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PHYTOCHEMISTRY AND TOXICITY EVALUATION OF DIFFERENT EXTRACTS OF *SMILAX CHINA* ON ALBINO RATS

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ABSTRACT
The present study aims to test the toxic effect of the rhizome extract of *Smilax china* using three different solvents *viz* ethanol, acetone and benzene by examine the changes in behaviour, body weight, food intake, water intake, haematological parameters and histological changes in the vital organs such as lungs, heart, liver and kidney. No behavioural changes or any toxic symptoms and mortality was observed throughout the experimental period. There is a slight variations in the body weight, food intake and water intake of the extract treated groups compared to control group rats. The haematological parameters showed significant difference among the different extract treated rats and control rats, but the levels are not exceeded from the normal range. The microscopic and macroscopic examination of the vital organs showed normal cell structures, blood vessels and nuclei. Thus the present study revealed that the different extracts of *S. china* did not produce any toxic effects at the high dose of 2000mg/kg body weight and is found to be safe, and to be used for further evaluation studies.

KEYWORDS: *Smilax china,* Albino rats, Toxicity, Behaviour, Haematology and Histological changes.

INTRODUCTION
Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical substance that produces a definite physiological action on the human body.[1] Many of the indigenous medicinal plants are sometimes added to food for medicinal for pregnant and nursing mothers.[2] Moreover, in developing countries, medicinal plants continue to be the main source of medication.[3] Despite substantial efforts by ethnobotanical researchers to document the majority of medicinal plants used in indigenous health systems.[4] The therapeutic potential of plants has surged over the years with volumes of scientifically documented information showing considerable potential for medicinal plants to be used in the treatment of several diseases.[5] According to WHO more than one million people rely on herbal medicines to some extents.[6] It is considered that because of the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovery of potential new drugs and biological entities.[7] The apparent reversal of trend from western to herbal medicine is partly due to the fact that synthetic drugs have always shown adverse reactions and other undesirable side effects. This has led to the belief that natural products are safe because they are more harmonious with biological systems. In traditional system of medicine, medicinal plants are used in many countries to control many diseases.[8,9]

Many plants synthesize substances that are useful for the maintenance of health in humans and other animals. These substances, most of which are phenols or their oxygen substituted derivatives such as tannins, secondary metabolites of plants which have been isolated are at least 1200.[10] Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients.[11] Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity.[12] Some of the main phytochemicals include: carotenoids, isoflavonoids, steroids, lignans, anthraquinones, gums and resins. Although it is not important to know the names and benefits of the large range of phytochemicals that exist, what is important is to understand that maintaining a diet that contains a variety of fruits and vegetables will combine the benefits of the phytochemicals and vitamins to help you achieve optimal health.[13] The other phytochemical generally present in plants include detoxifying agents like in doles, isothiocyanates, nonstarch polysaccharides (NSP) or dietary fiber like gums hemicelluloses, mucilage, pectin, tannins and also alkaloids like coffin and nonprotein amino acids.[14]

Treatment of various ailments using medicinal plants has been from prehistoric times.[15] Their use has been

considerably increased among the populations of developing countries because of their beneficial and having few significant side effects.[16] However, there is limited report of the proper evaluations of the toxicity of these medicinal plants. Thus, proper phytochemical screening of the plant is necessary because plants can synthesize toxic substances to protect themselves against infections, insects and other organisms which feed on them. Various groups of compounds are responsible for the toxic effects of these plants. Major bio-active compounds responsible for these toxic effects include alkaloids, cardiac glycosides, phorbol esters, lectins and cynogenic glycosides. Previous studies had reported the cases of acute poisoning of patients admitted to hospitals and resulted into death mainly due to ingestion of toxic medicinal plants.[17] Recent investigations have also revealed the presence of genotoxic, mutagenic and carcinogenic compounds in many plants used as traditional medicine or food.[18] However, some toxic plants are used by doctors for the treatment of diseases.[19] Toxicity data are required to predict the safety associated before the use of medicinal products. Toxicological studies help to decide whether a new drug should be adopted for clinical use or not depending on the duration of exposure of animals to drug, toxicological studies may be of three type viz. acute, sub-acute, and chronic. Toxicity depends not only on the toxic properties of the substance. The relationship between these two factors is important in the assessment of therapeutic dosage in pharmacology and herbalism.[20] Thus the present study focused on the preliminary phytochemical analysis to know the bio-active compounds of the medicinal plant *Smilax china* and in order to know biosafety of the plant extracts, acute toxicity test is to be performed through *in vivo* studies.

MATERIALS AND METHODS

Preparation of plant extract

The selected plant material for the present study is rhizome of *Smilax china*. The process of extraction and formulation of the traditional remedy is as described by Sohini and Bhatt (1996).[21] The powder of *S. china* was pulverized and extract as a whole preparation in a Soxhlet apparatus using polar (ethanol and acetone) and nonpolar (benzene) solvents. The extracts were concentrated to a dry mass by vacuum evaporator and stored in desiccator. The percentage yield was obtained using this formula W2-W1/W0× 100. Where, W2 is the weight of the extract and the container, W1 the weight of the container alone and W0 the weight of the initial dried sample. The powder of *S. china* was subjected to analyse the preliminary phytochemicals such as alkaloids, carbohydrates, Fixed oils and fats, flavonoids, glycosides, phenolic compounds, protein, steroids, saponins, tannins and terpenenoids according to the standard methods.[22,23,24,25]

Selection of Animal Model

Healthy adult female Wistar Albino rats, *Rattus norvegicus* (150-200 mg/kg b.wt.) were used for the present study. The rats were obtained from SASTRA University, Thanjavur and brought to the laboratory and maintained under controlled environment. All animals were fed with standard pellet feed and water *ad libitum*. The principles of animal care (Ethical Committee's Approval No.001/HCC/IAEC/DST-NPDF/2017) were followed throughout the experimental period. Drug dosage was calculated according to the body weight of the rats.[26]

Experimental design

Toxicity determination for each extract was conducted separately using modified method of Lorke.[27] Normal healthy female albino rats fasted for 12 hours were randomly divided in to control and extract treated groups. In each extract, 2000mg/kg were separately administered orally to the rats for 14 days by oral gavage needle.

The rats were observed for clinical signs and symptoms of toxicity and mortality from the time of extract administration to 14th day. Behavioural changes, changes in body weight, daily food intake and water intake were observed over a period of 14 days. Further at end of the experiment all animals were sacrificed and the vital organs such as lungs, liver, kidney and heart and heart tissues were removed and washed with ice cold saline and, weighed and preserved in 10% formalin solution for histological studies.

Statistical analysis

Values were represented as Mean ± Standard deviation. All statistical analyses were performed by using windows based SPSS package (Statistical Package for Social Sciences / Statistical Product and Service Solutions).

RESULTS AND DISCUSSION

Herbal drug is a chief constituent in traditional medicine and a common constituent in ayurvedic, homeopathic, naturopathic and other medicine systems.[28] Phytochemical constituents are secondary metabolites of plants that serve as a defence mechanism against many microorganisms, insects and other herbivores.[29] A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects.[30] The preliminary phytochemical analysis of the different extracts of the present study (rhizome of *Smilax china*) revealed the presence of many phytochemical compounds which possessed various medicinal activities. The ethanol, acetone and benzene extracts of *Smilax china* rhizome powder showed the presence of terpenoids, oil and carbohydrates. Steroids and triterpenoids showed the analgesic properties.[31] The yield of crude ethanol

extract of *Smilax china* is 12.48% and 4.28%, respectively whereas yield of crude acetone extract of *S. china* is 1.05%. The percentage yield of the benzene extract of *S. china* is 1.47%. Dosage calculation and stock solution preparation in preclinical studies, involving the use of experimental animals is important in screening and development of new drugs.[26]

Acute toxic effect of the plant extract

Acute toxicity is usually an initial study performed: to serve as the basis for classification and labeling, to provide initial information on the mode of toxic action of a substance, to help arrive at a dose of a new compound and to help in dose determination in animal studies.[32] In the present study, acute toxic effect of ethanol, acetone and benzene extract of *Smilax china* powder was evaluated. There were no noticeable changes in the general behaviour, toxicity signs and mortality observed in rats treated with test drug orally at 2000 mg/kg body weight for a period of 14 days. Weekly body weight changes, mean food intake and water intake of the different extract treated rats and control rats are given in the Table 1. Effect of treatment of the plant extract on relative organ weights of the albino rats and control rats are given in Table 2. Evaluation of hematological parameters represents an important and relevant risk evaluation as the changes in the hematological system have a higher predictive value for human toxicity, when the popular data are translated from animal studies.[33] In addition, hematological analyses provide information about the 360 mg of the extract, translating to an intake of 5.14 mg/kg per day hematopoietic system and immunological responses.[34] The haematological analysis of the present study showed slight changes between the control and extract treated groups but the values are not exceeded the normal range. Haematological parameters of control and extract treated rats are depicted in Table 3.

Photomicrography of lungs, heart, liver and kidney of control and different extract of plant powder treated rat groups are shown in Plates 1-4. There was no macroscopic change of central organ (such as appearance, colour and size) considered to be related to the treatment. The histopathological examinations of lungs, heart, liver and kidney revealed no remarkable morphological alteration in all treatment and control groups. The control and extract treated rats showed normal alveoli, alveolar duct and blood vessels. The normal bronchi lined by ciliated epithelium are observed in both extract treated groups. The muscle of the heart exhibited alternative light and dark bands and possessed normal central nucleus in all the extract treated rats. The liver of control rat and extract treated rats showed normal hepatic lobules, hepatocytes and central vein. The cell cords were separated by narrow blood sinusoids. Sinusoidal capillaries (sinusoids) separate the sheets of hepatic cells and empty into the central veins was observed in all the groups. The hepatic cells were thicker and the sinusoids appear as light areas between the cords of cells. The nuclei of hepatic cells were large and spherical, binucleated cells also found. Histological sections of kidney of all the groups showed that the glomeruli, tubules and blood vessels appear normal. No pathological changes were observed in test herbal drugs treated rat kidney.

Table 1: Toxic effect of test drugs on body weight, food and water intake in different groups of the albino rats. Values within the parentheses are range of respective mean.

Parameters	Groups	Week 1 (Mean ± SD)	Week 2 (Mean ± SD)
Body weight(g)	I	207.8 ± 8.59 (197.0 – 227.0)	216.2 ± 10.97 (206.0 – 237.0)
	II	191.8 ± 7.14 (183.0 – 203.0)	195.0 ± 6.57 (186.0 – 205.0)
	III	190.1 ± 3.29 (184.0 – 194.0)	192.9 ± 3.45 (187.0 – 200.0)
	IV	195.1 ± 6.76 (187.0 – 206.0)	198.6 ± 7.57 (188.0 – 210.0)
Food intake (g)	I	19.3 ± 2.59 (16.2 - 23.8)	18.9 ± 2.09 (15.8 - 22.2)
	II	17.1 ± 1.55 (15.0 - 19.2)	16.7 ± 1.47 (14.5 - 18.8)
	III	15.1 ± 1.08 (14.0 - 16.8)	18.2 ± 1.56 (15.0 – 20.0)
	IV	16.5 ± 1.56 (14.5 - 18.5)	17.2 ± 0.91 (16.0 - 18.8)
Water intake (ml)	I	22.1 ± 1.85 (18.8 - 24)	22.6 ± 2.57 (19 - 25.5)
	II	21.4 ± 2.52 (17.8 - 24.5)	20.6 ± 2.58 (17.0 – 24.0)
	III	21.7 ± 1.91 (18.8 – 25.0)	20.9 ± 2.48 (17.2 - 24.5)
	IV	21.9 ± 1.93 (19.0 - 24.2)	21.7 ± 2.01 (18.8 – 25.0)

Groups
I = Control
II = Ethanol extract of *S. china* treated rats
III = Acetone extract of *S. china* treated rats
IV = Benzene extract of *S. china* treated rats

Table 2: Toxic effect of test drugs on organ weight in different groups of the albino rats. Values within the parentheses are range of respective mean.

Groups	Organ weight (g/100g body weight)				
	Liver (Mean ± SD)	Heart (Mean ± SD)	Lungs (Mean ± SD)	Right Kidney (Mean ± SD)	Left Kidney (Mean ± SD)
I	3.98 ±0.39 (3.40 – 4.26)	0.4 ± 0.05 (0.31 – 0.44)	0.6 ± 0.09 (0.55 – 0.75)	0.5 ± 0.06 (0.38 – 0.53)	0.5 ± 0.07 (0.38 – 0.54)
II	3.39 ± 0.453 (2.92 – 4.01)	0.4 ± 0.02 (0.36 – 0.41)	0.7 ± 0.09 (0.53 – 0.74)	0.4 ± 0.04 (0.33 – 0.41)	0.4 ±0.06 (0.33 – 0.46)
III	3.48 ± 1.381 (2.27 – 5.40)	0.4 ± 0.18 (0.21 – 0.63)	0.7 ± 0.27 (0.47 – 1.09)	0.4 ± 0.17 (0.29 – 0.66)	0.4 ± 0.15 (0.27 – 0.62)
IV	4.51 ± 0.997 (3.54 – 5.81)	0.4 ± 0.07 (0.35 – 0.53)	0.9 ± 0.21 (0.59 – 1.07)	0.4 ± 0.07 (0.36 – 0.51)	0.5 ± 0.07 (0.37 – 0.54)

Groups
I = Control
II = Ethanol extract of *S. china* treated rats
III = Acetone extract of *S. china* treated rats
IV = Benzene extract of *S. china* treated rats

Table 3: Student-Newman-Keuls (SNK) post hoc test results show the toxic effect of ethanol, acetone and benzene extract of *S. china* on haematological parameters of different groups of the albino rats. Mean values are arranged in ascending order.

Parameters	Groups			
WBC (10^3/µL)	3.60 (IV)	4.48 (III)	9.03 (I)	11.65 (II)
Neutrophil (%)	0.21 (III)	0.21 (II)	0.23 (IV)	0.39 (I)
Eosinophil (%)	0.001 (III)	0.002 (II)	0.003 (IV)	0.006 (I)
Basophil (%)	0.001 (IV)	0.007 (III)	0.008 (II)	0.020 (I)
Lymphocyte (%)	0.09 (III)	0.12 (IV)	0.14 (II)	0.19 (I)
Monocyte (%)	0.007 (III)	0.008 (II)	0.010 (I)	0.017 (IV)
RBC (10^6/µL)	5.53 (III)	5.80 (I)	6.29 (II)	6.38 (IV)
Haemoglobin (g/dL)	13.38 (I)	13.60 (IV)	14.20 (II)	14.60 (III)
PCV/HCT (%)	40.48 (IV)	41.45 (I)	41.53 (II)	44.25 (III)
MCV (fL)	84.35 (I)	84.40 (II)	86.35 (III)	87.13 (IV)
MCH (pg)	27.48 (II)	27.63 (I)	28.70 (IV)	28.75 (III)
MCHC (g/dL)	32.55 (III)	32.75 (I)	33.18 (IV)	33.70 (II)
Platelet (10^3/µL)	1.53 (IV)	1.78 (II)	1.88 (III)	4.06 (I)

Horizontal lines connect similar means.

Groups

I = Control rats (Group I)

II = Ethanol extract of *S. China* treated rats (Group II)

III = Acetone extract of *S. China* treated rats (Group III)

IV = Benzene extract of *S. China* treated rats (Group IV)

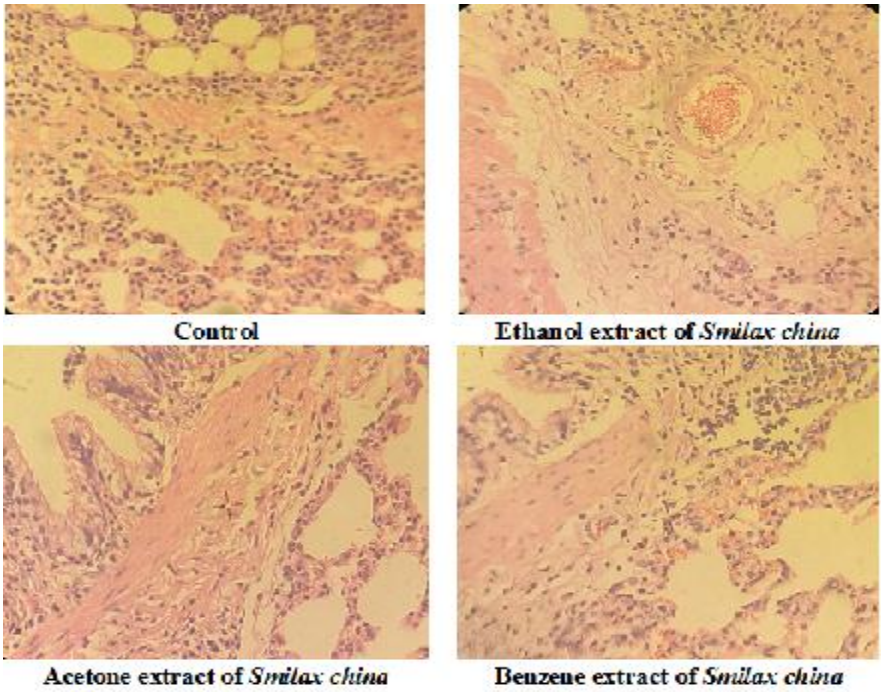

Plate 1: Acute toxic effect of different extracts of *Smilax china* on histoarchitecture of lungs.
(Images showed the normal alveolar cells in both control and extract treated groups)

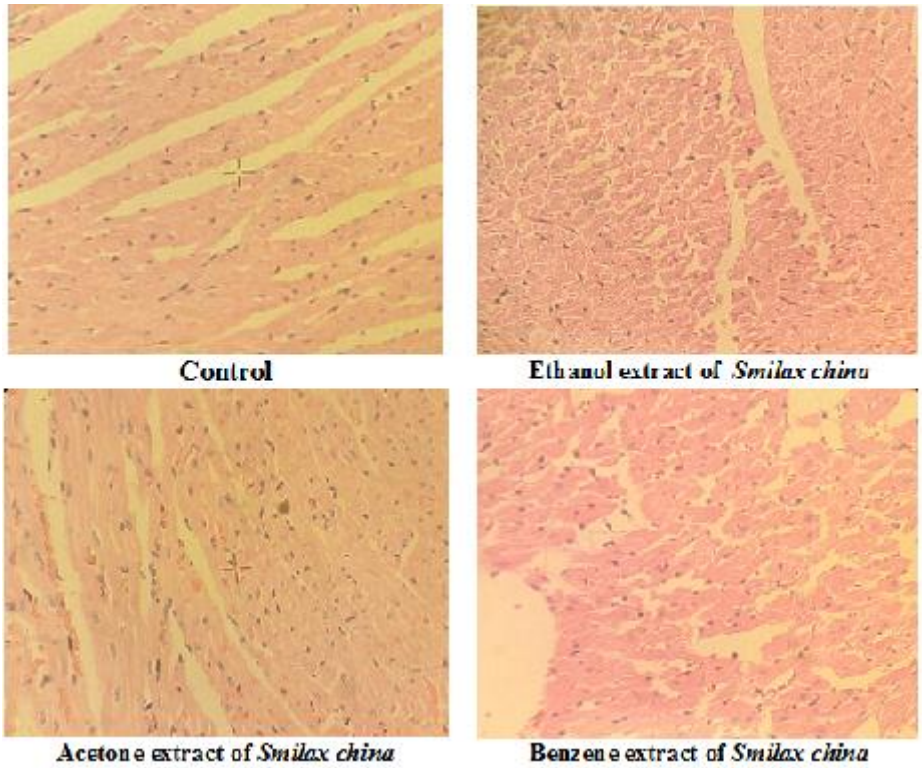

Plate 2: Acute toxic effect of different extracts of *Smilax china* on histoarchitecture of heart.
(Images showed the normal cardiac cells in all the groups)

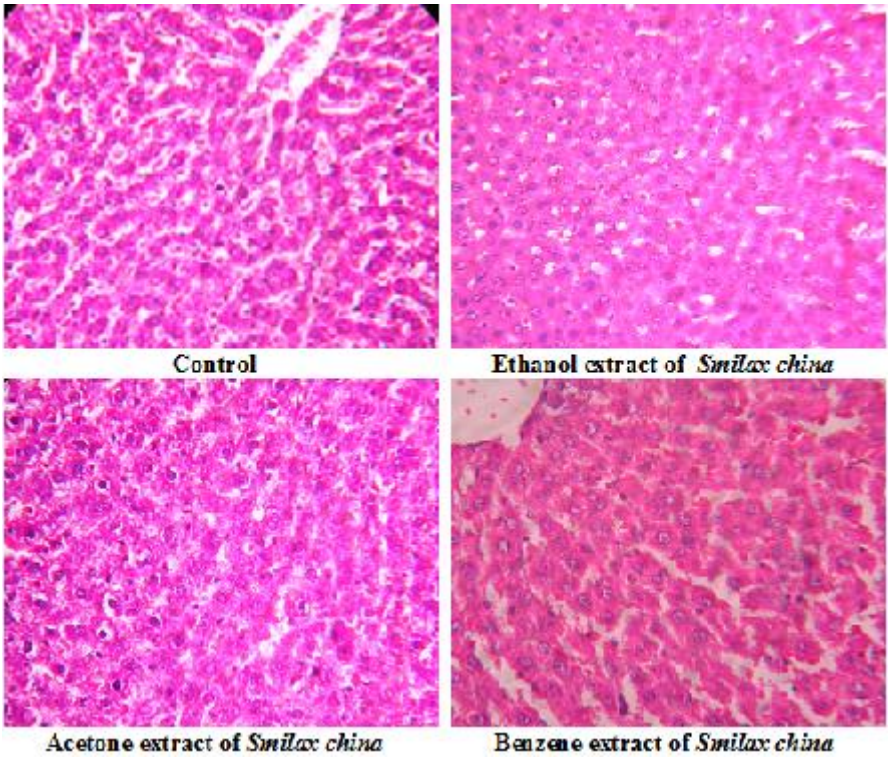

Plate 3: Acute toxic effect of different extracts of *Smilax china* on histoarchitecture of liver.
(Images showed normal hepatic lobules, hepatocytes, central vein and sinusoids in all the groups)

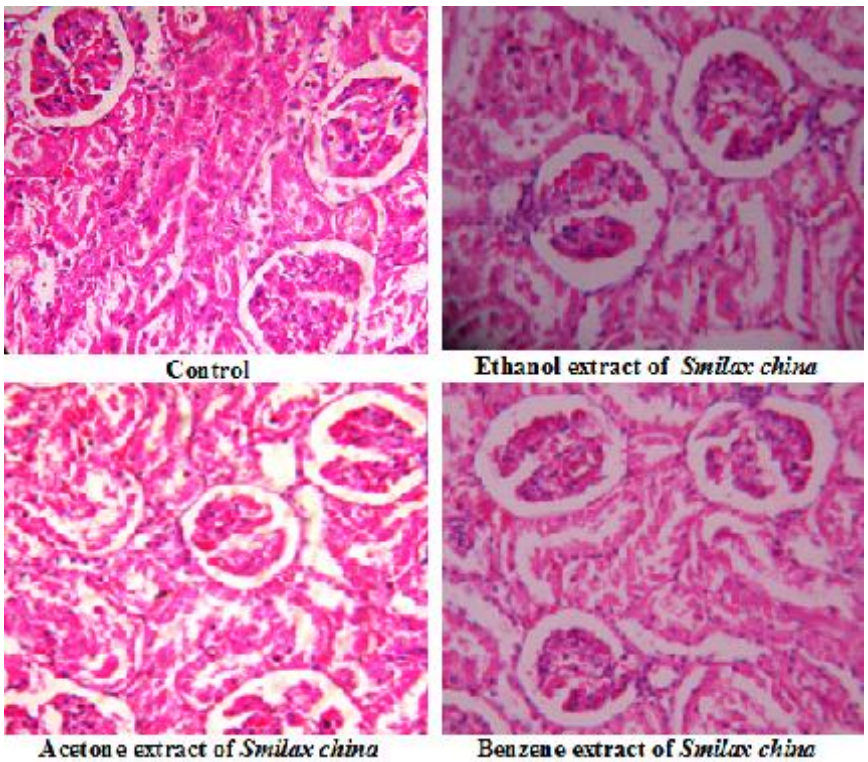

Plate 4: Acute toxic effect of different extracts of *S. china* on histoarchitecture of kidney.
(Images showed normal glomeruli, tubules and blood vessels in all the groups)

CONCLUSION

The results of this study showed no changes in the behaviour, no toxic symptoms, and changes in the body weight, food intake, water intake, and relative organ weight. However, the haematological parameters differed from each other but it does not exceed from the normal range. The histoarchitecture of the vital organs did not show any damaged cells, blood vessels and tubules in all the extract treated rats. Thus the present study revealed that the *Smilax china* rhizome extract at 2000 mg/kg

body weight does not produce any toxic effect in the ethanol, acetone and benzene extract treated rats.

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REFERENCES

1. Edoga HO, Kwa D, Mbaebia BO. (Phytochemical constituents of some Nigerian medicinal plants). African J. Biotechnol, 2005; (7): 685-8.
2. Okwu DE. (Evaluation of the chemical composition of indigenous spices and flavouring Agents). Global J. Pure Appl. Sci., 2001; 7(3): 455-9.
3. Mahidol C, Prawat H, Prachyawarakorn V, Ruchirawat S. (Investigation of some bioactive Thai medicinal plants). Phytochemistry Reviews, 2002; 1: 287–97.
4. Rukangira E. (Conserve Africa. The African Herbal industry constraints and challenges). Conserve Afr. Int., 2004; 126: 1-23.
5. Idris Bello, Mustapha W Shehu, Mustapha Musa, Mohd Zaini Asmawi, Roziahanim Mahmud. (*Kigelia africana* (Lam.) Benth. (Sausage tree): Phytochemistry and pharmacological review of a quintessential African traditional medicinal plant). Journal of Ethnopharmacology, 2016; 189: 253-76.
6. Elavarasi S, Saravanan K. (Ethnobotanical study of plants used to treat diabetes by tribal people of Kolli hills, Namakkal District, Tamilnadu, South India). International Journal of Pharm Tech Research, 2012; 4(1): 404-11.
7. Cordell GA. (Biodiversity and drug discovery-a symbiotic relationship). Phytochemistry, 2000; 55: 463–80.
8. Revathi G, Elavarasi S, Saravanan K. (Evaluation of toxic effect of traditionally used antidiabetic polyherbal formulation on albino rats). International Journal of Pharma and Biosciences, 2015; 6(4): 181-7.
9. Revathi G, Elavarasi S, Saravanan K. (Protective effect of *Daucus carota* (Carrot) and *Raphanus sativus* (Radish) on aspirin induced gastric ulcer in albino rat models). International Journal for Science and Advance Research in Technology, 2017; 3(9): 348-53.
10. Fabricant DS, Farnsworth NR. (The Value of plants used in traditional Medicine for Drug Discovery). *Environ. Health Perspec.*, 2001; 109 (1): 69-75.
11. Hasler CM, Blumberg JB. (Symposium on Phytochemicals Biochemistry and Physiology). Journal of Nutrition, 1999; 129: 756s-57s.
12. Vijayalakshmi R, Ravindran R. (Preliminary comparative phytochemical screening of root extracts of *Diospyrus ferrea* (wild.) Bakh and *Aerva lanata* (L.) Juss. Ex schultes). Asian J. plant Sci. Re., 2012; 2: 581-7.
13. Rajesh P, Latha S, Selvamani P, Rajesh Kannan V. (Phytochemical Screening and Toxicity Studies on the leaves of *Capparis sepiaria* Linn). A publication of Global Scientific Forum, 2010; 1-10.
14. Liu J, Sempos CT, Donahue RP, Dorn J, Trevisan M, Grundy SM. (Non–high-density lipoprotein and very-low-density lipoprotein cholesterol and their risk predictive values in coronary heart disease). American Journal of Cardiology, 2006; 98(10): 1363-8.
15. Yumnamcha T, Upendra Nongthomba, Damayanti Devi M. (Phytochemical screening and evaluation of genotoxicity and acute toxicity of aqueous extract of *Croton tiglium* L). International Journal of Scientific and Research Publications, 2014; 4(1).
16. Hoyos LS, Au WW, Heo MY, Morris DL, Legator MS. (Evaluation of the genotoxic effects of a folk medicine, *Petiveria alliacea* (Anamu)). Mutation Research, 1992; 280: 29–34.
17. Van Wyk BE, Van Heerden FR, Van Oudtshoorn B. (Poisonous Plants of South Africa). Briza Publications, Pretoria. 2002.
18. Demma J, Engidawork E, Hellman B. (Potential genotoxicity of plant extracts used in Ethiopian traditional medicine). Journal of Ethnopharmacology, 2009; 122(1): 136-42.
19. Botha CJ, Penrith ML. (Poisonous plants of veterinary and human importance in southern Africa). Journal of Ethnopharmacology, 2008; 119: 549–58.
20. Chanda S, Parekh J, Vaghasiya R, Dave R, Baravalia Y, Nair R. (Medicinal plants-from traditional use to toxicity assessment: A Review). International Journal of Pharmaceutical Sciences and Research, 2015; 6(7): 2652.
21. Sohini YR, Bhatt RM. (Activity of crude extract formulation in experimental hepatic amoebiasis and in immunomodulation studies). J Ethnopharmacol, 1996; 54: 119-24.
22. Kokate A. (Phytochemical methods). Phytotherapy, 1996; 78: 126-9.
23. Harborne JB. (Phytochemical methods. A Guide to Modern Techniques of Analysis). 3rd edition. Chapman and Hall, London. 1998; Pp. 40-96.
24. Rajpal V. (Testing and extraction methods of Medicinal Herbal, Standardisation of Botanicals). Estern Publishers, New Delhi, 2002; 2: 124.
25. Raaman N. (Phytochemical Techniques). New India Publishing Agency, ISBN-81-89422. 2006; 30-8.
26. Erhirhie Earnest Oghenesuvwe, Ekene, Nwoke E, Ajaghaku Daniel Lotanna. (Guidelines on dosage calculation and stock solution preparation in experimental animals' studies). Journal of Natural Sciences Research, 2014; 4(18).
27. Lorke D. (A new approach to practical acute toxicity testing). Arch Toxicol, 1983; 54: 275–87.
28. Maiti Atanu, Drohat AC. (Thymine DNA glycosylase can rapidly excise 5-formylcytosine and 5-carboxylcytosine potential implications for active

demethylation of CpG sites). Journal of Biological Chemistry, 2011; 286(41): 35334-8.
29. Rahman AU, Zaman K. (Medicinal plants with hypoglycemic activity). J. Ethnopharmacol., 1989; 26: 1-55.
30. Akindele AJ, Adeyemi OO. (Antiinflammatory activity of the aqueous leaf extract of *Byrsocarpus coccineus)*. Fitoterapia, 2007; 78(1): 25-8.
31. Malairajan P, Gopalakrishnan G, Narashiman S, Jessi Kala Veni K. (Analgesic activity of some Indian medicinal plants). Journal of Ethnopharmacology, 2006; 106(3): 425-8.
32. Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM. (Toxicological studies of hydromethanolic leaves extract of *Grewia crenata*). International Journal of Pharmaceutical Sciences and Drug Research, 2012; 4: 245–9.
33. Oslon H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B, Heller, A. (Concordance of the toxicity of pharmaceuticals in humans and in animals). Regulatory Toxicology and Pharmacology, 2000; 32: 56–67.
34. Igwebuike U, Obidike R. (Effects of Nigerian Qua Iboe Brent crude oil on rat spleen and haematological parameters). Veterinarski Arhiv., 2007; 77: 247–56.