organs except in the spleen. The changes in hematological parameter and clinical biochemistry analyses showed damage to metabolic organs (kidney and liver).

Anwarbaig C. et.al. 2019, performed Safety evaluation of syringic acid: subacute oral toxicity studies in wistar rat.[17]Changes in biochemical and haematological parameters were also observed. In haematological analysis, HB, PCV, PLT count and RBC showed no significant increase in male rat treatment group. All haematological parameter were increased in satellite group. Total WBC count in male treatment group showed no increase as compared to normal control group. Decrease in lymphocyte count and increase in neutrophil observed no recovery in satellite group. In female treatment group no significant changes were observed in WBC count. In biochemical parameter no significant changes were observed in treatment group and satellite group as compared to normal control group. In this study low level of ALP in treated group and no recovery in satellite group of female rats. Low level of ALP is an indicator of Wilson disease. In male treatment group, no significant changes in phosphate level, ALT,

ISSN: 2519-9889
Impact Factor: 5.9

AST, RBC count were observed, hence it may not be considered as severe toxic effect. Serum electrolyte (Na, Ca, P, C) level were estimated at the end of study, no significant changes were observed in treatment and satellite group as compared to control group. This effect showed that SA may not be affecting the digestive system, metabolism process, cardiac and renal functioning with no significant changes in renal and lipid parameters (HDL, TC, TG) and electrolyte balance. In histopathological studies no significant changes in relative kidney weight in treatment and satellite group of both male and female rats. The study showed no major alteration in the structural features of the liver. Heart and Spleen weight were increase in satellite group. There was no major alteration in relative weight for other organs in both male and female rats. The study concluded that SA not have toxic effect on internal body organs. Hence synergic acid could be considered safe.

Swapnil Y. Chaudhary et.al performed acute and sub chronic toxicity study of Tamara bhasma (increased copper) prepared with and without amritikarana.[18] In the present study, administration of Tamara bhasma showed significant increase in the haematological parameters (HB, RBC, PCV). At middle dose level of TB monocyte percentage was significantly increased. Copper supports absorption of iron from intestine also essential for the synthesis of Hb, which may be responsible for increase in RBC related parameter in rats. Significant decrease in fasting blood sugar at TED level of tamra bhasma and higher dose level of TBA suggest role of copper in glucose metabolism. Copper also plays role in carbohydrate metabolism by stimulating insulin binding and hexose transport. This is the reason behind the decrease in blood sugar level. TB (at TED) treated group significantly increased serum triglyceride, VLDL, LDL and HDL-Cholesterol compared to control group. Higher dose did not show any changes. No change was observed in SGPT, SGOT, ALP and Bilirubin in both treated groups compared to control group. Significant increase in albumin was observed at all dose level of tamra bhasma and higher dose level of TBA. Histopathological study indicated mild changes in four organs (heart, liver, thymus, and kidney) at higher dose level. These changes were not observed at low dose level of TB. Thus, bhasma was considered to be safe at TED level. The effect of both drugs TB and TBA at different dose levels were compared with control group. TB and TBA produce almost same effect except TB produced changes on ALP, total bilirubin, triglyceride and LDL- cholesterol. TBA showed less adverse changes compared to TB. Tamra Bhasma also produced fatty changes in heart, these changes were absent in TBA treated group. The study suggests that TB is not much toxic at therapeutic dose level it produces adverse effects when TB administered for longer duration at higher dose level. The study concluded that tamra bhasma can be relatively safe at therapeutic dose level in rats. Tamra Bhasma at TED x 10 dose level produce toxic changes in liver, kidney, and heart by haematological, biochemical and histopathological parameter in rats. Tamra Bhasma can be relatively safe at therapeutic dose level in rats. Amritikarana is must for internal administration of Tamra Bhasma in clinical study.

Lyoussi B. et.al, 2017, evaluated cytotoxic effects and studied the acute and chronic toxicity of aqueous extract of the seeds of *Calycotome villosa* (poiret) in rodents.[19]The present study showed the aqueous extract of *C. villosa* intermedia seeds, used in the traditional medicine for number of diseases. Sub-chronic oral administration *of C. villosa* seeds extract did not show any sign of hepatotoxicity nor nephrotoxicity as assessed by its biochemical parameter measurement and by histopathological examination. Significant decrease in body weight and plasma glucose level was observed in rats treated

ISSN: 2519-9889
Impact Factor: 5.9

with *C. villosa* seed extract at the highest dose (300 and 600 mg/kg body weight) as compared to control group. Reduction of body weight gain in this study was as a result of decreased appetite and thereby lower calorie intake by the animals. *C. villosa* seeds extract did not produce any toxic effect based on the relative organ weights of spleen, testes, liver and kidneys at all tested doses. Plasma glucose level and reduction of body weight was significantly decreased at the highest dose level of *C. villosa* seeds extract. The extract of *C. villosa* seeds did not affect the hemogram of the rats, which is source of reticulocytes. Study also showed that *C. villosa* seeds extract did not induce any significant changes in the histology of liver and kidneys. Result suggest that aqueous extract of *C. villosa* seeds could be considered as not cytotoxic and did not any cause signs of mortality or organ toxicity in both acute and chronic toxicity studies in rodents. Thus, it can be safe for consumption as traditional medicine. The study concluded that extract of *C. villosa* seed was non- toxic and did not produce any significant changes in the biochemical and haematological parameter.

Ravanbaksh A. et.al, 2016, performed acute and sub chronic toxicity study of medium septum of *Juglans regia* in Wistar rats.[20]The present study showed no mortality or acute toxicity in rats treated with 5000 mg/kg *Juglans regia* median septum extract (JRSME) which indicated that oral LD_{50} of JRSME is greater than the 5000 mg/kg body weight. *Juglans regia*is used for treatment of chronic disease such as gout, diabetes mellitus and cancer in traditional medicine, and their safety was evaluated through the subchronic toxicity study. Administration of JRSME was done for four weeks and antioxidant, biochemical and histopathological parameters were studied. No changes were observed in the blood SOD, serum PON-1 and GPx activities. No significant effects on the liver and kidney SOD, MDA activities were observed. In biochemical study urea, creatinine and uric acid level were observed. Serum urea level and creatinine serum concentration were significantly decreased. Administration of *Juglans regia* showed increase in glomerulus diameter which indicated improvement of kidney function and structure and lack of mechanical damage to the renal filtration. The plasma levels of AST and ALT were increased. Serum levels of AST and ALT did not significantly change with four weeks treatment with *Juglans regia*. It did not show any significant toxic effect on the heart tissue. Histopathological study indicated mild pathological changes in both liver and kidney tissue. In kidney tissue of the treated rats, mild degenerative changes such as fatty changes in urine tubes, in some areas, damage to the kidney cortex was observed along with interstitial nephritis. Heart, brain, eye tissues in control rats showed no pathological and structural changes.No pathological changes were observed in brain and eye tissue in the treated rats. The study concluded that no morphological changes regarding cell damage and biochemical disturbances were observed.

Gopi S.et.al. 2016, performed acute and subchronic oral toxicity studies of hydrogenated curcuminoid formulation 'CuroWhite' in rats.[21]'CuroWhite' is the blend of hydrogenated curcuminoids encapsulated with β-cyclodextrin. The present study showed results of the acute (single dose) and subchronic (repeatedly 90 days) oral toxicity of the CuroWhite in Sprague Dawley rats. In subchronic study, rats of both sexes divided into three group and each group orally treated with CuroWhite at daily doses 200, 400 and 800 mg/kg for 90 days. During treatment of acute and subchronic toxicity study mortality, body weight, feed consumption, clinical observations, haematology, organ weight and histopathological parameter are studied. In this study oral administration of Curo White showed no treatment related signs

ISSN: 2519-9889
Impact Factor: 5.9

of toxicity in any of the animals during the study. Subchronic oral toxicity test showed no significant treatment related adverse effects and no any changes in relative body weight and food consumption as compared to control group. The present study concluded that CuroWhite orally administered to rats was safe and no drug related toxicity was detected at the highest dose in both acute (2000 mg/kg) and subchronic toxicity (200, 400 and 800 mg/kg).

Mohan M. et al. 2018, performed pre-clinical toxicity study of tamra bhasma on albino wistar rats.[(22)]The present study evaluated behavioral, biochemical, haematological and histopathological changes in animals. Behavioral study on open field test and elevated plus maze test were performed and there was no significant changes in locomotion as compared to control group. Biochemical study shown that decrease in the serum ALP activity in all tamra bhasma treated group as compared to control group animals. In haematological study, no any significant changes in any of the treated groups in haemoglobin, red blood cell count, platelet counts as compared to control. The histopathological changes in tamra bhasma treated group showed normal structure of heart, liver, kidney, lung and brain. Histology of brain showed normal structure and neurons. Lung showed normal connective tissue, pulmonary veins, and kidney showed normal architecture of nephron, glomeruli and tubules. Tamra bhasma at dose 5.5 mg/kg showed non-toxic effect as indicated by its morphological, behavioral, biochemical, haematological and histopathological studies in wistar rats, and higher doses were found to have toxic effect.

CONCLUSION

The present study provides an overview of the safety toxicity of different plant extract in animals. By assessing its morphological, biochemical, haematological, and histopathological parameter. present study, it is concluded that all these extract treated animals may not show any observable toxic effects and mortality in acute and subchronic toxicity studies. The results of acute and subchronic toxicity of all these extract can be relatively safe at therapeutic dose level. Extract generally safe in rats, and may not cause severe treatment –related toxicity. However, further clinical investigations are needed to confirm its safety and effectiveness in humans.

REFERENCES

[1]. Scheanger T.F., What is Toxicology. Archived from the original on march, 2007.
[2]. Parshuman S.Toxicological screening, journal of pharmacology and pharmacotherapeutics, 2(2):74-79, 2011.
[3]. Committee on risk assessment of hazardous air pollutants, commission on life sciences, national research council (1994) science and judgement in risk assessment, the national academic press.
[4]. Human health toxicity assessment united states environmental protection agencies.
[5]. Ottoboni, Alice M., The dose makes the poison a plain language guide to toxicology, new yark, N.Y. Van nostrand reinhold,(2), 1991.
[6]. The environmental science of drinking water 143-196, 2005.
[7]. OECD Guideline for the testing of chemicals on Chronic Toxicity Studies 1-18, 2018.

ISSN: 2519-9889
Impact Factor: 5.9

[8]. Bhat S. Udupa, Kumarswamy, Toxinomical outline of bio-diversity of karnatka in a 14th century kannada toxicology text khagendra mani darpana asian pacific journal of tropical biomedicine, 3(8):668-672, 2013.
[9]. Biography of mathieu joseph bonaventure orfila, U.S. national library of medicine (1787-1853).
[10]. Mechanisms of toxicity 16:18, 2011. Available from: http://www.iloencyclopaedia.org
[11]. Akhindele A., Unachukwu E., Osiagwu D., 90 Days toxicological assessment of hydroethanolic leaf extract of *Ipomoea asarifolia* (Desr.) Roem. and Schult. (Convolvulaceae) in rats.Journal of ethanopharmacology1-13, 2015.
[12]. Amoateng, p.es administration of a hydro-ethanolic extract of *Synedrella nodiflora* (L) Gaertn in male Sprague Dawley rats: biochemical, haematological and histopathological changes, Ghana Med 50(3):163-171, 2016.
[13]. Kabbaoui, M.E., et.al, Acute and suc-chronic toxicity studies of the aqueous extract from leaves of *Cistus landniferus l.* in mice and rats. Journal of Ethanopharmacology 147-156, 2017.
[14]. Motiat B.et.al, et.al, Sub-acute and chronic toxicity profile of *Markhamia tomentosa* ethanolic ethanolic leaf extract. Journal of Ethanopharmacology.07.036, 2016.
[15]. Liang J., Evaluation of toxicity studies of flavonoid fraction of linthocarpus polystachyus Rehd in rodents, Regulatory and pharmacology.7;006, 2017.
[16]. Zhang Y.et.al, A subchronic toxicity study of ethanol root extract of baked *Aconitum flavum* in rats. Brazilian Journal of Pharmacognosy 438-445, 2016.
[17]. Mirza A.C, Shital S.Panchal, Safety evaluation of syringic acid: subacute oral toxicity studies in wistar rats. Heliyon (5):2129, 2019.
[18]. Chaudhari S.Y. et.al, Acute and subchronic toxicity study of Tamra Bhasma (incinerated copper) prepared with and without Amritikarana, Journal of Ayurveda and integrative medicine (7)23-29, 2016.
[19]. Badiaa L. et.al, Evaluation of cytotoxic effects and acute chronic toxicity of aqueous extract of the seeds of *Calycotome villosa* (poiret) link (subsp. intermedia) in rodents. Avicenna j phytomed, 2018:8(2):122-135.
[20]. Ravanbakhsh, A. et.al, Acute and Subchronic Toxicity Study of the Median Septum of Juglans regia in Wiatar Rats. J.Adv pharm Bull, 6(4), 541-549, 2016.
[21]. Gopi S.,Jacob J, Mathur K.Y., Acute and subchronic oral toxicity studies of hydrogenated curcuminoid formulation 'CuroWhite'in rats.Toxicological Reports 3 ; 817-825, 2016.
[22]. Mohan M, et al. Pre-clinical toxicity study of tamra bhasma on albino wistar rats. Int. Res. J. Pharm.9(1): 36-46, 2018.
[23]. OECD Guideline for the testing of Chemicals, Repeated Dose 90-day oral Toxicity study in Rodents, 408, September 1998.

