EXTERACTION, PHYTOCHEMICAL EVALUATION AND HEPATOPROTECTIVE ACTIVITY OF *PHOENIX SYLVESTRIS Linn.*

Sanjay Kumar Dhaker*, Dr. Hemant Swami[1], Dr. Raghvendra Dubey[2]

*Department of pharmacy, Research scholar, Institute of Pharmaceutical Sciences, SAGE University, Indore 452020 Madhya Pradesh.

[1]Department of pharmacy, Professor, Institute of Pharmaceutical Sciences, SAGE University, Indore 452020 Madhya Pradesh.

[2]Department of pharmacy, HOD, Institute of Pharmaceutical Sciences, SAGE University, Indore 452020 Madhya Pradesh.

*Corresponding Author: Sanjay Kumar Dhaker, Department of pharmacy, Research Scholar, Institute of Pharmaceutical Sciences, SAGE University, Indore 452020 Madhya Pradesh.

Abstract:
*Phoenix sylvestris*is a globally recognized and historically significant plant with high nutritional value. Carbohydrates, phenols, amino acids, flavonoids, tannins, alkaloids, terpenoids, dietary fibers, and vital vitamins and minerals are all abundant in it. The group received only paracetamol exhibited significant (P<0.001) rise in serum AST, ALT and ALP values bymean values of 94.33±1.11, 127±1.21 and 221±1.93 respectively, when compared withthe corresponding values of 74.15±1.14, 34.17±1.07 and 117.8±1.81 in normal group.Marked reduction in ALP, ALT and AST i.e., P<0.001 was seen in animals treated withLiv52with mean values of 78.67±1.49, 39.83±1.32 and 144.7±2.52 respectively .*P. sylvestris* Linn showed significantly (P<0.01, 0.001) reduction in total and direct bilirubin respectively with the mean estimations of 0.77±0.33 and 0.22±0.005 in *P. sylvestris* Linn alcoholic concentrate; 1.74±0.04 and 0.17±0.004 in *P. sylvestris* Linn aqueous extract and 0.73±0.06 and 0.22±0.04 in isolated compound of *P. sylvestris* Linn. *P. sylvestris* Linn with respective mean values of 0.87±0.03, 88.04±7.88 and 36.84±3.41 in EtOH E-PS; 32.73±2.92, 35.89±0.90 and 0.78±0.03 in AqE-PS; 80.35±7.00, 36.72±3.00 and 0.82±0.01 in isolated compound of *P. sylvestris* Linn group. HE-PS and $CHCl_3$E-PS of thisplant failed to produce significant changes in hepatic SOD,GSH and CAT levels ascompared to that of positive controls, with mean values of 31.14±4.91, 22.03±1.45 and0.72±0.01inHE-PSgroup;32.73±3.15,24.14±3.50and0.61±0.02in$CHCl_3$E-PSgroup.

Key words: *Phoenix sylvestris*, SOD, GSH, Hapatotoxicity.

INTRODUCTION:

The liver is the most important organ in the body. It plays a vital role in the production and digestion of substances required for the body's upkeep. The liver performs the metabolism of biomolecules such as proteins, lipids, and carbs. The liver is the final stop of the metabolic

process, which is a very important bodily function. The liver also metabolizes xenobiotics, or medications, which are given to patients with certain illnesses. Hepatotoxicity is the term for the harm that occurs to liver cells when these substances or xenobiotics are metabolized.

The majority of medications that cause liver toxicity also cause hepatic damage. The liver is the second largest organ in the body and weighs about 1.5 kg in adults, or 2% of the total weight. It is located in the upper quadrant of the stomach, under the rib cage. Glisson's capsule, a visceral extension of the peritoneum, covers it. The liver is made up of the left and right first lobes as well as the caudate and quadrate accent lobes. Compared to the right lobe, the left lobe is six times smaller. The proper lobe performs amazing roles in our body, including hormone generation, drug excretion, bile formation, cholesterol, bilirubin, and fat, protein, and carbohydrate metabolism. carbs. It interacts with nearly every metabolic process involved in the creation of energy, the delivery of nutrients, growth, illness, and the prevention of reproduction. Additionally, it serves as the hub for the metabolism of nutrients like proteins, fats, and carbohydrates as well as the excretion of waste products. Our liver filters harmful toxins out of our blood. Detoxification is the process of eliminating toxic compounds (Towseef H. et al., 2019).Liver damage caused by chemicals found in medications and other substances may additionally virtually mimic any shape of evidently going on liver ailment. To set off hepatotoxicity, several chemicals were recognized. Galactosamine, Carbon tetrachloride (CCl4), lipopolysaccharide or D-Galactosamine (LPS/GalN), Anti tubercular drugs, Thioacetamide (TAA), To cause experimental hepatotoxicity, substances such as arsenic, alcohol, paracetamol, rifampicin, isoniazid, and aflatoxin are utilized. The majority of inorganic chemicals that cause hepatotoxicity are phosphorus, iron, and copper.

MATERIALS AND METHODS:

Entire plants of *Phoenix sylvestris (L.)* were collected from local area, Bhopal, Madhya Pradesh (India). The plant was authenticated by Dr. S. N. Dwivedi Department of Botany, Janata PG College, A.P.S. University, and Rewa (M.P.) India. A voucher specimen is preserved in our laboratory for future reference (Voucher Specimen Number/ JC/B/PAN/537). The leaves of *Phoenix sylvestris (L.)* plant was characterized by its morphological features like colour, shape, size and surface characteristics [6] has been studies.

Preparation of plant material:

The Leaves of plant were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use.

Extraction and fractionation:

The extraction yield of the extracts from plant species is vastly depends on the solvent polarity, which find out both qualitatively and quantitatively the extracted compounds. Ethanol and water are the commonly used solvent for the extraction because of their low toxicity and high extraction yield with the advantage of modulating the polarity of the solvent by using mixtures at different ratios (Jackson et al, 1996). The plant materials (1 kg) were initially defatted with petroleum ether and then extracted with n-hexane (E1), chloroform (E2), ethyl acetate (E3), alcohol (E4) and water (E5) using a Soxhlet apparatus. The yield of the plant extracts ethanol (70%) and aqueous measured about 20 g each after evaporating the solvent using water bath. The standard extracts obtained from *Phoenix sylvestris (L.)* were then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening (Akueshi et al, 2002).

Hepatotoxicity Study

The Institutional Animal Ethics Committee (IAEC vide protocol number IRDA/IAEC/M02/08/2024-25, dated 06/01/2024) has reviewed and approved the protocol for general procedures and the use of animals for performing this rat study (IAEC vide protocol number IRDA/IAEC/M02/08/2024-25, dated 06/01/2024). All procedures related to animal experiment was performed as per the recommendations of the Guide for the Care and Use of Laboratory Animals and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines [6, 7] following all the ethical practices as laid down in the CPCSEA guidelines for animal care.

Safety Precautions:

Personnel working with animals, test item and its formulation were wear personal protection such as gloves, head cap, face mask.

Test System

Species	:	Rat (*albino*)
Strain	:	Wistar
Sex	:	Males and Females (Females will be nulliparous and non-pregnant)

Body Weight Range	:	150 – 220 grams at the start of treatment
Number of Animals	:	50* (25 males + 25 females) - at receipt 40 (20 males + 20 females) - for study
No. of Animals/group	:	5
Total No. of Groups	:	4
Source	:	INVITOX R&D Institute, Pune (CPCSEA Registration NoIRDA/IAEC/M02/08/2024-25, dated 06/01/2024)

Environmental Conditions

Animals were maintained under the following environmental conditions:

Temperature	: 22°C (± 3° C)
Relative humidity	: 50% (± 20%)
Light/dark cycle (photoperiod)	: 12 h light & 12 h dark cycle

Hepatoprotetive activity of various extracts of *Phoenix sylvestris* Linn

Male Wistar rats were starved over night with tap water and on the day of experiment they were randomly divided into 8 groups to receive different treatments of paracetamol and alcohol (Devaki T. et. al., 2004) hepatotoxicity studies. All the extracts of *Phoenix sylvestris* were suspended in required volume of 0.1% Tween 80 so as to get 100mg/ml concentration of the extract and the maximum volume of administration was 10ml/kg p.o. [17] Before administration, the extract suspension was prepared freshly. Group-I (Negative control) received only vehicle, while Group-II (positive control) received only hepatotoxicant. Group-III received Liv52 (Sandhir R. and Gill K. D. 1999) [18] in the dose of 5ml/kg. Group-IV received isolated compound in the dose of 100mg/kg. Remaining 4 groups received orally various extracts in the dose of 200mg/kg once daily for seven days and paracetamol 2gm/kg was administered only on 5th day single dose. The duration of study for paracetamol induced hepatotoxicity was for 7 days and theanimals were sacrificed on 8th day for further study. Similarly the duration of alcohol induced hepatotoxicity was for 90 days and the animals will be sacrificed for further study on 91st day [19]. Details of hepatotoxicants and extracts treatment are shown below in the treatment schedule.

In the present study *Phoenix sylvestris* Linn whole plant extracts with n-hexane, chloroform, ethanol, water and isolated compound from alcoholic extract were used for the evaluation of

their hepatoprotective activity in experimentally induced hepatotoxicity in male Wistar rats by hepatotoxicants i.e. paracetamol and alcohol [20]. A day after the last portion of treatment animals were anesthetized with halothane and the blood was pulled back by cardiac puncture of all the animals individually and the liver was dissected out for enzymatic as well as histopathological studies.

Isolated sera from the blood tests gathered were utilized for biochemical examination viz, alanine aminotransaminase (ALT), aspartate aminotransferase (AST) [21], soluble phosphate (ALP), all out protein, bilirubin (all out and direct) and Some portion of liver was utilized for the MDA estimation (Malondialdehyde), GSH (Glutathione), Super Oxide Dismutase (SOD) and CAT (Catalase) [22] by homogenizing with tris-support and another part was prepared promptly for histological examinations.

Table 1: Treatment schedule of hepatotoxicants and extracts treatment

Group	Treatment	Paracetamol in0.1%Tween 80	Ethanol (20%v/v)
1	Vehicle treated	2gm/kg b.w.Orallyon5thday. Standard drug/extracts treatment from day1to7. Animals will be sacrificed for further study on8th day	25ml/kg orally daily from day 1to90. Standard drug/extracts treatment from day 1to90. Animal will be sacrificed for further study on91stday
2	Positive Control (paracetamol/Ethanol)		
3	Liv52®5ml/kg.		
4	Standard		
5	n-hexane extract of *Phoenix sylvestris* Linn		
6	Chloroform extract of *Phoenix sylvestris* Linn		
7	Alcohol extract of *Phoenix sylvestris* Linn		
8	Aqueous extract of *Phoenix sylvestris* Linn		

RESULT AND DISCUSSION:

The group received only paracetamol exhibited significant (P<0.001) rise in serum AST, ALT and ALP values bymean values of 94.33±1.11, 127±1.21 and 221±1.93 respectively,

when compared withthe corresponding values of 74.15±1.14, 34.17±1.07 and 117.8±1.81 in normal group.Marked reduction in ALP, ALT and AST i.e., P<0.001 was seen in animals treated with Liv52 with mean values of 78.67±1.49,39.83±1.32 and 144.7±2.52 respectively. Similarly, groups treated with 200 mg/kg of alcoholic, aqueous and isolated compound of *P. Sylvestris* Linn showed significantly (P<0.05, 0.01, 0.001) lowered ALP, ALTand AST levels with individual mean estimations of 84.15±1.11, 52±1.23and138.7±1.30 for alcoholic extract. 86.83±0.70, 115±4.03 and 211±0.91 for aqueous extract. 79±1.48,46±1.42 and 136.5±1.56 for isolated compound. n-hexane and chloroform extracts of *P. Sylvestris* Linn plant failed to produce significant changes in serum AST, ALT and ALP levels as compared to that of positive controls. In the groups treatedwith n-hexane of *P. Sylvestris* Linn the mean values of AST, ALT and ALP92.67±2.02, 122.2±1.62 and 215.5±2.23 respectively. Similarly insignificantly changed values of AST, ALT and ALP respectively were 88.17±1.07, 122.3±1.17 and 215±2.23inchloroform extract of *P. Sylvestris* Linn.

Effects of various treatments on paracetamol induced changes in AST, ALT, ALP, Bilirubin and Protein

Group Dose/Kg^{-1}	AST IU/L	ALT IU/L	ALP IU/L	Bilirubin mg/dl		Protein mg/dl
				Total	Direct	
Normal Vehicle treated	74.15 ±1.14	34.17 ±1.07	117.8 ±1.815	0.28 ±0.01	0.13 ±0.006	198.3 ±2.431
Paracetamol 2 gm	94.33# ±1.11	127 ±1.21	222 ±1.932	2.29 ±0.08	0.14 ±0.007	67.17 ±0.94
Paracetamol 2 gm+Liv52 5ml	78.67* ±1.49	39.83* ±1.32	144.7* ±2.525	0.5* ±0.08	0.22* ±0.006	198.7* ±3.80
Paracetamol 2gm+ PS-EtOHE 200mg	84.15 ±1.11	52 ±1.23	138.7 ±1.308	0.77 ±0.13	0.22 ±0.005	180.7 ±4.28
Paracetamol 2gm +PS-AqE200mg	86.83 ±0.70	115 ±4.03	211.3 ±0.918	1.74 ±0.04	0.17 ±0.004	82.33 ±2.60
Paracetamol 2gm + PS-HE	92.67 ±2.02	122.2 ±1.62	215.5 ±1.72	2.117 ±0.09	0.12 ±0.006	62 ±0.57

200mg			7			
Paracetamol 2g+CH-CL_3E200mg	88.17 ±1.07	122.3^{ns} ±1.17	215.3 ±2.232	2.18 ±0.12	0.13^{ns} ±0.007	62.67 ±0.84
Alcohol + Rhamnetin 30 mg	79 ±1.48	46 ±1.42	136.5 ±1.56	0.73 ±0.06	0.22 ±0.004	195.5 ±3.42

One way ANOVA pursued by Turkeys' different examination tests. #P<0.001 in comparison with control normal group *P<0.001 in comparison with paracetamol intoxicated group AST (Aspartate amino transferase), (ALT) Alanine amino transferase, (ALP) Alkaline phosphates. PS-EtOHE- *P. sylvestris* ethanolic extract, PS-AqE-*P. sylvestris* aqueous extract, PS-HE- *P. sylvestris* n-hexane extract, CH-CL_3E- *P. sylvestris* chloroform extract.

Effect on hepatic antioxidant (SOD µ/mg, GSH µ/mg and CAT U/mg of protein) enzymes:

Effects of various treatments on paracetamol induce changes in antioxidant and oxidant

Dose/Kg^{-1} Group	**SOD U/ mg Protein**	**GSH µg/ mg Protein**	**CAT U/ mg Protein**	**MDA nmol/ g Protein**
Normal Vehicle treated	76.51 ±10.79	36.57 ±3.008	0.925 ±0.04	13.06 ±1.03
Paracetamol 2 gm	27.7 ±4.45	19.89 ±1.506	0.5783 ±0.03	24.05 ±0.76
Paracetamol 2gm +Liv525ml	75.67*** ±7.34	39.49*** ±2.578	0.861*** ±0.04	13.18*** ±1.14
Paracetamol 2 gm+ PS-EtOHE 200mg	79.33*** ±6.52	36.97*** ±2.46	0.8567*** ±0.03	12.25*** ±1.27
Paracetamol 2 gm +PS-AqE 200mg	64.63** ±7.07	36.49** ±2.66	0.766** ±0.006	18.85** ±0.35
Paracetamol 2gm +	31.15^{ns} ±4.18	22.16^{ns} ±1.88	0.706^{ns} ±0.01	20.18^{ns} ±0.31

HE 100mg				
Paracetamol 2gm+ $CHCL_3E$ 200 mg	35.59^{ns} ±3.351	24.9^{ns} ±3.34	0.722^{ns} ±0.01	20.19^{ns} ±0.64
Alcohol + Rhamnetin 30mg	78.67*** ±6.83	38.07*** ±2.73	0.816*** ±0.01	11.97*** ±1.12

One way ANOVA pursued by Turkeys' different examination tests. #P<0.001 when compared with Normal control group*P<0.001 when compared with paracetamol intoxicated group SOD-Superoxide dismutase, GSH-Glutathione, CAT-Catalase, MDA Malondialdehyde, PS-Et OHE-*P. sylvestris* ethanolic extract, PS-AqE- *P. sylvestris* aqueous extract, PS-HE-*P. sylvestris* n-hexane extract, CH-CL_3E-*P. sylvestris* chloroform extract.

Animal administered by 200mg/kg of watery, alcoholic and segregated compound of *P. Sylvestris* Linn showed significantly (P<0.01, 0.001) reduction in total and direct bilirubin respectively with the mean estimations of 0.77±0.33 and 0.22±0.005 in *P. Sylvestris* Linn alcoholic concentrate; 1.74±0.04 and 0.17±0.004 in *P. Sylvestris* Linn aqueous extract and 0.73±0.06 and 0.22±0.04 in isolated compound of *P. Sylvestris* Linn. Serum bilirubin was not significantly affected by treatment with HE and $CHCl_3$E of*P. Sylvestris* plant. The mean values of total and direct bilirubin respectively were 0.04±2.14 and 0.12±0.006 in HE-PS and 2.18±0.12 and 0.13±0.007 in $CHCl_3$E.

Effects of various treatments on alcohol induced changes in AST, ALT, ALP, Bilirubin and Protein

Group Dose/Kg^{-1}	**IU/L AST**	**IU/L ALT**	**IU/L ALP**	**Bilirubin mg/dl**		**Protein mg/dl**
				Total	Direct	
Normal Vehicle treated	139.3 ±1.20	87.17 ±1.90	118.3 ±1.11	0.5217 ±0.01	0.125 ±0.007	7.713 ±0.18

Alcohol	214.2# ±3.88	163.8# ±3.02	221.3# ±1.87	1.013# ±0.03	0.1917# ±0.007	4.662 # ±0.17
Alcohol+Liv52 5ml	150.8* ±1.01	123.2* ±1.22	144.7* ±2.52	0.823* ±0.01	0.136* ±0.006	7.008 * ±.052
Alcohol+PS-EtOHE 200mg	123.8 ±2.30	123.8 ±2.30	138.8 ±1.85	0.5617 ±0.02	0.125 ±0.004	6.567 ±0.08
Alcohol+PS-AqE 200mg	202 ±1.03	153 ±1.30	210.2 ±0.54	0.8783 ±0.01	0.15 ±0.007	5.513 ±0.12
Alcohol+PS-HE 200mg	207.3 ±1.53	165.5 ±1.72	215.5 ±1.72	0.96 ±0.007	0.19 ±0.007	4.29 ±0.16
Alcohol+PS-$CHCL_3$E 200mg	205.8 ±1.95	163.5 ±1.87	215.5 ±2.23	0.945 ±0.008	0.198 ±0.009	4.518 ±0.21
Alcohol + Rhamnetin30mg	149 ±1.39	121 ±0.79	137.2 ±1.88	0.773 ±0.08	0.116 ±0.004	6.742 ±0.08

One way ANOVA pursued by Turkeys' different examination tests. With normal control group, #P<0.001when compared*P<0.001when compared with Alcohol intoxicated group

PS-HE-*P. sylvestris* n-hexane extract, Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), PS-EtOHE- *P. sylvestris* ethanolic extract, PS-Aq E-*P. sylvestris* aqueous extract, Alanine amino transferase (ALT), CH-CL_3E- *P. sylvestris* chloroform extract.

Effects of various treatments on alcohol induced changes in antioxidant and oxidant

Dose/Kg^{-1} Group	**Protein SOD U/mg**	**Protein GSH µg/mg**	**Protein CAT U/mg**	**Protein MDA n mol/gm**

Normalvehicle treated	81.65 ±10.72	37.2 ±3.40	0.9267 ±0.044	13.44 ±1.122
Alcohol	29.62# ±4.70	19.56# ±2.08	0.6083# ±0.03701	24.51# ±0.871
Alcohol+Liv525ml	77.06* ±7.16	50.05* ±1.34	0.916* ±0.0.856	13.68* ±1.043
Alcohol+ PS-EtOHE 200mg	88.04 ±7.88	36.84 ±3.41	0.871 ±0.034	12.45 ±1.311
Alcohol+PS-AqE 200mg	32.73 ±2.92	35.89 ±0.90	0.78 ±0.030	19.21 ±0.595
Alcohol+PS-HE 200mg	31.14 ±4.91	22.03 ±1.45	0.728 ±0.011	21.3 ±0.365
Alcohol+PS-$CHCL_3$E 200mg	32.73 ±3.15	24.14 ±3.50	0.616 ±0.024	21.39 ±0.294
Alcohol+Rhamnetin 30mg	80.35 ±7.004	36.72 ±3.003	0.825 ±0.016	11.98 ±0.994

One way ANOVA pursued by Turkeys' different examination tests. With normal control group, #P<0.001when compared*P < 0.001when compared with alcohol intoxicated group SOD - Superoxide dismutase, GSH - Glutathione, CAT Catalase, MDA - Malondialdehyde, PS-EtOHE-*P. sylvestris* ethanolic extract, PS-AqE-*P. sylvestris* aqueous extract, PS-HE-*P. sylvestris* n-hexane extract, CH-CL_3E- *P. sylvestris* chloroform extract.

CONCLUSION:

Based on the results of the current experimental study, it can be concluded that *P. sylvestris* Ethanolic extracts (EEPS) at doses of 200 and 400 mg/kg have the potential to be useful Hepatoprotective agents because they effectively reduce liver damage in rats that have been induced to become hepatoxic using paracetamol and ethanol. The outcomes shown may be advantageous and lower hepatotoxicity risk factors.

REFERENCES:

1. DotiWahyuningsih ,YudiPurnomo. Antidiabetic Effect of *Urenalobata*: Preliminary Study on Hexane, Ethanolic, and Aqueous Leaf Extracts. JurnalKedokteranBrawijaya, Vol. 30, No. 1, Februari 2018. 1-6.
2. Purnomo, Y. Purnomo, S.B. Sumitro, M.A. Widodo. Incretin effect of Urenalobata leaves extract on structure and function of rats islet b-cells. Journal of Traditional and Complementary Medicine. 2017, 7(3), 301-306.
3. Inadera H, Shirai K and Saito Y. The enzymes related to lipoprotein metabolism (Hmg-coAreductase, 7-alpha hydroxylase). *Japanese J ofClinl Med* 1990; 48(11): 2483-91.
4. Vinay Kumar, Abul K Abbas and Nelson F. Pathological basis of disease.7thEdn. New Delhi: Elsevier; 2002:576.
5. Nawrocki J W, Weiss S R, Davidson M H, Sprecher D L, Schwartz S L, Lupien P J, Jones P H, Haber H E, Black D M. *J.Pharmacol*. 1995; 3(4):58.
6. Assy N, Kaita K, Mymin D, *et al.* Fatty infiltration of liver in hyperlipidemic patients. *Dig Dis Sci*. 2000; 45:1929 -1934.
7. Ridker PM, Danielson E, Fonseca FA, *et al*. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N *Engl J Med*. 2008; 359:2195-2207.
8. Glynn RJ, Danielson E, Fonseca FA, *et al*. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. *N Engl J Med.* 2009; 360:1851-61.
9. Riordan M .The side effects of statins: Heart healthy and head harmful? *The Wall Street Journal. 2010:10*-24.
10. Demacker PN, Reijnen IG, Katan MB, et al. Increased removal of remnants of triglyceride rich lipoproteins on a diet rich in polyunsaturated fatty acids. *Eur J Clin Invest*. 1991; 21:197.
11. Prasad K. Hypocholesterolemic and antiatherosclerotic effect of flax lignin complex isolated from flaxseed. Atherosclerosis. 2005; 179: 269-275.
12. Jain GC, Agarwal S. Favourable effect of *Cleome viscosa* L. on serum and hepatic lipid in hyperlipidemic rats. *Asian J Exp Sci*. 2006; 20: 331-336.
13. India: Ministry of Social Justice and Empowerment; 2000. Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), OECD Guidelines for the testing of chemicals, revised draft guidelines 423: Acute Oral toxicity- Acute toxic class method, revised document.

14. India: Ministry of Social Justice and Empowerment; 2000. Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), OECD Guidelines for the testing of chemicals, revised draft guidelines 423: Acute Oral toxicity- Acute toxic class method, revised document.
15. Pande VV, Dubey S. Antihyperlipidemic activity of *Sphaeranthusindicus* on atherogenic diet induced hyperlipidemia in rats. Int J Green Pharm. 2009;3:159–61.
16. Allian CC, Poon LS, Chan CS, Richmond W, Paul CF. Enzymatic determination of total serum cholesterol. Clin Chem. 1974;20:470–5.
17. Pari L, Latha M. Effect of *Cassia auriculata* flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. Singapore Med J. 2002;43:617–21.
18. .P. Lissy, Thara K Simon, M.S. Latha Antioxidant potential of *Sidaretusa, Urenalobata* and *Triumfettarhomboidea*AncSci Life. 2006, 25(3-4): 10–15
19. Mazumder UK, Gupta M, Manikandan L, Bhattacharya S. Antibacterial activity of Urenalobata root. Fitoterapia2001,72(8):927-9
20. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Wright-Scientechnica, Bristol, pp. 4-9 (1975)
21. Nirmal SA, Pal SC, Mandal SC. Pharmacognostic Evaluation of Nyctanthesarbortristis bark. Asian Pacific Journal of Tropical Biomedicine. 2012, 5494-5500.
22. Hussain M. Recent Trends in Medicinal Plant Science. Akinik Publications, New Delhi, 2019, Vol-5, p. 28.