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Evaluation Of Acute and Subacute Toxicity Studies of Herbal Formulations of *Sphaeranthus Indicus Linn* (Gorakh Mundi) In Swiss Albino Mice

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ABSTRACT

Background: -

Sphaeranthus Indicus Linn or East Indian Globe Thistle is a multi-branched aromatic herb consisting of various medicinal properties, and is used in the treatment of skin conditions, as an antimicrobial, immunomodulator, immunostimulant and in wound healing traditionally.[9] All the parts of the Sphaeranthus Indicus have been utilized for the treatment of various disease conditions out of which the marketed herbal formulations of Sphaeranthus Indicus were investigated for its toxicity profile which showed dose dependent toxicity. Toxicity profile of this marketed formulation was not investigated in previous studies

Aim: -

Explore the acute and subacute toxicity profile of the following two marketed herbal formulation of Sphaeranthus indicus linn for the safe use of traditional medicine and explore its potential for further effectiveness.

Method: -

In Acute toxicity study of both herbal formulations, Total no. of animals used were 24 male Swiss albino mice, weighing 25-30g, randomly assigned to experimental groups of 5mg/kg,50mg/kg, 300mg/kg and 1000mg/kg with 3 mice each and receiving single dose of the extract at 5mg/kg b.w,50mg/kg,300mg/kg and 1000mg/kg b.w respectively. Animals were monitored for 14 days for signs of toxicity, behavioral changes, mortality etc.

In Sub-acute toxicity study, Total no of animals used were 70 Swiss albino mice weighing 25-30 g consisting of male and female Swiss albino mice randomly assigned to experimental groups of 50mg/kg, 1000mg/kg ,2000mg/kg with control group been given distilled water. The extract of herbal formulation of Sphaeranthus indicus was given at the doses of 50mg/kg,1000mg/kg,2000mg/kg per body weight of the mice for 28 consecutive days. The studies were conducted in compliance with OECD guidelines 423 and 407.

Results: -

Acute toxicity study showed no mortality up to the dose of 1000mg/kg, but mortality rates increase with higher doses. In subacute toxicity study, there was no mortality, up to the dose of 2000mg/kg with increase in deaths at higher doses. The hematological and biochemical analysis of the blood parameters don't show much significant differences. The histopathological analysis of liver shows marked lesions at higher doses.

Conclusion: -

It is concluded that the oral administration of doses of herbal formulation of Sphaeranthus indicus in acute study don't cause any mortality or other changes up to the level of 1000mg/kg. At higher doses there are possibilities of finding gross changes. In subacute study, no significant changes in the hematological, biochemical, and histopathological parameters were observed. There is mild grade of lesion with repeated and daily dosing of the drug at higher dose above 2000mg/kg indicating that at higher doses with repeated dosing there is mild level development of toxicity signs.

Keywords: -Sphaeranthus indicus Linn, Hematology, Biochemical, Histopathology, toxicity.

INTRODUCTION

Medicinal plants are an important part of our daily lives and are frequently used as therapeutic agents for various diseases and conditions. In many developing countries ,80% population use some or other forms of natural sources. In India, Ayurvedic system of medication contains various such use of medicinal plants. [9] The whole part of the Sphaeranthus indicus plant are used for the treatment of variety of the disease conditions. Sphaeranthus indicus Linn. is known by a variety of names such as Mundi in Hindi, Gorakh Mundi in Gujarati, Sravani in Sanskrit and East Indian Globe Thistle in English. These herbs and its species are native to tropical Asia, Africa and Australia. The plant Sphaeranthus indicus Linn belongs to the family Asteraceae and is abundantly grown in the plains all over India, uphill to an altitude of 1500 m in the hills, especially as a weed in the rice fields. [7]

The whole parts of the plant are widely used in traditional medicine for the treatment of various disorders. It is an important indigenous medicinal plant used for the treatment of styptic gastric disorders, skin diseases, Anti syphilitic, anthelmintic, glandular swelling, nervine tonic, analgesic, antifungal and laxative properties. [8] The juice of the plant is styptic, and diuretic and it is said to be useful against liver and gastric disorders. It is reported that flowers are highly alterative, depurative, cooling tonic and blood purifiers in skin diseases. Dried and powdered leaves of plant are useful in the treatment of chronic skin diseases, urethral discharges and jaundice. [8] Extract of S.Indicus has been reported for mast cell stabilizing activity and exhibited tremendous antibacterial action against gram positive as well as gram negative bacteria. [9] The phytochemical analysis of the plant showed that it contains eudesmanolide type of sesquiterpene possessing immunoprotective and anti-inflammatory actions. It also reported to possess anxiolytic activity, neuroleptic activity, antioxidant activity, wound healing activity, analgesic and antipyretic activity, thus based on these characteristics, it is believed that S.indicus can be used as a safe nutraceutical for the treatment of various disorders. [6] Although numerous pharmacological studies have been carried out with this herb, there is no experimental evidence on its toxicity. Hence in the present study, single oral dose toxicity and 28-day subacute oral dose toxicity studies were conducted in the mice. In this study, acute and 28-day subacute oral dose toxicity and LD50 values were performed in experimental mice.[11]

MATERIALS AND METHODS

Plant materials: -

The herbal formulation containing pure extract of Sphaeranthus indicus Linn (Gorakh Mundi) in aqueous base is procured from Dindayal Ayurved Bhawan (Haryana)-**Herbal formulation I** and Shree Baidyanath Ayurved Bhawan (Uttar Pradesh)-**Herbal formulation II**

Animals and housing: -

Healthy Swiss albino mice of both sexes (6-8 weeks old/20-25g) were used for the study. Animals were maintained in standard household conditions of temperature, light and humidity. All animals were stored in standard cage and maintained at $(23\pm2)^0$C under 12h dark/light cycle. Healthy young adult animals are randomly assigned to the control and treatment groups. Cages are arranged in such a way that possible effects due to cage replacement are minimized. The animals were uniquely identified and kept in their cages for at least 14-15 days prior to the start of the treatment study to allow for acclimatization to the laboratory conditions. They were fed with standard rodent pellet diet food and water freely available. Animals are fasted prior to oral administration of the herbal formulation. Studies were conducted as per CCSEA and OECD guidelines 423,407 for acute and subacute toxicity study. The Institutional Animal Ethics Committe of Viva Institute of Pharmacy, Virar, India (IAEC/VIP/2024/FEB/P/03), approved the study.

Preparation of extract doses: -

The herbal formulation doses were prepared accordingly by calculating the total quantity of the Sphaeranthus Indicus linn present in each herbal formulation and actual body weight of the mice with doses given as 5mg/kg b.w of the mice,50mg/kg,300mg/kg, 1000mg/kg for acute toxicity study and 50mg/kg b.w ,1000mg/kg,2000mg/kg b.w of the mice in subacute toxicity study respectively.. The herbal formulation extracts were stored under cold conditions.

Acute Toxicity Studies: -

Acute toxicity test was performed as per guidelines of the Organization for Economic Co-operation and Development (OECD) 423(Test No.423: Acute Oral toxicity –Acute Toxic Class Method,2002). Total 24 mice were divided into 4 groups of each herbal formulation with total groups counting to 8 and each group containing 3 male mice each. Groups 1,2,3,4 of Herbal formulation I and Groups 5,6,7,8 of Herbal formulation II received 5mg/kg,50mg/kg,300mg/kg and 1000mg/kg b.w of the mice and 50mg/kg,1000mg/kg and 2000mg/kg b.w of the mice respectively as single dose. The animals were monitored for mortality and other visual changes during the first 30 min, then at regular intervals over the next 24 hrs., with close monitoring during the first four hours. Animals were watched once a day for 14 days. Animals were provided food and

water, and their daily intake was recorded. Body weight, food intake and water consumption were all recorded and compared weekly to see if there were any changes. Deaths and other physical changes were recorded. At the end of experiment, blood samples were collected for subsequent hematological and biochemical analysis. The animals were sacrificed with anesthesia (Di-ethyl ether) and organs (lungs,spleen,heart,liver, brain, kidney) were dissected and collected in 10% formalin for further histopathological studies.

Subacute Toxicity Studies: -

Sub-acute toxicity study of the herbal formulation of Sphaeranthus indicus Linn was performed on mice as per OECD guideline 407 (OECD 2008). A total of 70 animals were divided into 12 groups for both herbal formulation of Sphaeranthus indicus Linn, each with 5 male and 5 female animals. For 28 days, Control group received vehicle, whereas Groups 1,2,3,4 received extract dose of 50mg/kg, Groups 5,6,7,8 received extract dose of 1000mg/kg, Groups 9,10,11,12 received extract does of 2000mg/kg of b.w ,respectively. Body weight, food and water intake, and the development of any side effects were all observed during the study. Weekly weight, food and water intake was compared. Blood samples were taken at the end of the study for hematological and biochemical analysis. After blood collection, animals were sacrificed using di-ethyl ether, and the target organs (liver, brain, kidney,lungs,spleen and heart) were dissected and collected in 10% formalin for histopathological studies.

Histopathological analysis

Organs that had been kept in formalin were imbedded in paraffin. A microtome cutter was used to slice embedded tissues with a thickness of 5 µm. The samples were stained with hematoxylin and eosin, and tissues section were examined under an inverted microscope at 400x to observe histological changes in the organs. Histopathological analysis was performed by Unique Biodiagnostic enterprises, Veterinary pathology laboratory.

Hematological and biochemical analysis

K2EDTA tubes were used to collect the blood for hematological analysis. Hb(g/dL), PCV (%), RBC count(mill/mm3), MCV(fL),MCH(pg),RDW(%), TLC (thou/mm3), segmented neutrophils (%),lymphocytes, monocytes, eosinophils, basophils, neutrophils (thou/mm3),lymphocytes(thou/mm3), monocytes(thou/mm3),eosinophils(thou/mm3),basophils(thou/mm3), platelets (thou/mm3) and mean platelet volume(fL) were estimated in hematological analysis by Unique Biodiagnostics enterprises, Veterinary Pathology Laboratory. Biochemical analysis was performed in blood samples in which albumin, urea, creatinine, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were analyzed by a Veterinary pathology laboratory.

Statistical analysis

Statistical tool one way ANOVA followed by Tukey's multiple comparisons test was performed. The values were significantly different when $p<0.05$. Results were expressed as Mean ± SEM (Standard error of the mean). Data analyses were performed by the Software GraphPad Prism 10.3.1

Results

Acute toxicity study

The body weight gain of treatment groups was comparable. During the 14-day study, there was no mortality, and no behavioral abnormalities were seen at dosages of 5,50,300,1000 mg/kg b.w. The body weight gain of the treatment groups was similar, but no statically significant difference was seen. Food and water intake were slightly lower in the treated groups than in the control groups, but no significant differences were identified in the individual groups. During the 14-day period following the treatment no deaths were recorded. The acute toxicity test indicated that a single dose of the herbal formulation (2000mg/kg) administrated orally did not produce morbidity or mortality in treated animals over a 14-day period.

Body weight gain, food and water consumption of mice treated orally with herbal formulation

During the entire study, there was no death recorded. There were no behavioral changes seen in either the treated or control groups. As shown in Table 1, the body weight of all groups of animals increased in the first and second weeks, but no significant changes observed. Food and water consumption did not alter considerably

Table 1

Body weight gain of mice treated orally with herbal formulation in acute toxicity study.

Herbal Formulation	Parameters	5mg/kg	50mg/kg	300mg/kg	1000mg/kg
	Groups	Group I	Group III	Group V	Group VII
Herbal	Initial weight(g)	16.2 ±1.62	23.4 ±0.91	35.3 ±2.54	26.2 ±2.15
Formulation	One week(g)	26.3 ±0.86	28.9 ±0.72	39.6 ±2.71	28.3 ±1.47
I	Two weeks(g)	27.3 ±0.89	28.2 ±0.79	38.6 ±2.69	28.1 ±1.45

	Groups	Group II	Group IV	Group VI	Group VIII
Herbal	Initial weight(g)	24.8 ±0.49	23.8 ±1.55	21.3 ±1.82	23.4 ±0.91
Formulation	One week(g)	28.6 ±0.77	22.2 ±1.53	29.1 ±1.85	28.9 ±0.72
II	Two weeks(g)	28.1 ±0.73	22.0 ±1.50	28.9 ±1.80	28.8 ±0.71

Values are expressed as mean ± SEM. Statistical tool one way ANOVA followed by Tukey's multiple comparisons test was performed. There was no significant change in body weight, food intake, and water intake in the treatment groups ($p < 0.05$).

Hematological parameters

All hematological parameters were estimated and found to be within normal limits, with no significant differences. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant.

Table 2

Haematological and Biochemical analysis of experimental mice treated orally with herbal formulation for 14 days

Observation:

Group	Hb gm	RBC x 10^6 /cmm	WB C X 10^3 /cmm	PLT X 10^5 cmm	PCV %	MCV fl	MCH pg	MCH C gm/dl	N %	E %	L %	M %
Group – I (3Mice)	0.4±0.416	5.55±0.34	7.43±0.39	515.67 ±64.19	26.37± 0.58	47.77± 1.11	15.13± 0.46	33.90± 1.40	64.67 ±4.84	1.00± 0.58	27± 2.61	0.33± 0.27
Group – II (3Mice)	9.77± 1.13	6.87±0.94	6.60±0.40	533.00 ±50.11	27.30± 0.58	47.23± 0.58	15.67± 0.72	35.60± 2.03	57.33 ±3.96	1.00± 0.58	44± 1.46	0.33± 0.27
Group – III (3Mice)	10.37 ±1.07	6.81±1.17	7.28±0.46	473.00 ±36.35	33.17± 0.87	46.27± 1.89	14.53± 0.61	32.33± 1.19	53.33 ±2.89	1.00± 0.58	18± 0.577	0.33± 0.27
Group – IV (3Mice)	10.73 ±1.10	7.37±0.54	8.00±0.84	511.33 ±11.82	32.67± 0.67	47.27± 0.49	15.33± 0.32	33.87± 1.54	60.67 ±2.03	1.00± 0.58	33.33±2.34	0.33± 0.27
Group – V (3Mice)	10.00 ±1.63	6.56±0.62	11.20± 0.47	378.33 ±27.47	38.30± 0.47	42.57± 1.49	16.67± 0.75	35.17± 1.75	54.33 ±5.80	0.33± 0.33	44± 1.25	0±0
Group – VI (3Mice)	11.63 ±1.49	6.17±1.23	10.63± 1.99	560.33 ±33.11	38.43± 0.70	46.87± 0.30	15.60± 0.78	32.60± 2.14	56.33 ±4.10	1.00± 0.58	32.33±1.77	0.33± 0.27
Group – VII (3Mice)	10.23 ±1.20	6.01±0.80	8.97±0.97	562.67 ±34.35	33.37± 0.78	47.90± 0.84	15.33± 0.67	32.37± 1.05	59.00 ±5.79	0.67± 0.67	23± 2.08	0.33± 0.27
Group – VIII (3Mice)	8.67± 0.32	6.17±1.03	7.51±0.42	493.33 ±15.87	23.10± 0.61	47.57± 0.70	15.17± 0.58	32.10± 0.81	47.00 ±5.58	0.33± 0.33	35.67±2.04	0.67± 0.66

Abbreviations: -

Hematology

Abbreviations	Parameter	Units	Abbreviations	Parameter	Units
HB	Hemoglobin	g /dl	MCHC	Mean Corpuscular Hemoglobin Concentration	g/dl
RBC	Red Blood Cell	Million/cmm	MCH	Mean Corpuscular Hemoglobin	Mg/dl
WBC	White Blood Cell	Thousand/cmm	H	Heterophile	%
PLT	Platelets	Lakhs/cmm	E	Eosinophils	%
PCV	Packed Cell Volume	%	L	Lymphocytes	%
MCV	Mean Corpuscular Volume	g/dl	M	Monocytes	%

One way ANOVA followed by Tukey's multiple comparison test was performed. The values were significantly different when $p < 0.05$.

Serum biochemical analysis

The results of biochemical analysis of all treatment groups (5 mg/Kg,50mg/kg 300 mg/Kg, and 1000 mg/Kg) were no significant difference detected ($p < 0.05$). The data were expressed using the mean ± SEM ($p < 0.05$).

Alterations in serum concentration of creatinine were observed. A slightly decrease difference was observed in the levels of serum total cholesterol and triglyceride level.

The results of biochemical analysis of all treatment groups

Groups	AST IU/L	ALT IU/L	ALP IU/L	BUN mg/dl	CHOL mg/dl
Group – I (3 mice)	34.33±6.61	37.67±3.51	43.67±3.50	12.00±0.96	47.00±5.36
Group – II (3 mice)	40±3.46	47.67±4.34	52.00±4.49	17.20±0.54	51.67±3.95
Group – III (3 mice)	47.67±5.72	34±2.19	42.67±6.80	13.73±0.56	45.67±4.56
Group – IV (3 mice)	45±4.30	37.00±5.47	45.00±4.71	13.20±0.87	59.67±2.67
Group – V (3 mice)	138.33±5.04	56.67±5.74	40.67±8.69	9.47±0.90	37.00±5.47
Group – VI (3 mice)	45.67±6.09	77.00±5.70	35.33±4.79	27.77±1.11	97.33±5.13
Group – VII (3 mice)	59±3.21	61.37±0.22	151.33±13.17	22.03±0.64	81.33±7.44
Group -VIII (3 mice)	35.67±4.45	52.33±3.44	15.67±1.82	12.50±1.10	55.00±4.25

Values are expressed as mean ± SEM. Statistical tool one way ANOVA followed by Tukey's multiple comparisons test was performed. The values were considered significantly different when p value <0.05. There was no significant change in the parameters during the treatment.

Abbreviations: -

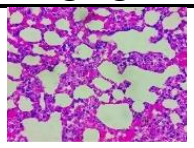
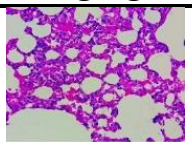
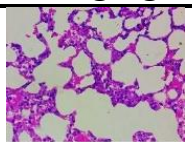
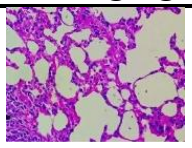
Serum Biochemistry

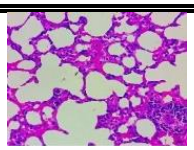
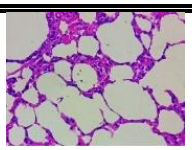
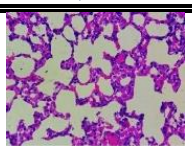
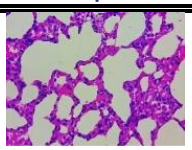
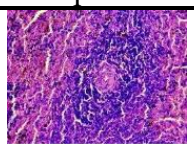
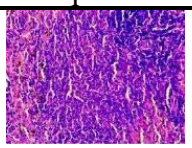
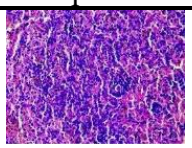
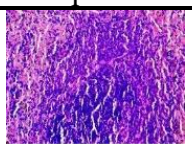
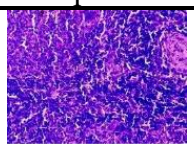
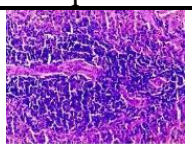
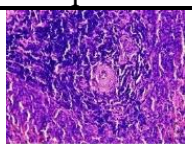
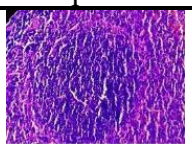
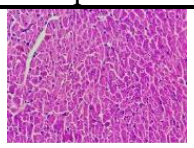
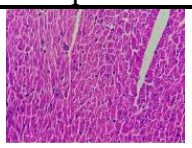
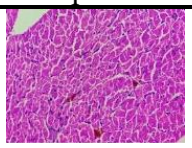
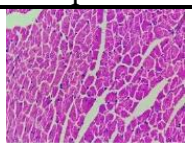
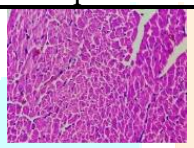
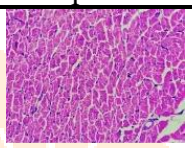
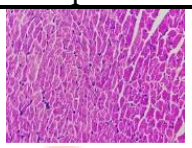
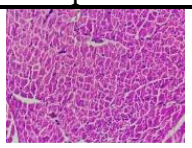
Abbreviations	Parameter	Units
Bili	Serum Bilirubin	Mg/dl
SGOT	Serum Glutamic Oxaloacetic Transaminase (AST = Aspartate Amino Transferase)	IU / L
SGPT	Serum Glutamic Pyruvic Transaminase / (ALP = Alanine Amino Transferase)	IU / L
ALP	Serum Alkaline Phosphatase	IU / L
PRO	Serum Total Proteins	g/dl
ALB	Serum Total Albumin	g/dl
BUN	Blood Urea Nitrogen	Mg/dl
CREAT	Serum Creatinine	Mg/dl

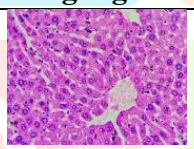
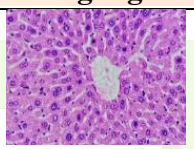
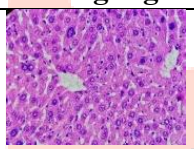
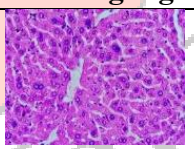
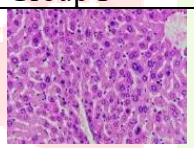
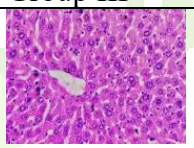
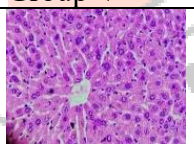
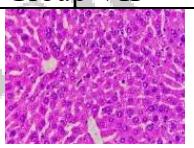
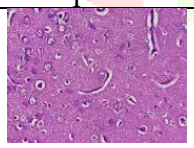
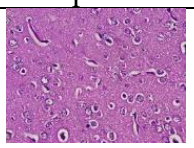
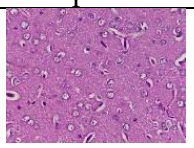
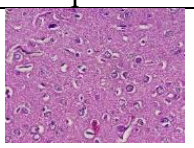
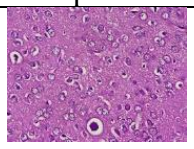
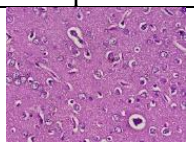
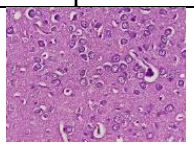
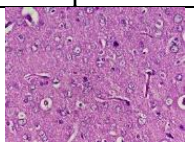
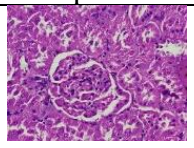
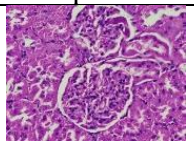
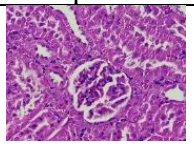
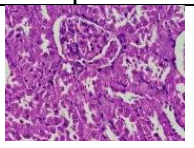
Histological analysis

The liver,kidney,brain,lungs,spleen and heart were sectioned for histological study to observe the changes in organs at the dosages of 5mg,50 mg, 300 mg, and 1000 mg/kg. Observation by the microscope showed no remarkable histological changes. Apoptosis was not seen in the liver images. The kidney glomeruli and tubules were normal in histology. Brain showed normal architecture.

H.F. I-Herbal Formulation I **H.F.II**-Herbal Formulation II

Organ		**Groups**			
		5 Mg/Kg	**50 Mg/Kg**	**300 Mg/Kg**	**1000 Mg/Kg**
Lungs	**H.F I**	 Group I	 Group III	 Group V	 Group VII

	H.F II	 Group II	 Group IV	 Group VI	 Group VIII
Spleen	H.F I	 Group I	 Group III	 Group V	 Group VII
	H.F II	 Group II	 Group IV	 Group VI	 Group VIII
Heart	H.F I	 Group I	 Group III	 Group V	 Group VII
	H.F II	 Group II	 Group IV	 Group VI	 Group VIII

Organ		Groups			
		5 Mg/Kg	50 Mg/Kg	300 Mg/Kg	1000 Mg/Kg
Liver	H.F I	 Group I	 Group III	 Group V	 Group VII
	H.F II	 Group II	 Group IV	 Group VI	 Group VIII
Brain	H.F I	 Group I	 Group III	 Group V	 Group VII
	H.F II	 Group II	 Group IV	 Group VI	 Group VIII
Kidney	H.F I	 Group I	 Group III	 Group V	 Group VII
	H.F II	Group II	Group IV	Group VI	Group VIII

Histopathological images of organs (Lungs, Spleen, Heart, Liver, Brain, Kidney) in mice after oral administration of herbal formulation of Sphaeranthus indicus linn for 14 days at the doses of 5 mg, 50mg, 1000 mg/Kg ,2000mg/kg Bw. Apoptosis was seen in the liver images at higher doses. The Lungs, Spleen, Heart, kidney glomeruli and tubules were normal in histology. Brain showed normal architecture.

Subacute toxicity study

Mortality and behavioral observations

In the 28-day subacute toxicity study, there was no behavioral change, and no mortality was recorded at the doses of 50, 1000, and 2000 mg/Kg b.w. of herbal formulation. There were no adverse events which was recorded in any of the treated groups.

Body weight gain of mice treated orally with herbal formulation for 28 days

The body weight of animals in all groups was increased in the first and second weeks, but no significant change was recorded among all the groups. Food consumption and water intake were similar in the control and treated groups without any significant change.

Herbal Formulation	Parameters	M/F	Control	50mg/kg	1000mg/kg	2000mg/kg
	Groups		Control	Group I/II	Group V/VI	Group IX/X
	Initial weight(g)	M	26.8 ±1.58	24.3 ±1.523	24.8 ±1.62	26.9 ±0.98
		F	23.3 ±2.10	25.2 ±0.96	22.4 ±0.71	24.7 ±1.88
Herbal	One week(g)	M	28.9 ±1.94	25.6 ±1.69	26.1 ±0.48	29.2 ±0.80
		F	26.2 ±1.48	29.4 ±1.27	26.2 ±1.24	26.6 ±1.63
Formulation I	Two weeks(g)	M	28.7 ±1.93	25.7 ±1.68	27.6 ±0.35	30.3 ±0.95
		F	29.9 ±1.39	29.8 ±1.29	26.9 ±1.27	27.7 ±1.61
	Three weeks(g)	M	28.3 ±1.89	26.1 ±1.69	27.2 ±0.32	29.1 ±0.75
		F	29.4 ±1.32	29.3 ±1.21	25.5 ±1.69	26.6 ±0.95
	Final weight(g)	M	29.1 ±1.97	25.3 ±1.64	25.7 ±1.61	29.5 ±0.75
		F	29.5 ±1.31	29.1 ±1.19	25.3 ±1.65	26.3 ±1.54

Values are expressed as mean ± SEM. Statistical tool one way ANOVA followed by Tukey's multiple comparisons test was performed. There was no significant change in body weight, food intake, and water intake in the treatment groups ($p < 0.05$).

Herbal Formulation	Groups	M/F	Control	Group III/IV	Group VII/VIII	Group XI/XII
	Initial weight(g)	M	26.8 ±1.58	23.8 ±1.56	21.7 ±1.31	29.9 ±2.03
		F	23.3 ±2.10	19.1 ±1.048	25.9 ±0.94	27.2 ±0.63
Herbal	One week(g)	M	28.9 ±1.94	32.2 ±0.914	23.9 ±1.47	33.2 ±0.87
		F	26.2 ±1.48	23.5 ±2.21	27.9 ±0.80	27.3 ±1.69
Formulation II	Two weeks(g)	M	28.7 ±1.93	32.8 ±0.91	24.5 ±1.69	31.3 ±0.83
		F	29.9 ±1.39	25.5 ±2.20	27.6 ±0.79	27.8 ±1.61
	Three weeks(g)	M	28.3 ±1.89	31.1 ±0.92	22..9 ±1.43	32.1 ±0.75
		F	29.4 ±1.32	24.5 ±1.59	26.7 ±0.56	27.1 ±0.56
	Final weight(g)	M	29.1 ±1.97	30.5 ±0.81	24.8 ±1.65	29.8 ±2.01
		F	29.5 ±1.31	24.8 ±1.89	25.8 ±0.93	26.3 ±1.62

Values are expressed as mean ± SEM. Statistical tool one way ANOVA followed by Tukey's multiple comparisons test was performed. There was no significant change in body weight, food intake, and water intake in the treatment groups ($p < 0.05$).

Hematological parameters

All hematological parameters were estimated and found to be within normal limits, with no significant differences between the control and treated groups ($p < 0.05$). Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant

Observations: -

SUBACUTE TOXICITY STUDY-HEMATOLOGICAL

Groups	Sex	Dose	Herbal Formulation	Hb gm	RBC x 10^6 cmm	WBC X 10^3 cmm	PLT X 10^5 cmm	PCV %	MCV fl	MCH pg	MCHC gm/dl	N %	E %	L %	M %
Group I 5 Mice	M	50 mg/kg	H.F I	8.04 ± 0.51	5.35 ± 0.48	7.16 ± 0.50	507.0 ± 27.60	22.44 ± 0.72	41.24 ± 1.36	18.18 ± 0.86	36.02 ± 1.23	54.6 ± 1.50	0.8 ± 0.37	45.2 ± 3.17	0.4±0.245
Group II 5 Mice	F	50 mg/kg	H.F I	8.60 ± 0.72	5.81 ± 0.45	8.00 ± 0.43	412.8 ± 42.29	23.00 ± 0.44	45.18 ± 1.50	16.62 ± 0.45	33.08 ± 0.84	55.2 ± 3.69	0.8 ± 0.37	42.8 ± 3.72	0.4±0.245
Group III 5 Mice	M	50 mg/kg	H.F II	8.24 ± 0.41	4.63 ± 0.37	4.62 ± 0.51	522.0 ± 41.82	23.98 ± 0.91	44.08 ± 0.82	13.96 ± 0.66	32.94 ± 1.02	44.8 ± 2.97	0.4 ± 0.24	49.8 ± 2.60	0.2±0.200
Group IV 5 Mice	F	50 mg/kg	H.F II	9.38 ± 0.26	6.74 ± 0.34	7.23 ± 0.78	427.6 ± 49.15	29.10 ± 0.81	42.76 ± 0.42	14.22 ± 0.60	32.66 ± 0.21	50.4 ± 3.47	0.4 ± 0.24	35.0 ± 2.02	0.4±0.245
Group V 5 Mice	M	1000 mg/kg	H.F I	8.10 ± 0.62	6.95 ± 0.62	8.14 ± 0.22	539.2 ± 38.30	33.66 ± 1.08	52.76 ± 0.80	16.90 ± 0.95	34.56 ± 0.52	81.8 ± 3.29	0.4 ± 0.24	25.0 ± 1.95	0.4±0.245
Group VI 5 Mice	F	1000 mg/kg	H.F I	8.76 ± 0.39	6.87 ± 0.62	5.34 ± 0.99	692.8 ± 81.03	26.94 ± 1.64	44.44 ± 0.89	16.70 ± 0.96	38.90 ± 1.90	49.6 ± 4.23	0.8 ± 0.37	44.6 ± 6.41	0.4±0.245
Group VII 5 Mice	M	1000 mg/kg	H.F II	7.36 ± 0.44	5.39 ± 0.80	6.00 ± 0.36	447.0 ± 46.10	23.42 ± 0.65	42.88 ± 2.23	18.30 ± 0.55	42.70 ± 2.69	49.2 ± 4.42	0.8 ± 0.37	49.2 ± 6.56	0.2±0.200
Group VIII 5 Mice	F	1000 mg/kg	H.F II	8.52 ± 0.56	7.59 ± 0.72	6.08 ± 0.75	361.8 ± 37.59	28.10 ± 2.08	46.32 ± 0.79	15.94 ± 0.97	35.32 ± 0.86	47.4 ± 2.20	0.6 ± 0.40	48.6 ± 3.06	0.2±0.200
Group IX 5 Mice	M	2000 mg/kg	H.F I	7.06 ± 0.85	4.79 ± 0.31	5.86 ± 0.66	491.2 ± 61.76	18.32 ± 0.69	44.50 ± 0.87	15.90 ± 0.69	35.02 ± 1.07	52.2 ± 3.61	0.6 ± 0.40	45.0 ± 6.40	0.2±0.200
Group X 5 Mice	F	2000 mg/kg	H.F I	8.26 ± 0.65	7.55 ± 0.48	7.00 ± 1.06	486.4 ± 20.20	15.55 ± 6.17	52.24 ± 2.11	17.74 ± 0.61	41.28 ± 4.49	67.2 ± 8.53	0.4 ± 0.24	50.4 ± 4.93	0.4±0.400
Group XI 5 Mice	M	2000 mg/kg	H.F II	9.10 ± 0.63	6.65 ± 0.37	13.58 ± 0.97	421.8 ± 44.07	33.86 ± 0.62	46.60 ± 0.64	17.20 ± 1.46	36.40 ± 0.80	57.6 ± 2.66	0.2 ± 0.20	50.8 ± 4.49	0.2±0.200

Group XII 5 Mice	F	2000 mg/kg	H.F II	8.08 ± 0.71	5.78 ± 0.83	6.14 ± 0.71	569.0 ± 51.65	27.96 ± 2.90	45.86 ± 5.24	17.42 ± 0.84	45.14 ± 3.90	54.0 ± 5.79	0.4 ± 0.40	47.6 ± 12.93	0±00

Data are expressed as mean ± SEM. One way ANOVA followed by Tukey's multiple comparison test was performed. The values were significantly different when $p < 0.05$.

Hematology

Abbreviations	Parameter	Units	Abbreviations	Parameter	Units
HB	Hemoglobin	g /dl	MCHC	Mean Corpuscular Hemoglobin Concentration	g/dl
RBC	Red Blood Cell	Million/cmm	MCH	Mean Corpuscular Hemoglobin	Mg/dl
WBC	White Blood Cell	Thousand/cmm	H	Heterophile	%
PLT	Platelets	Lakhs/cmm	E	Eosinophils	%
PCV	Packed Cell Volume	%	L	Lymphocytes	%
MCV	Mean Corpuscular Volume	g/dl	M	Monocytes	%

One way ANOVA followed by Tukey's multiple comparison test was performed. The values were significantly different when $p < 0.05$.

Serum biochemical analysis

The results of biochemical analysis of all treatment groups (50 mg/Kg, 1000 mg/Kg, and 2000 mg/Kg) where no significant difference was detected ($p < 0.05$). The data were expressed using the mean ± SEM ($p < 0.05$). Statistical tool one way ANOVA followed by Tukey's multiple comparisons test was performed. The values were considered significantly different when p value < 0.05. There was no significant change in the parameters during the treatment.

SUBACUTE TOXICITY STUDY- BIOCHEMICAL ANALYSIS

Groups	**AST IU/L**	**ALT IU/L**	**ALP IU/L**	**BUN mg/dl**
Group I (5 Mice) 50 mg/kg	219.6 ± 34.3	174.6± 21.44	92.6 ± 3.23	12.78 ± 0.55
Group II (5 Mice) 50 mg/kg	304.4 ± 37.5	147.8± 37.64	174.2 ± 35.34	14.26 ± 0.64
Group III (5 Mice) 50 mg/kg	353.4 ± 44.1	714.8± 76.44	161.2 ± 8.00	13.00 ± 1.11
Group IV (5 Mice) 50 mg/kg	276.4 ± 26.7	297.4 ± 37.8	117.6 ± 26.23	12.16 ± 0.60
Group V (5 Mice) 1000 mg/kg	213.6 ± 27.2	362 ± 31.75	163.2 ± 10.34	12.00 ± 0.64
Group VI (5 Mice) 1000 mg/kg	370 ± 50.9	175.6± 36.54	197.2 ± 13.84	12.34 ± 0.69
Group VII (5 Mice) 1000 mg/kg	395.2 ± 37.5	310.8± 33.51	170.2 ± 58.00	20.16 ± 1.47
Group VIII (5 Mice) 1000 mg/kg	367.8 ± 37.9	110.4± 11.40	173.4 ± 31.73	14.50 ± 0.53
Group IX (5 Mice) 2000 mg/kg	296.2 ± 61.8	305.6± 77.43	334.8 ± 58.09	14.86 ± 0.71
Group X (5 Mice) 2000 mg/kg	244.4 ± 44.0	148.4± 54.89	327.6 ± 39.18	15.16 ± 0.62
Group XI (5 Mice) 2000 mg/kg	418.4 ± 41.9	255 ± 59.14	208.2 ± 26.56	17.26 ± 1.18
Group XII (5 Mice) 2000 mg/kg	237.4 ± 63.1	64 ± 19.85	76.2 ± 8.00	16.92 ± 2.06

Data are expressed as mean± SEM. Statistical tool one way ANOVA followed by Tukey's multiple

comparisons test was performed. The values were considered significantly different when $p < 0.05$. There was no significant change was found in biochemical parameters.

Serum Biochemistry: -Values are expressed as mean ± SEM. Statistical tool one way ANOVA followed by Tukey's multiple comparisons test was performed. The values were considered significantly different when p value <0.05. There was no significant change in the parameters during the treatment.

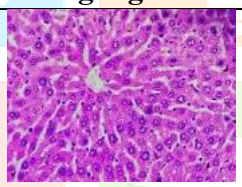
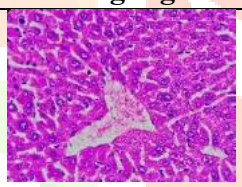
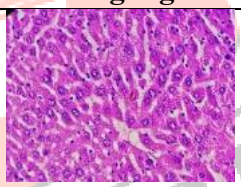
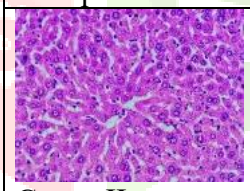
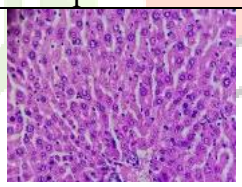
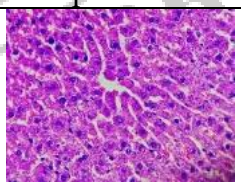
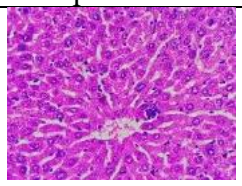
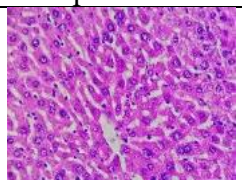
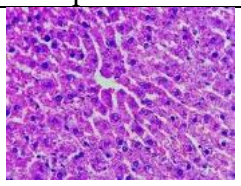
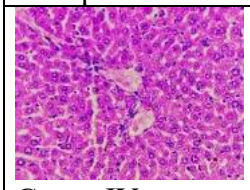
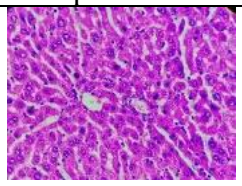
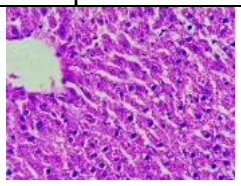
Abbreviations	**Parameter**	**Units**
Bili	Serum Bilirubin	Mg/dl
SGOT	Serum Glutamic Oxaloacetic Transaminase (AST = Aspartate Amino Transferase)	IU / L
SGPT	Serum Glutamic Pyruvic Transaminase / (ALP = Alanine Amino Transferase)	IU / L
ALP	Serum Alkaline Phosphatase	IU / L
PRO	Serum Total Proteins	g/dl
ALB	Serum Total Albumin	g/dl
BUN	Blood Urea Nitrogen	Mg/dl
CREAT	Serum Creatinine	Mg/dl

Subacute toxicity study

Histological analysis

The liver, kidney, brain, lungs, spleen and heart were sectioned for histological study to observe the changes in organs at the dosages of 50 mg, 1000 mg, and 2000 mg/kg. Observation by the microscope showed no remarkable histological changes. Apoptosis was not seen in the liver images.

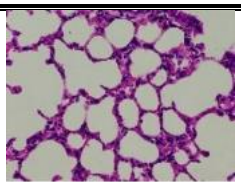
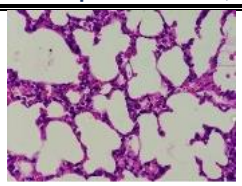
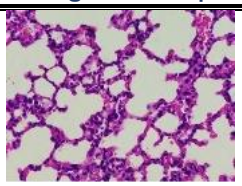
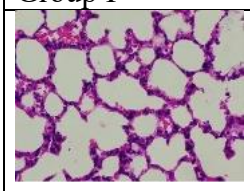
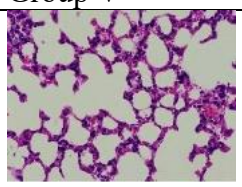
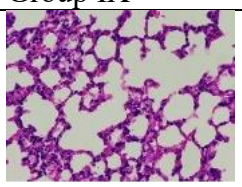
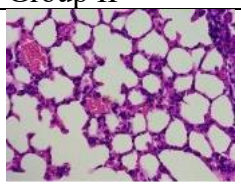
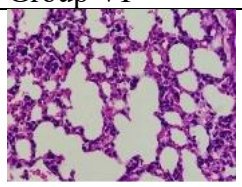
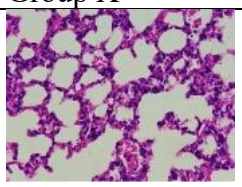
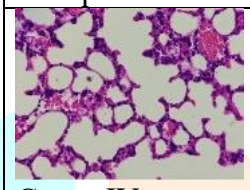
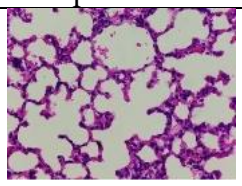
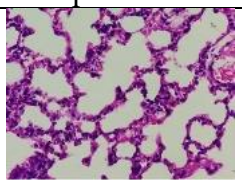
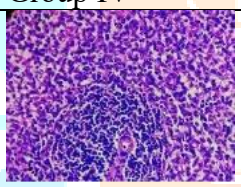
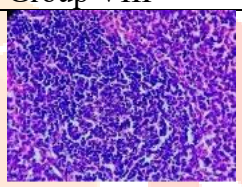
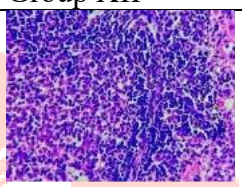
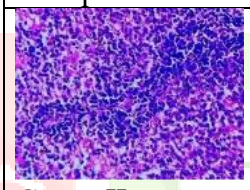
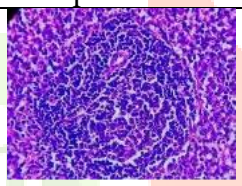
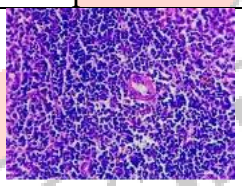
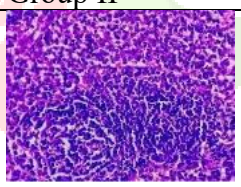
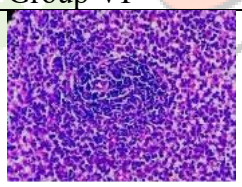
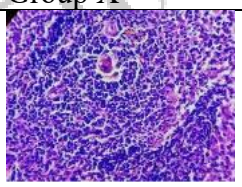
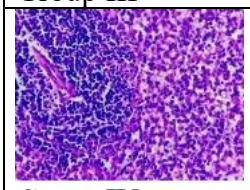
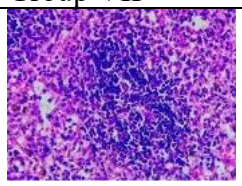
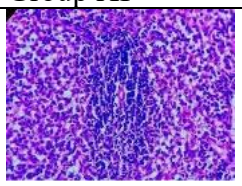
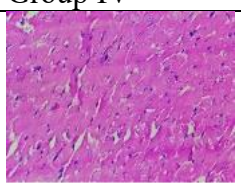
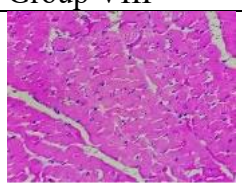
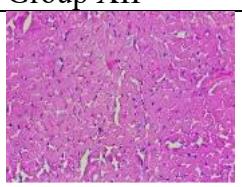
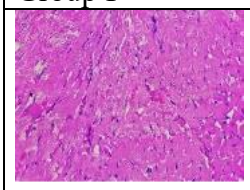
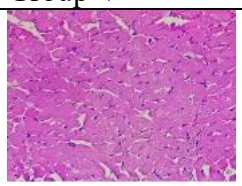
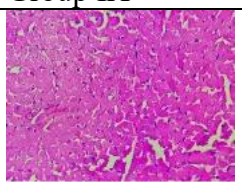
H.F. I-Herbal Formulation I **H.F.II**-Herbal Formulation II

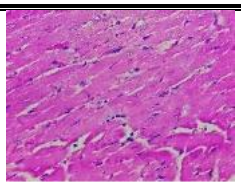
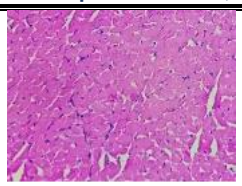
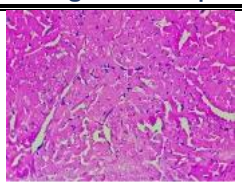
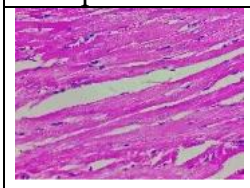
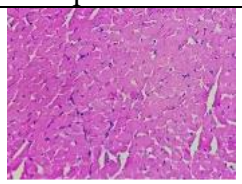
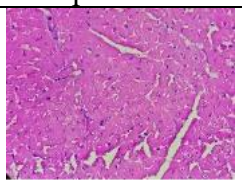
Organ		**Groups**		
		50 Mg/Kg	**1000 Mg/Kg**	**2000 Mg/Kg**
Liver	**H.F I**	 Group I	 Group V	 Group IX
		 Group II	 Group VI	 Group X
	H.F II	 Group III	 Group VII	 Group XI
		 Group IV	 Group VIII	 Group XII

Subacute Toxicity Study

H.F. I-Herbal Formulation I **H.F.II**-Herbal Formulation II

Organ		**Groups**		
		50 Mg/Kg	**1000 Mg/Kg**	**2000 Mg/Kg**

Lungs	**H.F I**	 Group I	 Group V	 Group IX
		 Group II	 Group VI	 Group X
	H.F II	 Group III	 Group VII	 Group XI
		 Group IV	 Group VIII	 Group XII
Spleen	**H.F I**	 Group I	 Group V	 Group IX
		 Group II	 Group VI	 Group X
	H.F II	 Group III	 Group VII	 Group XI
		 Group IV	 Group VIII	 Group XII
Heart	**H.F I**	 Group I	 Group V	 Group IX
		 Group II	 Group VI	 Group X

	H.F II	 Group III	 Group VII	 Group XI
		 Group IV	 Group VIII	 Group XII

DISCUSSION

Herbal medicines are used throughout developing countries and play a key role in the management of various chronic diseases and in recent times have received a great preference by researchers as alternative source to allopathic pharmaceutical drugs. Sub-acute toxicity is a repeat-dose study performed to expose any deleterious changes in body weight, haematological and biochemical indices that may arise during repeated administration of a test substance. In acute toxicity study, mice treated at graded doses did not show any signs of adverse reactions and no changes in animals' behaviours during monitoring.[11]

In the sub-acute toxicity study, the observed no significant difference in the body weights at doses 50 mg/kg ,1000mg/kg ,2000mg/kg body weight in mice during 28-day oral administration of herbal formulations may indicate that the animals were having healthy growth based on their food intake as well as the plant extract. In the subacute toxicity study, the mean body weight and the percentage body weight gain of mice in both treated were like those of the control group at the end of the experiment. The haematological analysis is very important because hematopoietic system, being one of the most sensitive targets of toxic chemicals, is an important index of physiological and pathological status of animals and human. In this study, the test group of animals did not show any significant deviation in the haematological parameters. The prolong administration of herbal formulation appeared to have beneficial effects on their hematopoietic system. Blood is the main medium of transport for many nutrients and foreign bodies in the body. Due to this reason, components of the blood such as red blood cells, white blood counts, platelets and haemoglobin are first exposed to significant concentrations of toxic compounds. Damage to the blood cells has an adverse effect on the normal functioning of the body, since the administration of extract did not cause any significant change in the haematological parameters, therefore the herbal formulation can be suggested non-toxic. Transaminases such as Aspartate and alanine aminotransferase are distinguished indicator of liver function and used as biomarkers. ALP is most often measured to indicate bile duct obstruction. Liver parameters such as, AST, ALT were within normal range as like control group suggesting that sub-acute administration of extract did not cause deleterious effect on liver functions. The elevated levels of these enzymes are clinically measurable indications of potential risks to normal liver function. In addition, observed a significant protective effect by lowering the serum AST, ALT and ALP, might be an indication that herbal formulation may have some hepatoprotective properties. Creatinine is most often measured for the indication of renal function and any rise in the levels of these parameters indicates a marked renal damage.[11]

CONCLUSION:

Acute and subacute toxicity was performed on mice orally. Oral administration of single dose of the extract 2000 mg/Kg did not cause mortality. 28-day treatment did not show mortality at the dose of 50, 1000, 2000 mg/Kg. Biochemical and haematological parameters were within the normal range and there was no significant difference recorded. Histopathology of liver, kidney, brain, lungs, spleen and heart revealed that no major toxicity was found. Therefore, it is concluded that acute and subacute treatment by herbal formulation of Sphaeranthus indicus linn did not cause significant toxicity.

In conclusion, the present study provides valuable data on the acute and subacute toxicity profile of the herbal formulation extracts of Sphaeranthus Indicus Linn in Swiss albino mice. The present investigation

demonstrated that the extract at level up to 2000 mg/kg body weight has no harmful effects and considered as non-toxic and safe.[11]

REFERENCES

1. OECD (2002), *Test No. 423: Acute Oral toxicity - Acute Toxic Class Method*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264071001-en
2. OECD (2008), *Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris,https://doi.org/10.1787/9789264070684-en.
3. Bose, S., Datta, R., Kirlin, W.G. **(2021)**. Toxicity Studies Related to Medicinal Plants. In: Mandal, S.C., Chakraborty, R., Sen, S. (eds) Evidence Based Validation of Traditional Medicines. Springer, Singapore.
4. Srivastava, S., Misra, A. (**2018**). Quality Control of Herbal Drugs: Advancements and Challenges. In: Singh, B., Peter, K. (eds) New Age Herbals. Springer, Singapore.
5. NG. Mahajan, MZ. Chopda, RT. Mahajan "A Review on Sphaeranthus indicus Linn: Multipotential Medicinal Plant" Int. J. of Pharm. Res. & All. Sci. **2015**;4(3):48-74
6. Jain A, Basal E. Inhibition of Propionibacterium acnes-induced mediators of inflammation by Indian herbs. Phytomedicine. **2003** Jan;10(1):34-8. doi: 10.1078/094471103321648638. PMID: 12622461.
7. K P, Dintu & Ks, Vinayaka & Basumatary, Maofungsar & Das, Kadambini & Hamdy, Rim & Mishra, Sweta & Kumar, Sanjeet & Sharma, Bhagwati. **(2024)**. Sphaeranthus indicus L.: a medicinal herb of rural and tribal India. 10.5281/zenodo.11465075.
8. Shirode, Devendra & Shevkar, Pranav & Ram, Bindurani. **(2021).** Sphaeranthus indicus Linn.: A Pharmacological Review. Asian Pacific Journal of Health Sciences. 8. 32-35. 10.21276/apjhs.2021.8.4.04.
9. Ramachandran S. Review on Sphaeranthus indicus Linn. (Koṭṭaikkarantai). Pharmacogn Rev. **2013** Jul;7(14):157-69. doi: 10.4103/0973-7847.120517. PMID: 24347924; PMCID: PMC3841994.
10. Emani, Lakshma Reddy, Suryachandra Rao Ravada, Machi Raju Garaga, Bharani Meka, and Trimurtulu Golakoti. **2017.** "Four New Sesquiterpenoids from *Sphaeranthus Indicus*." *Natural Product Research* 31 (21): 2497–2504. doi:10.1080/14786419.2017.1315576.
11. Gajendra Pratap Choudhary, Ashutosh Pal Jain. Evaluation of Acute, Subacute and LD50 values of Methanolic extract of Sphaeranthus indicus leaves in Albino mice. Research Journal of Pharmacy and Technology. **2021**; 14(5):2487-2.
12. Singh S, Semwal BC, Kr Upadhaya P. Pharmacognostic study of Sphaeranthus indicus Linn.: A Review. Pharmacog J. **2019**;11(6):1376-85.
13. M. Anitha, G. Deepika, K. Sandhiya, G. Sneha, M. Soniya, A Review of Phytopharmacological Aspects of Sphaeranthus Indicus Linn, Int. J. of Pharm. Sci., **2024**, Vol 2, Issue 9, 1341-1357.
14. Selvamoorthy, H.; Elumalai, K.; Madhavan, M. K.; Veerapathiran, V.; Yuvan Sankar, S. B.; Ranganathan, S. A Review of Phytopharmacology and Formulation of Sphaeranthus Indicus. *World Journal of Current Med and Pharm Research* **2024**, *6*, 34-50.
15. Dr. Saraswathidevi HN; Dr. Mahesh CD; Dr. Seema Pradeep. A Classical Review on Mundi (Sphaeranthus Indicus Linn.). *J Ayurveda Integr Med Sci* **2019**, *4*, 300-310.
16. Joshi S, Megha, Bhide BV, Ghildiyal S, Nesari TM. Physico-chemical and Phytochemical Analysis of Sphaeranthus indicus Linn. (Whole Plant). Pharmacog Res. **2023**;15(3):492-6.
17. Ramachandran S. Review on Sphaeranthus indicus Linn. (Kot.t.aikkarantai). Phcog Rev **2013**; 7:157-69.
18. Garg, M.; Dwivedi, N. Physicochemical and Phytochemical Studies on Sphaeranthus Indicus Linn. With HPTLC Fingerprinting. *J. Drug Delivery Ther.* **2021**, *11* (2), 100-107.
19. Dr. Seema Pradeep,Dr.Mahesh CD, Dr. Saraswathidevi HN. A Classical Review on Mundi (Sphaeranthus Indicus Linn.). J Ayurveda Integr Med Sci **2019**; 4:300-310.
20. Sharma A and Sisodia S: Pharmacognostical evaluation and standardization of aerial parts of Sphaeranthus indicus Linn. (Asteraceae). Int J Pharmacognosy **2019**; 7(4): 116-20.
21. Nestmann ER, Alluri VK, Dodda S, Davis BA. Toxicological studies on the botanical supplement LI12542F6 containing extracts of *Sphaeranthus indicus* flower heads and *Mangifera indica* (mango tree) bark. Food Sci Nutr. **2019** Jan 29;7(2):817-833.
22. Chellappandian M, Thanigaivel A, Vasantha-Srinivasan P, Edwin ES, Ponsankar A, Selin-Rani S, Kalaivani K, Senthil-Nathan S, Benelli G. Toxicological effects of Sphaeranthus indicus Linn. (Asteraceae) leaf essential oil against human disease vectors, Culex quinquefasciatus Say and Aedes aegypti Linn., and impacts on a beneficial mosquito predator. Environ Sci Pollut Res Int. **2018** Apr;25(11):10294-10306.
23. Emani LR, Ravada SR, Garaga MR, Meka B, Golakoti T. Four new Sesquiterpenoids from Sphaeranthus indicus. Nat Prod Res. **2017** Nov;31(21):2497-2504.
24. Galani VJ, Patel BG, Rana DG. Sphaeranthus indicus Linn.: A phytopharmacological review. Int J Ayurveda Res. **2010** Oct;1(4):247-53.

25. Saiyed ZM, Sengupta K, Krishnaraju AV, Trimurtulu G, Lau FC, Lugo JP. Safety and toxicological evaluation of Meratrim®: an herbal formulation for weight management. Food Chem Toxicol. **2015** Apr; 78:122-9.
26. Mathew JE, Joseph A, Srinivasan K, Dinakaran SV, Mantri A, Movaliya V. Effect of ethanol extract of Sphaeranthus indicus on cisplatin-induced nephrotoxicity in rats. Nat Prod Res. **2012**;26(10):933-8.
27. Mishra BB, Yadav SB, Singh RK, Tripathi V. A novel flavonoid C-glycoside from Sphaeranthus indicus L. (family Compositae). Molecules. **2007** Oct 20;12(10):2288-91
28. Akshata M. Girase, Bhupendra M. Mahale, Sulbha G. Patil, Sunila A. Patil. The Study on Sphaeranthus Indicus. Research & Reviews: A Journal of Pharmacognosy. **2024**;11(1): 37–43.
29. Sundaresan, P. K.; Kesavan, K. P. In Vitro Evaluation of Antibacterial Activity in Ethanolic Extract of Whole Plant Sphaeranthus Indicus Linn. *Int J Basic Clin Pharmacol* **2020**, *9*, 1730-1734.
30. Pawar Harshal A & Therani Deepika (**2012**), A comprehensive review on Sphaeranthus indicus Linn., Global J Res. Med. Plants & Indigen. Med., Volume 1(9), 404–410
31. Sundaresan, P. K.; Prabhakaran, S. S.; Palappallil, D. S.; Chellappan, D. Diuretic Activity of Ethanolic Extract of Whole Plant of Sphaeranthus Indicus Linn in Albino Rats. *Int J Basic Clin Pharmacol* **2017**, *6*, 265-270.
32. Roop Ganguly et al. Evaluation of efficacy of herbal preparation in the management of Oral Submucous Fibrosis: A Study. Int. J. Res. Ayurveda Pharm**. 2020**;11(4):152-160
33. Sangale PP; Devikar SD; Naikawadi VB; Ghawate AN. IN VITRO REGENERATION OF SPHAERANTHUS GOMPHRENOIDES: A ETHNOMEDICINAL HERB SPECIES. *Ierj* **2023**, *9*.
34. Tandon, D., Gupta, A.K. Bioautography, synergistic effect and HPTLC-MS and SEM analysis of antimicrobial and antioxidant compounds of inflorescence extract of *Sphaeranthus indicus*. *Futur J Pharm Sci* **9**, 72 **(2023).**
35. Ajoy Bhakat & Sumana Saha: A Critical Review of Mundi (Sphaeranthus Indicus Linn). International Ayurvedic Medical Journal {online} **2018** {cited June 2018}
36. Chauhan MS, Yadav S, Qualitative and Quantitative Determination of Secondary Metabolites of *Sphaeranthus indicus* and *Spathodea campanulata* Flowers Extracts**,** Journal of Drug Delivery and Therapeutics. **2023**; 13(4):85-89
37. Ghildiyal S, Kumar V, Bidhuri Y, Nesari TM, Sircar D. Micromorphology, Physiognomies and Bio-Element Analysis of Launaea nudicaulis (L.) Hook. f. Pharmacog Res. **2024**;16(2):414-22.
38. Sharwan G, Jain P, Pandey R, Shukla SS. Toxicity profile of traditional herbal medicine. J Ayu Herb Med **2015;**1(3):81-90.
39. Acute and subacute toxicity study of ethanolic extract of Calotropis procera (Aiton) Dryand flower in Swiss albino mice, Kumar A., Kumar B., Kumar R., Kumar A., Singh M., Tiwari V.Trigunayat A. Singh P. (2022) Phytomedicine *Plus, 2 (*2), art. no. 100224