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TOXICITY ASSESSMENT OF ETHYL ACETATE EXTRACTS FROM AERIAL PARTS OF *Ipomoea horsfolliae* HOOK IN RODENTS

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ABSTRACT

Various species of the genus *Ipomoea* are mostly used in traditional medicine. *Ipomoea horsfolliae* Hook were selected because there is nosystematic toxicity studies for this plant were described. So the present study was undertaken to assess acute and sub-acute toxicity of this plant in mice and rats. The acute oral toxicity study were carried out as per the OECD guidelines 425 in mice and the sub-acute toxicity were carried out at as per OECD guidelines 407 in male and female rats. Mice administered up to 2000 mg/kg as a single dose orally not caused any signs of toxicity or mortality in mice. In sub-acute toxicity study in rats, ethyl acetate extract of *Ipomoea horsfolliae* Hook (EAIH) at three different daily doses of 150, 300 and 600 mg/kg for 28 days did not cause any significant change including the hematological and biochemical parameters. Histopathological examinations showed normal architecture suggesting no morphological disturbances. No deaths or any signs of toxicity was observed after oral administration in acute and toxicity studies according to OECD guidelines concluded that EAIH can use for *in vivo* biological activity studies in laboratory animals to explore its various medicinal activity prior to study in human subjects.

Keywords: *Ipomoea horsfolliae* Hook; mice; acute toxicity; sub-acute toxicity; rats

INTRODUCTION

In the most recent decade, the medicinal plants characterize a significant and rich source of novel synthetic drugs **[1]**. Massive number of the world's residents depends medicinal plants as a substitute and complimentary remedies for various illnesses. Lacks of experimental reports on their safety make it essential to perform toxicological analysis **[2]**. The beneficial uses of herbal preparations have been limited because of shortage of healthy-clear chemical classification, dose, and toxicity data to assess plant safety concern **[3]**. The plant based products consist of secondary metabolites which undergoes preclinical studies including toxicological investigation followed by experimental investigation in laboratory animals and the successful molecules will become novel molecules with a definite therapeutic activity **[4]**. The medicinal plants contain bioactive components which act as protective mechanisms against numerous ailments but the plant themselves might be poisonous in nature **[5]**. It must be obviously assumed that all plant products should not be incorrectly measured as safe or harmless as they are naturally obtained. Some secondary metabolites in plants may be harmless to the plant but it might be poisonous or even can be lethal when used in human. The information from toxicity studies on secondary metabolites or other plant derivatives must be obtained to document their safety for the usage in humans which is important in drug discovery courses **[6]**. In addition, certain chemical ingredients existing as naturally harmless may show poisonous properties at a definite dose or sustained contact **[7]**. The chief principle of toxicity investigation is to study and document adverse effects of the test substance that are proposed to be used or consumed by humans. The progress of toxicity from herbal medicine could be extrinsic, intrinsic, or any other additional causative aspects **[8]**. Herb-herband herb-drug interactions including sustained ingestion and excess dosage than therapeutic dose could also play vital responsibility in activating adverse reaction **[9]**.

The genus Ipomoea consist of about 500-600 species all over the world and honored as the largest genus of family Convolvulaceae. This family is dominated by twining or climbing woody or herbaceous plants that regularly have heart-shaped leaves and funnel-shaped flowers **[10]**. *Ipomoea horsfalliae* Hook. Convolvulaceae, well known as "morning glory," has simple and

dark green alternate leaves; its inflorescences have deep fuchsia color and grow on the terminal part of the branches **[11]**.

There is no scientific proof or reports obtainable in the literature for the oral acute and sub-acute toxicity study for *Ipomoea horsfolliae* Hook. Hence to determine and establish the safety for its use in traditional practice, acute and sub-acute toxicity studies were carried out as per OCED guidelines.

MATERIAL AND METHODS

Plant material and preparation of extracts

The plant materials were identified and authenticated by Dr. A.K. Pradeep., Assistant Professor -Department of Botany, Calicut University (Calicut, India). Voucher specimens were deposited in the same department herbarium as specimen No.148253. The aerial parts of *Ipomoea horsfolliae* Hook was dried properly in shade for 3 weeks, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. About 1 kg of air-dried plant material was extracted in soxhlet assembly successively with petroleum ether, chloroform, ethyl acetate, ethanol and water (order of increasing polarity). Each time before extracting with the next solvent, the powdered material was dried at room temperature. Each extract was concentrated by using rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material. The color and consistency of the extract were also noted. All the solvents used for this entire work were of analytical grade (Merck, Mumbai). The percentage yield was calculated for the extracts and major compounds with reference to the crude material taken.

EXPERIMENTAL DESIGN

Animals

Adult healthy young female Swiss albino mice, nulliparous, non-pregnant and weighing 25-30 and wistar rats of either sex(10-12 weeks old, 125-175 g) were used and kept in the animal house of the Department of Pharmacology, K.M. College of Pharmacy, Madurai, Tamil nadu, India. The experiments were designed and conducted in accordance with ethical norms approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and Institutional Animal Ethical Committee (IAEC). The animals were kept in plastic cages (34×47×18 cm3) at animal house, in an air conditioned environment with five mice in each cage and maintained at room temperature of (25 ± 2)^{0}C with relative humidity (60% ± 10%) under 12 h night and light cycle. They were

fed with standard laboratory animal diet and water *ad libitum*. Animals were acclimatized to laboratory conditions before the test. The experimental protocol were approved by institutional animal ethical committee under reference number IAEC/MUHAMMED ASHRAF VK/Ph.D/AU/ 1661130022/KMCP/91/2020**.**

Acute toxicity

The oral acute toxicity study of the of ethyl acetate extract of *Ipomoea horsfolliae* Hook (EAIH) was carried out using the 'Up-and- Down' method of testing in mice and rats at single doses of 175, 500 and 2000 mg/kg in accordance with the Organization for Economic Development (OECD) guideline no. 425 **[12]**. Five female mice were used for each dose level in the study. An animal was picked at a time, weighed and dosed with the equivalent volume of extract dissolved in distilled water. The extract was administered orally using gastric feeding tube. Each animal was observed after dosing for the first 5 min for signs of regurgitation and kept in a metallic cage. Each was then observed every 15 min in the first 4 h after dosing, every 30 min for 6 h and daily for 48 h for behavioral signs of toxicity (changes in skin, hair, eyes, mucous membranes, and respiratory, circulatory, autonomic and central nervous systems, motor activity, convulsion, tremors, salivation, diarrhea, lethargy, or sleep) according to the specifications of the OECD (2001). The animals were monitored for a total of 14 days for the long-term possible lethal outcome. The body weights of the animals were measured on days 1, 7, and 14.

Sub-acute toxicity study

Wistar rats of both sexes were assigned randomly to four groups (n=12/group: sixmales and six females).The test was performed according to the OECD guidelines 407[13].Group's I-III received 150, 300 and 600 mg/kg of the extract respectively while group IV received distilled water (5 ml/kg) only. The rats were dosed by oral gavage, using a curved, ball tipped stainless steel feeding needle for 28 days. Daily feed and water intake were recorded for the female rats. The weights of all animals were measured weekly .On day 29, the rats were weighed and sacrificed. Blood samples were collected via cardiac puncture for biochemical and hematological analysis. The heart, liver, lungs, kidneys, brain and spleens, were excised, weighed, trimmed and then fixed in Bouin's solution for histological analysis.

Body weight, food, and water consumption

Body weight of the rats in all the groups was recorded before administration of

doses, further body weight was taken weekly during the treatment and finally on the day of sacrifice. The amount of food andwater intake was recorded daily. The consumed amount of food and water were measured before they provided to each group, their remnants were calculated next day to get the differences, which were recorded as daily food (g./rat/day) and water consumption (ml/rat/day).

Blood analysis

Blood (1.5 ml) was collected from the retro-orbital region of the rats for measurement of hematological (EDTA-coated tubes) and biochemical (dry tubes) parameters after 14 days and 28 days.

Hematological analysis

The blood samples collected in heparinized tubes were used for the hematological analyses. The following parameters: red blood cell count (RBC), white blood cell count (WBC), neutrophils (NP), lymphocytes (LC), monocytes (MC), eosinophils (EP), hemoglobin (Hb), platelets (PL) and packed cell volume (PCV) were evaluated by automated analyzer (KX-21-Hematology-analyzer, Sysmex Corporation, USA).

Biochemical analysis

Dry tubes containing collected blood were centrifuged at 3000 rpm at 25 °C for 15 min to obtain the serum, which was stored at −20 °C until the measurement of biochemical parameters (Erba chem 5 semiauto analyzer) Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) (Coral clinical system), Alkaline phosphatase (ALP) (Arkray health care Pvt ltd.), Urea, Creatinine (Coral clinical system) and BUN analysis was performed

Histological analysis

The fixed tissues were dehydrated in an ascending series of alcohol, cleared inxylene, and embedded in paraffin wax melting at 60 ^{0}C. Serial sections (5-mm thick) obtained by cutting the embedded tissue with microtome, were mounted on 3-aminopropyl triethsilane coated slides and dried for 24h at 37 ^{0}C **[14]**. The sections on the slides were deparaffinised with xylene and hydrated in a descending series of alcohol. They were thereafter stained with Mayer's haematoxylin and eosin dyes, dried and mounted on a light microscope (X40, 100 and 200) for histopathological examination.

STATISTICAL ANALYSIS

The results of the study are expressed as the mean ±SEM (standard error of the mean). The results were analyzed using Graph Pad Prism version 6 software. Comparison in all the groups was made using

one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. The $p < 0.05$ were considered significant.

RESULTS

Acute toxicological evaluation

The acute toxicity test using the Up and Down method at an oral limit doses of 175, 500 and 2000 mg/kg of the ethyl acetate extract of *Ipomoea horsfalliae* Hook triggered no death in the mice. No lethal effects were observed entire course of the short and long-term observation period. No poisonousness signs were detected in the animals throughout the 14 days study period. Therefore, the extract may be harmless at these doses and the oral LD_{50} considered greater than 2000 mg/kg.

Sub-acute toxicity

All the treated rats of both sexes at the doses of 150, 300 and 600 mg/kg survived throughout the 28 days of treatment. No observable toxicity signs were noticed in the extract treated rats compared to the control.

Effect of EAIH on body weight, food intake and water consumption in rats

No significant change was observed in the animal's body weight during the study **(Figure 1)**. After 28 days of oral administration of EAIH, the food and water intake also not affected. It indicated that the extract did not show any significant change in appetite and harmful consequence on the growth of the animal. No significant variations were detected in rat's physiological as well as metabolic activity when comparing with control **(Table 1)**.

Effect of EAIH on hematological parameters

The hematological analyses of 14th and 28th days are given in **Table 2 and 3**. Results showed that there is no significant variation in hematological parameters.

Effect of EAIH on biochemical parameters

To measure the toxicity of any new substance it is very necessary to identify the status of kidney and liver function, which can be tested through biochemical assessment without sacrificing the experimental animals. The parameters such as SGOT, SGPT and ALP are very important for liver function test. Serum urea and creatinine are most important parameters for the assessment of kidney function. Any variations in above parameter after the intake of any test compound from the normal ranges indicate toxicity of that compound in animals. The results showed that the biochemical parameters in animals after 14th & 28th days have no significant deviations happened in SGPT, SGOT, ALP, urea and creatinine

levels at every tried dose in contrast to control **(Tables 4 and 5)**.

Histopathology

In histopathological study there is no changes at the cellular level in comparison to the control were observed after 14 and 28 days **(Figure 2 and 3 respectively)**.

The histopathological slides also confirmed that the EAIH treated group up to the dose of 600 mg/kg showed no toxicity in 28 days.

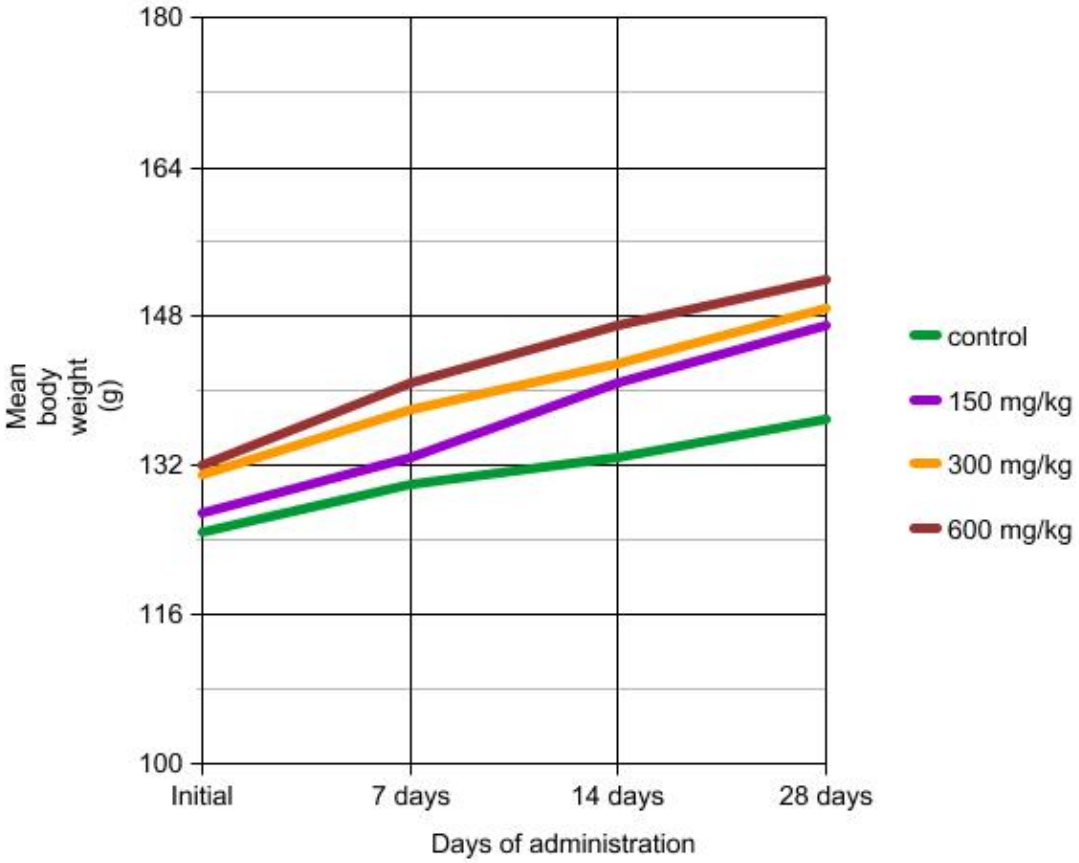

Figure 1: Body weight assessment of treated rats in sub-acute toxicity study

Table 1: Effect of EAIH on food intake and water consumption by rat during 28 days treatment

Treatment	Average food intake (g/day/rat)	Average water intake (ml/day/rat)
Control	16.27±1.23	19.34±1.34
150 mg/kg	15.13±1.43	17.79±1.45
300 mg/kg	16.34±1.34	18.54±1.74
600 mg/kg	15.44±198	19.42±1.22

Values are expressed in Mean ± SEM, n=6 animals/group, p < 0.05 (ANOVA/ Dunnett's test)

Table 2: Hematological parameters of rat treated with different dose level of EAIHin sub-acute toxicity after 14th day

Parameters	Normal ranges	Control	150 mg/kg	300 mg/kg	600 mg/kg
Hemoglobin (%)	10.2-16.6	14.43±0.34	15.13±1.34	12.14±1.22	13.43±1.36
Total RBC (10^6/µL)	5-10	5.12±1.64	6.12±0.34	9.13±1.15	7.12±1.65
WBC (10^3/µL)	6-15	9.34±0.36	13.44±0.56	12.34±1.76	11.36±1.86
Platelets(10^3/L)	782-985	839±1.45	939±0.49	765±1.44	879±0.35
PCV (%)	39-49	42.34±1.32	47.14±0.12	43.35±1.22	42.64±1.52
LC (%)	55-95	63.12±0.94	86.32±1.95	73.42±194	71.12±0.94
NP (%)	10-40	32.32±1.96	39.42±0.16	37.32±0.92	34.72±1.44
MC (%)	1-4	3.23±1.38	2.23±0.28	3.83±0.33	2.33±1.47
EP (%)	0-4	2.11±0.72	3.41±1.78	2.13±1.42	1.11±0.72

All values are expressed in Mean ± SEM, n=6 animals/group, p< 0.05 (ANOVA/ Dunnett's test)

Table 3: Hematological parameters of rat treated with different dose level of EAIH in sub-acute toxicity after 28th day.

Parameters	Normal ranges	Control	150 mg/kg	300 mg/kg	600 mg/kg
Hemoglobin (%)	10.2-16.6	15.67±1.24	17.23±0.19	14.48±0.52	12.53±0.46
Total RBC (10^6/µL)	5-10	7.62±0.26	8.12±1.82	6.13±1.57	7.12±0.25
WBC (10^3/µL)	6-15	10.34±1.76	14.34±1.33	9.24±0.46	10.36±1.36
Platelets(10^3/L)	782-985	919±0.26	942±0.34	895±0.35	879±0.33
PCV (%)	39-49	47.14±0.29	44.34±1.42	40.35±0.72	42.34±0.62
LC (%)	55-95	79.76±1.98	67.42±1.78	83.72±1.54	76.42±1.94
NP (%)	10-40	39.32±0.26	12.42±1.36	28.32±1.62	18.67±0.88
MC (%)	1-4	3.23±1.22	1.33±1.48	3.83±1.63	1.98±1.98
EP (%)	0-4	2.81±0.29	2.44±0.42	2.63±0.92	1.91±1.62

All values are expressed in Mean ± SEM, n=6 animals/group, p< 0.05 (ANOVA/ Dunnett's test)

Table 4: Biochemical estimation from blood serum of rats after 14th day's treatment at different dose level in sub- acute toxicity study

Parameters	Normal ranges	Control	150 mg/kg	300 mg/kg	600 mg/kg
SGOT (U/L)	54-298	176.12±19.45	253.77±25.13	149.56±12.56	227.26±23.76
SGPT (U/L)	17-77	56.23±1.86	47.25±2.36	33.56±2.56	59.49±1.76
ALP (U/L)	64-128	102.35±2.76	99.73±1.67	121.54±2.86	88.85±2.56
Creatinine (mg/dL)	0.2-0.9	0.25±0.12	0.72±0.17	0.43±0.37	0.37±0.65
Urea (U/L)	35-96	44.39±1.39	57.22±1.76	79.54±2.54	54.65±2.55
BUN (mg/dl)	8-33	22.95±1.94	20.56±0.35	18.34±1.76	13.54±1.67

All values are expressed in Mean ± SEM, n=6 animals/group, p < 0.05 (ANOVA/ Dunnett's test)

Table 5: Biochemical estimation from blood serum of rats after 28th day's treatment at different dose level in sub- acute toxicity study

Parameters	Normal ranges	Control	150 mg/kg	300 mg/kg	600 mg/kg
SGOT (U/L)	54-298	176.12±19.45	253.77±25.13	149.56±12.56	227.26±23.76
SGPT (U/L)	17-77	56.23±1.86	47.25±2.36	33.56±2.56	59.49±1.76
ALP (U/L)	64-128	102.35±2.76	99.73±1.67	121.54±2.86	88.85±2.56
Creatinine (mg/dL)	0.2-0.9	0.25±0.12	0.72±0.17	0.43±0.37	0.37±0.65
Urea (U/L)	35-96	44.39±1.39	57.22±1.76	79.54±2.54	54.65±2.55
BUN (mg/dl)	8-33	22.95±1.94	20.56±0.35	18.34±1.76	13.54±1.67

All values are expressed in Mean ± SEM, n=6 animals/group, p < 0.05 (ANOVA/ Dunnett's test)

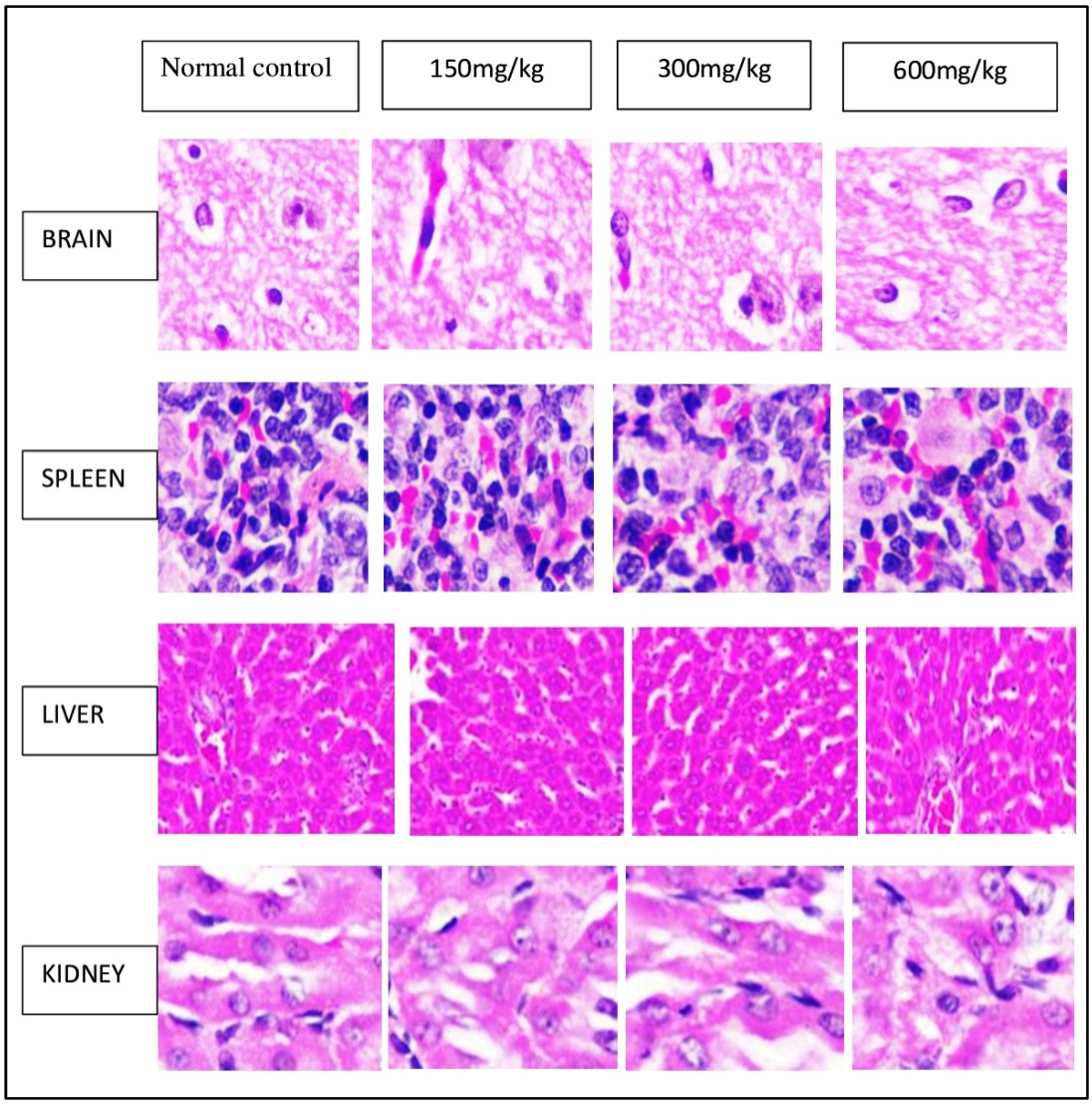

Figure 2: Histopathology (20X) of rat tissues of treated and control groups after 14th days

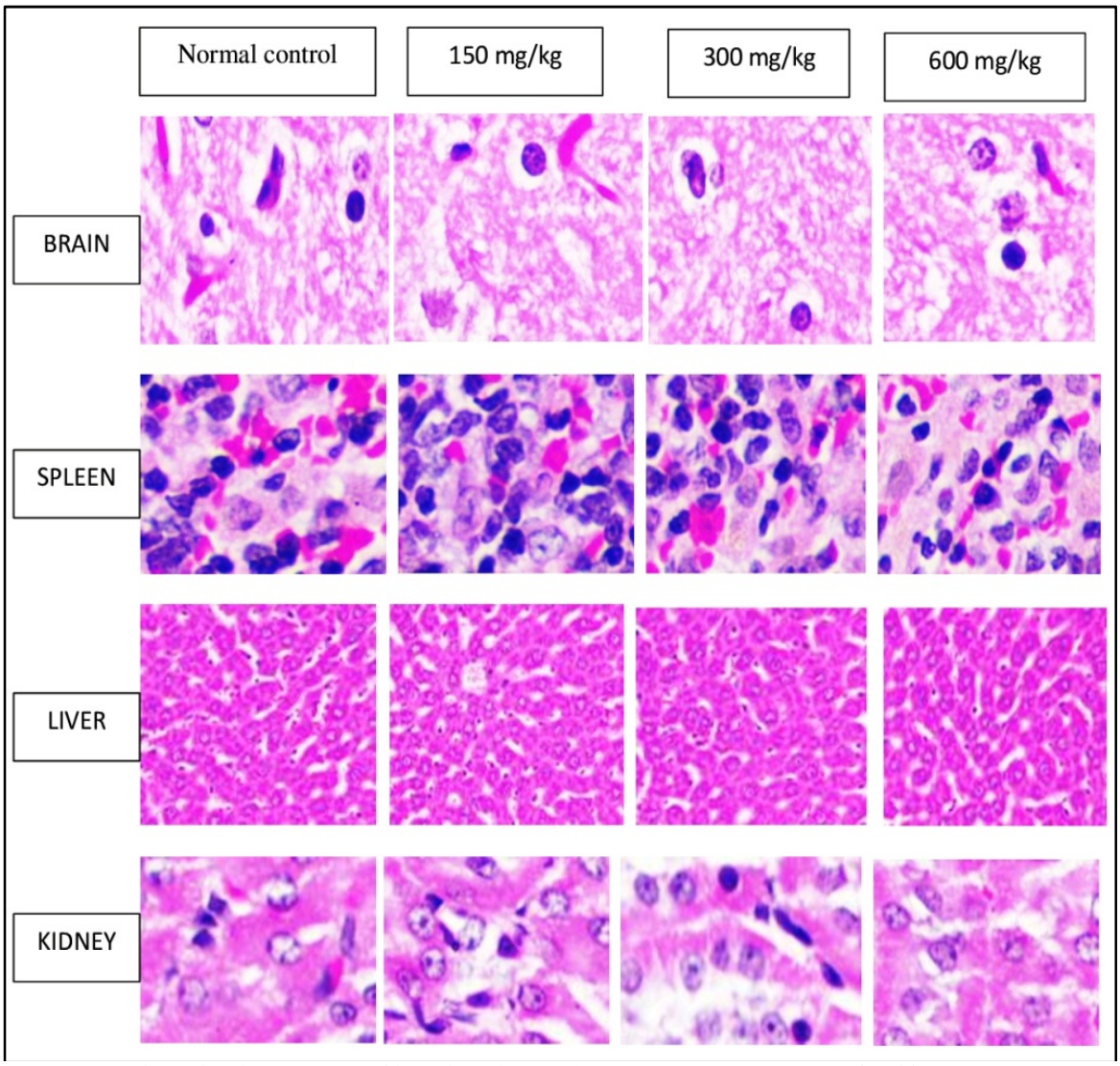

Figure 3: Histopathology (20X) of rat tissues of treated and control groups after 28th days

DISCUSSION

The chief point of assessing the safety of any remedial plant is to recognize the nature and depth of side effects and to make sure the exposure level at which any toxic consequences are detected **[15]**. Prior to conducting pharmacological activity study of secondary metabolites from any plants acute and sub-acute toxicity studies is compulsory according to the standard protocol like OECD guidelines **[16]**. Toxicity studies will help the researcher to determine the dose conformation of the test sample which is considered as one of important step in the drug discovery journey **[17]**. The outcomes of the acute toxicity study in our study specify that the aerial parts of EAIH administered by oral route to mice at 175, 500 and 2000 mg/kg using the up and down technique of acute toxicity testing did not cause any indication of toxicity and mortality in the animals used according to OECD criteria under its Globally Harmonised Classification System (GHS) any substances with LD50 greater than 2000 mg/kg considered as safe. Final and regular clinical monitoring in repeated dose analysis has foremost significance **[18]**. 28 days long sub-acute toxicity study found no harm or any mortality in any treated animals. Food intake and water consumption were not affected during the study period which point out that the EAIH did not disrupt the appetite and poisonous result on the growth of animals. No major variations were detected in rat's physiological, metabolic activity in contrast to control.

Changes in hematological parameters considered as indication of poisonous properties of test compound in rat blood either at pathological or physiological level. If the test sample displays the toxicity in the body, it will disturb blood components such as hemoglobin, white blood cells, red blood cells and platelets. Any alteration in the blood components will directly affect the body immune system and the of organs functions as well **[19]**. Our test results revealed that EAIH produced no significant changes hematological components when compared with control in sub-acute studies **(Tables 3 & 4)**. The liver and kidney are greatest vital organs which is accountable for metabolism and excretion respectively **[20]**. To evaluate the toxicity of any novel compound it is very crucial to know the status of these two vital organs, which can be tested by biochemical assessment without killing of rats.

In the histopathological examination, we observed that in all treated groups after 14 and 28days **(Figure 2 and 3 respectively)**

the organs displayed no major variations at the cellular level in contrast to control.

CONCLUSIONS

The oral $LD_{50}$of extract of aerial parts of Ipomoea *horsfolliae* Hook (EAIH) has been shown to be greater than 2000 mg/kg and is generally considered safe. This study clearly indicates that the EAIH can be used for further evaluation pharmacological activities on the above-mentioned doses of extract.

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Authors' contributions

All authors contributed to the manuscript. Muhammed Ashraf VK performed the works, VK Kalaichelvan and V.V Venkatachalam supervise the works, R. Regunathan has done data analysis. All the authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that there are no conflicts

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