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Safety assessment of Ethanolic extract of *Strychnos potatorum* on wistar rat's in accordance with regulatory guideline

Pitchiah Kumar Murugan[*1], Kaliyamurthi Venkatachalam [2]

[1]*Part time Research Scholar, Centre for Research and Development, Prist University, Thanjavur, Tamil Nadu, India cum State Drug Licensing Authority (Indian System of Medicine), Arumbakkam, Chennai – 600106, Tamil Nadu, India.*

[2]*Assistant professor, Department of Basic sciences, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India.*

Abstract

Global attention towards herbal remedies is exponentially increasing in recent times due to its high level of safety and versatile pharmacological activity in treating several dreadful disorders. Several herbs have been used in traditional system of medicine for many years. Some do seem to have remarkable therapeutic potential although there may not be sufficient scientific data. *Strychnos potatorum* (SP) is one such potential medicinal herb which is been traditionally used as an ailment in treating diabetes, gastropathy, bronchitis, ulcer, renal disorder etc. Still now there is no proper documentary evidence on exploring the efficacy of its epicarp. The main objective of the present investigation is to establish the safety profile of the ethanolic extract of *strychnos potatorum* epicarp (EESP) in female wistar rats in accordance with organization for economic co-operation and development (OECD) guideline 423.In the acute study, a single dose of 5000 mg/kg of EESP was orally administered and animals were monitored for 14 days for the possible signs of toxicity. Results of the study revealed that EESP exerts no signs of potential toxicity in the experimental animal. It was further evident that no significant changes were observed with respect to body weight, urine biochemistry, fecal consistence analysis, mortality and clinical signs. No significant toxicity was observed in the histological examination of vital organs such as brain, heart, lung, stomach, liver, kidney, spleen, uterus and ovary tissues at the highest dose of EESP. Outcome of the study provides an evidence based data on safety nature of the drug EESP and its tolerability on tested animals. Hence it was concluded that herb like *strychnos potatorum* has wide safety margin and may be used for treating chronic diseases in humans subjected to proper preclinical validation.

Key words: Medicinal herb, Safety profile, *Strychnos potatorum*, OECD, Clinical signs, Histological examination

1.INTRODUCTION

Toxicological profiling of drugs and other pharmaceuticals become inevitable as it deals with primary concern of life saving in several aspects. Several regulatory agencies have like WHO, OECD, ICH, AYUSH-ICMR and ISO have paved a clear methodology of which certain studies needs to be carried out. Even in-silico model of predicting toxic kinetic parameters are emerging in recent days to shorten the time involved in some predictions relating to receptor binding of drugs and chemicals. In general emergence of toxicity in animals and humans have been enumerated by observing the adverse effects relating to CNS, CVS, ANS and other core biological systems. Still now certain biologically active modern medicines are subjected to claim of potential adverse effects. To overcome this issue shift of research towards alternative traditional medicines are increasingly focused.

Medicinal plants have been used as potential therapy for thousands of years, and; some specific plants have been used for particular ailments [1]. Medicinal plants served as source of raw materials for both traditional systems of medicine and modern medicine. These medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and fewer lesser side effects. Traditionally, herbal supplements are considered to be safe and efficacious for treating certain diseases, whereas recent research outcome suggesting the need of safety studies for some of the traditional preparations as the biologically active components present in the herbs may have full-fledged activity similar to that of the purified drug entities hence it become a mandate requirement of carrying out toxicity studies even in herbal preparations to ensure the level of safety before its clinical application [2].

Strychnos potatorum is a medium-sized, glabrous tree. Stem is fluted and covered with black, thick, square to rectangular scales. Seeds are globose in shape. Fruit is a berry, black when ripe, globose, 12 cm in diameter, whitish, shining, with short addressed yellow silky hairs [3]. A dried seed was found to have diuretic and antidiarrheal activities. The seed powder was found to possess antidiabetic activity. Mannogalactans isolated from the seeds of *Strychnos potatorum* showed anti-hyperlipidemic activity in experimental rats [4]. Powdered stem bark mixed with lime juice given in cholera. The paste of seed is reported to be consumed internally along with little tender coconut milk in urinary disorder and retention of urine [5].As per the literature research it was strongly evident that the plant *Strychnos potatorum* has wide range of pharmacological activity which includes Anti-diabetic [6], Anti-inflammatory [7][8], Anti-ulcerogenic [9], Hepatoprotective [10], Antioxidant activity, Anti-arthritic [11], Anti-nociceptive [12], Anti-pyretic, Anti-diarrheal [13], Diuretics [14] and Antimicrobial properties [15].

The target organ of toxicity most frequently involved in systemic toxicity is the CNS (brain and spinal cord). Even with many compounds having a prominent effect elsewhere, damage to the CNS can be demonstrated by the use of appropriate and sensitive methods. Next in order of

frequency of involvement in systemic toxicity are the circulatory system; the blood and hematopoietic system; visceral organs such as the liver, kidney, and lung; and the skin. Muscle and bone are least often the target tissues for systemic effects. With substances that have a predominantly local effect, the frequency with which tissues react depends largely on the portal of entry (skin, gastrointestinal tract, or respiratory tract). The safety of using most of herbal preparations are not well established due to its complexity in composition, although most of this information comes from case reports rather than systematic preclinical investigation. Hence the present research work aimed at evaluating the short term safety assessment of ethanolic extract of *strychnos potatorum* epicarp (EESP) by acute toxicity study in rodent model.

2. MATERIALS AND METHODS

2.1.Animal

Healthy adult Wistar albino rat weighing between 180-200 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit (AHU). A 12 light / dark cycle were maintained. Room temperature was maintained between 22 $\pm$ 2^0 C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India.

2.2.Acute toxicity Study

Acute toxicity study carried out in accordance with OECD guideline 423 [16]. The animals were fasted overnight with free access to water. The study was conducted with single oral dose administration of *EESP*. Two group consist of 6 female rats each were used for this study. The dose utilized for evaluation of acute toxicity study is about 5000 mg/kg higher than that of the therapeutic dose. The study was conducted with single oral administration of study drug *EESP* 5000mg/kg (p.o). The animals were observed continuously for first 72 h and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality.

Occurrence of toxicity in animals were observed continuously for the first 4 to 24 h and observed periodically for the next 14 days. Observation includes the change in skin, fur, eyes and mucus membrane. Appearance of C.N.S, C.V.S and A.N.S related toxicity such as tremors, convulsions, sedation, steric behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation, diarrhea, lethargy, sleep, coma and mortality were observed with special attention. Body weight was recorded periodically. At the end of the experiment all animals were subjected for gross necropsy and observed for pathological changes.

2.3.Fecal Pellet Analysis

Rats of control and treatment group were allowed to explore to open field on clean and sterile environment. The collected pellets were analyzed for consistency, color, Shape, Presence of blood cells etc.

2.4.Urine Biochemistry

Rat of control and treatment group was placed individually in metabolic cage with free access to feed and water. Urine dropping from the animal was collected using specialized wire mesh system fixed at the base of the cage having provision to trap the fecal pellet mixed with urine sample. The collected urine sample was subjected to analysis with respect to colour, pH, glucose, ketone bodies, pus and blood cells [17].

2.5.Histopathological evaluation

Organs included of heart, brain, lungs, spleen, kidneys, liver, stomach, testes and ovary. Histological slides of organs were made and observed under the microscope. The pathological observations of cross section of these organs were performed on gross and microscopic bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes [18].

2.6.Statistical analysis

The statistical analysis was carried by oneway ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error. A statistical comparison was carried out using the Dunnet's test for the control and treatment group [19].

3.RESULTS

3.1. Effect of EESP on clinical signs of rats in Acute Oral Toxicity Study

Acute toxicity study was carried out in accordance with OECD guideline 423. EESP at the dose of 5000mg/kg were been used for the study. Results of the study has shown no signs of CNS, CVS, ANS related toxicities were observed in the tested rats. No mortality observed at this dose level, further no significant change with respect any of the clinical signs such as locomotion, sensory response, urination, muscle grip, posture etc. Close observation was made for (24-48 h) and a long period (14 days). The results were tabulated in Table 1.

3.2.Effect of EESP on Urine Biochemistry of rats in acute toxicity study

No significant changes were observed from the results of the urine biochemistry on its color and pH. Further the results shown the absence of glucose, pus cells, bilirubin, ketones and other blood cells. The results were tabulated in Table 2.

3.3. Effect of EESP on Fecal Consistency of rats in acute toxicity study

Results of fecal consistency analysis reveals no significant changes with respect to consistency, shape and colour. Further no signs of blood cell traces, mucous shedding and infections were observed in control and test drug treated group as well. The results were tabulated in Table 3.

3.5. Gross organ observation of control and EESP treated rats in acute toxicity study

Results of gross organ observation analysis reveals that there is no significant difference in organ morphology and weight in treatment groups rats when compared to that of the control group animals. As shown in figure 1 and 2.

3.4. Effect of EESP on Body weight of rats in acute toxicity study

Body weight measurement is one of the important parameter in enumerating the drug toxicity, significant change of about 20% increase or decrease in body weight denotes potentiality of toxicity. Data's obtained from the present study indicates that there is no significant change was observed in body weight of female rats treated with EESP the dose of 5000mg/ kg. The results were tabulated in Table 4.

3.5. Histopathology of vital organs in control and EESP treated rats in acute toxicity study

Histopathological observation of brain reveals no degeneration and with heart there is no signs of infarction, whereas histological examination of liver reveals no signs of inflammation and spleen reveals normal morphology. Similarly, no signs of ulceration in stomach and appearance of bronchi and alveoli seems normal in lungs. Appearance of glomeruli appears normal in kidney and morphology of reproductive organs like uterus and ovary reveals perfect normal histology. As shown in figure 3 and 4.

Table 1:Effect of EESP on clinical signs in Acute Oral Toxicity Study

Parameter	Group I	Group II
Clinical Signs Parameters for the duration of 14 days	Normal Saline	Test Drug EESP 5000mg/ Kg
Lacrimation	Absence	Absence
Salivation	Absence	Absence
Animal appearance	Normal	Normal
Tonic Movement	Absence	Absence
Clonic Movement	Absence	Absence
Laxative action	Absence	Mild
Touch Response	Normal	Normal
Response to Sound	Normal Response	Normal Response
Response to Light	Normal Response	Normal Response
Mobility	Normal Response	Normal Response
Respiratory Distress	Nil	Nil
Skin Color	Normal	Normal
Stereotype behavior	Absence	Absence
Piloerection	Absence	Absence
Limb Paralysis	Absence	Absence
Posture	Normal	Normal
Open field behavior	Normal	Normal
Gait Balancing	Normal	Normal
Freezing Behavior	Absent	Absent
Sings of Stress and Anxiety	None Observed	None Observed
Muscular coordination	Normal	Normal
Muscle grip	Normal	Normal
Sedation	Absence	Absence
Social Behavior	Normal	Normal
Mortality	Nil	Nil

Table 3:Effect of EESP on Fecal Consistency of rats in acute toxicity study

Urine Analysis	Control	EESP 5000 mg/kg
Urine Colour	Yellowish Orange	Yellowish
Urine pH	6	6
Urine -Glucose	Absence	Absence
Urine -Ketones	Absence	Absence
Urine- Bilirubin	Absence	Absence
Urine-Blood Cells	Negative	Negative
Urine - Pus cells	Negative	Negative

Table 3: Effect of EESP on Fecal Consistency of rats in acute toxicity study

Analysis	Control	EESP 5000 mg/kg
Consistency	Soft	Soft
Shape	Oblong	Pointed Head
Colour	Greenish	Greenish brown
Mucous Shedding	Absent	Absent
Blood Cells	Absent	Absent
Signs of Infection	None Observed	None Observed

Table 4:Effect of ESSP on Body weight of rats in acute toxicity study

DOSE	DAYS		
	1	7	14
Control	180.8± 1.47	183.7 ± 1.506	187 ± 2.191
EESP 5000 mg/kg	181.3± 1.211	184.5± 1.643	189.5 ± 1.871

Values are mean ± S.D (n = 6 per group). Control and treatment group were compared statistically using one-way ANOVA followed by Dunnett's test.

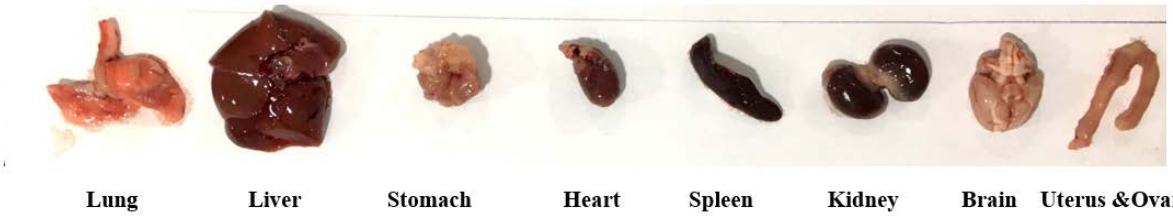

Figure 1: Gross organ observation of Control group

Figure 2: Gross organ observation of EESP treated group

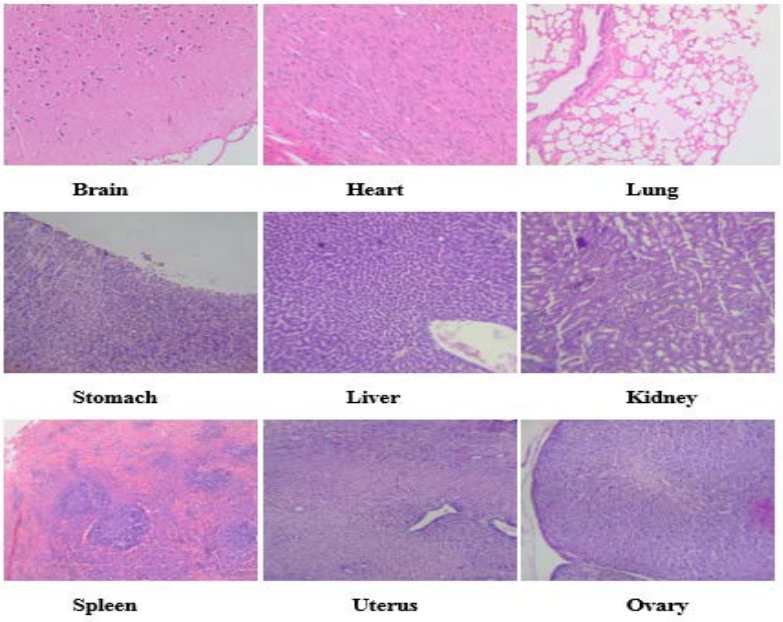

Figure 3: Histopathology of control group rats in acute toxicity Study

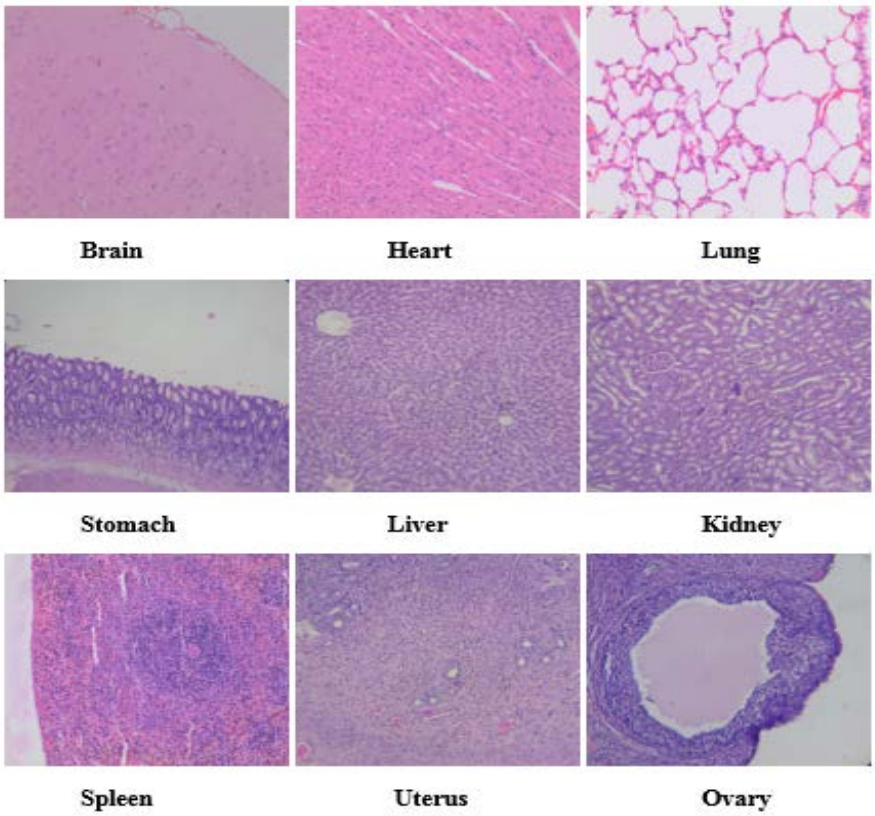

Figure 4: Histopathology of EESP treated rats in acute toxicity Study

4. DISCUSSION

Acute toxicity study provides reliable data's on toxicity pertains to CNS, CVS, ANS and also with respect to hematological profiling. Potential drug induced toxicity in rodents may observed by its change in skin coloration, pilo erection, body posture, open exploration, alteration in sleep pattern, convulsion, sensory and motor responses, social coordination, body weight, food and water intake etc. EESP at the dose of 5000mg/kg were been used for the study. Results of the study has shown no signs of CNS, CVS, ANS related toxicities were observed in the tested rats. No mortality observed at this dose level, further no significant change with respect any of the clinical signs such as locomotion, sensory response, urination, muscle grip, posture etc. Close observation was made for (24-48 h) and a long period (14 days) also reveals its safety. Medicines from natural origin have been used as a source of remedy for the prevention, cure and treatment of different ailments in traditional medicine [20]. Therapeutically important plants have been extensively explored recently, for their benefits and various applications in herbal health supplements [21] [22]. World health organization –traditional medicine strategy published recently states that traditional and Complementary medicines have gained a growing economic importance worldwide [23].

Monitoring of body weight index were highly essential as there is any deviation in body weight may also be considered as precipitation of toxicity. Not more than or less than 20% requires attention on cumulative drug induced toxicity. Similarly, neurotoxic potential of the drugs shall be clearly identified by change in behavioral pattern, signs of sedation and hyperactivity, neuromuscular coordination and importantly muscle grip strength index. Increase or decrease in gripness alerts the ganglionic degeneration. Results of the present investigation reveals that treatment with EESP at the dose of 5000mg/kg has shown no significant change in body weight and muscle grip strength.

Herbs are considerably safe as per the belief since ancient days but there as some potential possibilities that usage of some herbs may turn potentially lethal. This assessment is based on their usage in the treatment of diseases over centuries. However, some medicinal plants must be used with caution because they can cause adverse reactions, especially if they are taken in excessive doses, or if they interact with conventional drugs. Several official medical and scientific researchers strongly recommends the focus of research towards exploration of toxicity profiling of medicinal herbs based on the public health concerns [24].

Reflection of toxicity projects more on the vital metabolic organs in particular to liver and kidney. Toxicants that may tend to induce liver injury will damage is parenchyma and may leads to leakage of enzymes in to the blood stream. Hence increased serum SGOT/ SGPT level advice the researchers on prediction of drug induced liver toxicity. Histologically these finding are distinguished by margination of inflammatory cells, Shrinkage of hepatocytes followed by increased sinusoidal space. Kidney a master excretory organ which possess high vascular turnover reflects toxicity by its glomerular

infiltration. Toxic metabolite mostly damage the epithelial lining of the kidney micro filters followed by change in its permeability. This could be one of the prime reason for increase in blood urea nitrogen content. Further nephrotoxicity also monitored on alteration in urine composition like presence of glucose, ketone bodies, proteins, appearance of blood and pus cells. No significant changes were observed from the results of the urine biochemistry on its color and pH. Further the results shown the absence of glucose, pus cells, bilirubin, ketones and other blood cells.

5.CONCLUSION

Rodents are considered as reliable model for prediction of toxicity because of their genomic, physiological resemblance, drug metabolism and response towards toxic chemicals and drugs are almost similar to that of the humans. It was observed from the results of the acute toxicity study that treatment with EESP at the dose of 5000mg/kg did not alters any of the physiology and behavioral pattern of the rats. Further there was no mortality observed for the period of 14 days. Clinical signs of treatment and control group rats seems normal and histological observation of all vital organs projects regular histology with no signs of abnormality or degeneration in both treatment and control group. Furthermore, the data of acute toxicity studies on *Strychnos potatorum* (SP) were obtained in order to increase the confidence in its safety to humans for the use in the development of traditional pharmaceuticals towards ailment on treating specific disease of interest.

6.REFERENCES

1. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Sci. 2005;78(5):431–441.
2. Sharwan G, Jain P, Pandey R, Shukla SS. Toxicity profile of traditional herbal medicine. J Ayu Herb Med. 2015;1(3):81–90.
3. Kirtikar KR, Basu BD. Vol. 3. Allahabad: L.M. Basu 1647; 1933. Indian medicinal plants.
4. Kavita N. Strychnos potatorum: Phytochemical and pharmacological review. Pharmacogn Rev. 2014 Jan-Jun; 8(15): 61–66.
5. Asima C, Satyesh CP. Vol. 4. New Delhi: CSIR; 2001. The treatise on indian medicinal plants. Publications and Information Directorate; 857. Ayurvedic Pharmacopoeia of India. 2004:41–2.
6. Dhasarathan P, Theriappan P. Evaluation of antidiabetic activity of Strychonous potatorum in alloxan induced diabetic rats. J Med Med Sci. 2011;2:670–4.
7. Sanmuga EP, Venkataraman S. Anti-inflammatory effect of Strychnos potatorum. Seeds on acute and subacute inflammation in experimental rat models. Pharma Biol. 2007;45:435–9.
8. Yin W, Wang TS, Yin FZ, Cai BC. Analgesic and anti-inflammatory properties of brucine and brucine Noxide extracted from seeds of Strychnos nuxvomica. J Ethnopharmacol. 2003;88:205–14.
9. Sanmugapriyaa E, Venkataraman S. Antiulcerogenic potential of Strychnos potatorum Linn seeds on Aspirin plus pyloric ligationinduced ulcers in experimental rats. Phytomedicine. 2007;14:3605.
10. Sanmugapriya E, Venkataraman S. Studies on hepatoprotective and antioxidant actions of Strychnos potatorum Linn. Seeds on CCl4induced acute hepatic injury in experimental rats. J Ethnopharmacol. 2006;105:154–60.
11. Ekambaram SP, Perumal SS, Subramanian V. Evaluation of antiarthritic activity of Strychnos potatorum Linn seeds in Freund's adjuvant induced arthritic rat model. BMC Complement Altern Med. 2010;10:56.
12. Sanmugapriya E, Venkataraman S. Antinociceptive and antipyretic effects of Strychnous potatorum Linn. Seeds on experimental Rats. Int JPharm. 2010;6:681–5.
13. Biswas S, Murugesan T, Sinha S, Maiti K, Gayen JR, Pal M, et al. Antidiarrhoeal activity of Strychnos potatorum seed extract in rats. Fitoterapia. 2002;73:43–7.
14. Biswas S, Murugesan T, Maiti K, Ghosh L, Pal M, Saha BP. Study on the diuretic activity of Strychnos potatorum Linn. Seed extract in albino rats. Phytomedicine. 2001;8:469–71.
15. Mallikharjuna PB, Seetharam YN. In vitro antimicrobial screening of alkaloid fractions from Strychnos potatorum. EJChem. 2009;6:1200–4.
16. OECD guideline for testing of chemicals. Guideline 423 ,17th December 2001.
17. Jain N, Sharma P, Sharma N, Joshi S C. Haemato-biochemical profile following sub-acute toxicity of malathion in male albino rats. Pharmacologyonline. 2009;2:500–506.
18. Suvarna SK, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. 7th edn, Churchill Livingstone, London.2013.
19. Visweswara Rao. Biostatistics., A manual of statistic methods for use in Health, Nutrition and Anthropology, Rajkamal Electrical press, Delhi, 2007.226-312.
20. Rates S.M.K. Plants as source of drugs. Toxicon. 2001;39:603–613.
21. Gurib-Fakim A. Medicinal plants: tradition of yesterday and drugs of tomorrow. Mol. Aspects Med. 2006;27:1–93.
22. Katiyar C. Drug discovery from the plant sources: an integrated approach. AYU. 2012;33:10.
23. Anon. World Health Organization; 2013. WHO Traditional Medicine Strategy: 2014–2023.
24. Hind Lakmichi. Toxicity Profile of the Aqueous Ethanol Root Extract of Corrigiola telephiifolia Pourr. (Caryophyllaceae) in Rodents. Evid Based Complement Alternat Med. 2011:317090.