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Research Article

TOXICOLOGICAL STUDIES OF *AGERATUM CONYZOIDES* IN RATS

[1]*Harisha. C. B, [2]Shridhar, N.B., [3]Sunilchandra, U., [4]Prashant Kumar Waghe, [5]Kavitha Rani, B., [6]Patel Suresh Revanna, [7]Vinuta M.H., [8]Niranjan.D

[1]Veterinary Dispensary Aralehalli, Shikaripura, Department of Animal husbandry and Veterinary services, Government of Karnataka
[2]Department of Veterinary Pharmacology and Toxicology, Veterinary College, Shivamogga
[3]Department of Veterinary Pharmacology and Toxicology, Veterinary College, Shivamogga
[4]Department of Veterinary Pharmacology and Toxicology, Veterinary college, Bidar, KVAFSU, Bidar
[5]Department of Veterinary Pathology, Veterinary College, Gadag, KVAFSU, Bidar
[6]Department of Veterinary Clinical Complex, Veterinary College, Gadag, KVAFSU, Bidar
[7&8] Department of Veterinary Pharmacology and Toxicology, Veterinary college, Shivamogga

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ABSTRACT

The study was taken up to evaluate the pharmacological and toxicological property of *A. conyzoides* plant. The yield of methanolic extract was found to be 6.8 %. The pH of methanolic extract of *A. conyzoides* (MEAC) was 5.86. It was dark green in colour and the was dry and powdery in consistency with aromatic odour. Acute oral and repeated dose 28-day oral toxicity studies of methanolic extract was carried out in Wistar albino rats as per the OECD guidelines 423 and 407 respectively. In sub-acute oral toxicity dose levels of 500, 1000 and 1500 mg/kg were administered daily for 28 days and compared with the control rats. In toxicity studies, no mortality and toxicity signs were observed. The MTD value was found to be more than 5000 mg/kg. The general condition of the animals did not change and all the animals remained in normal health condition throughout the experiment. The histopathological changes were dose dependent and the changes were very mild to moderate and are not the major signs to conclude the extract are toxic in acute stage, in repeated dose toxicity study showed the alarming signs of toxicity. Haematological and biochemical parameters showed significant effect of the extract on different organs which are evident by histopathological changes observed in extract treated groups.

Keywords: *A. conyzoides*, phytochemical analysis, Anti-inflammatory activity, toxicity study and Wistar albino rats.

INTRODUCTION

The Indian subcontinent has a vast repository of medicinal plants that were being used in traditional treatments. Though only medicinal plants are utilised to make herbal pharmaceuticals, alternative medicines in traditional systems are made from herbs, minerals, and organic matter. In India, the utilisation of plant material as a source of therapeutic substances has existed for centuries and plays a significant role in the health care system of the nation. (Pande *et al*., 2013). The earliest known record in writing of the utilisation of herbal medicines for drug manufacture was discovered on a Sumerian clay slab, which is thought to be around 5000 years old. It had 12 drug preparation instructions citing more than 250 different plants, such as mandrake, henbane, and poppies (Petrovska, 2012). Traditional knowledge of herbal medicine can serve as a powerful approach to drug discovery. Plants are an important source of medicines to have a highly significant role in indigenous pharmacopoeias (Singh *et al*., 2014). As per the survey of the World Health Organization (WHO), about 80% of the world population is using herbs and other traditional medicines for their primary healthcare with three kinds of herbal medicines: raw plant material, processed plant material, and herbal products. Natural resources of enormous value, medicinal plants are essential to the world's primary healthcare system in distant, emerging, and underprivileged regions (Shinwari and Gilani, 2003). Unfortunately, the increasing acceptance to herbal drugs

*Corresponding Author: Harisha.C.B Senior veterinary officer, Veterinary dispensaryAralehalli, Shikaripura, Department of Animal husbandry and veterinary services, Government of Karnataka, Email: harishavet515@gmail.com, Mobile: +91 9632038059.

has rendered a hike in price of useful medicines and the plant material is depleting vigorously due to their over exploitation. Furthermore, in the wake of modernization and acculturation the communities had lost their familiarity with natural medicinal plants used by their ancestors as remedies for various ailments (Srivastava, 2018). *Ageratum conyzoides* is a perennial upright plant that has been identified as an invasive plant that has spread to farmland, colonised open fields, and negatively impacted biodiversity. There are over 30 species in the genus Ageratum, although only a few have been investigated phytochemically (Burkill, 1985).

The herbal medicine provides a ray of hope through its cocktail of phyto-compounds, which are believed to act in a synergistic manner, providing excellent healing touch with practically fewer side effects. *A. conyzoides* has been reported to possess anti-inflammatory and analgesic activity in mice (Rahman *et al*., 2012). Thus, the present study also included the study on evaluation of the anti - inflammatory and analgesic effect of *A. conyzoides* aerial parts in rats. On the contrary, several cases of sheep death, suspected to be due to consumption of the plant *A. conyzoides* in various villages of Maharashtra state have also been reported. The sheep had exhibited the clinical signs of onset of sudden bloat, hematuria, melena and recumbence before death. The suspected toxicity episodes with the same plant have been reported in Uttara Kannada district of Karnataka state. Thus, there was the necessity of exploring the toxic nature of *A. conyzoides*, apart from the several folklores claims of medicinal properties of the aerial parts of the plant. Hence, the present study was designed to study the pharmacological and toxicological properties of the aerial parts of *Ageratum conyzoides* with the following objectives. To study the anti-inflammatory and analgesic activity of *Ageratum conyzoides* in rats. To conduct acute and sub-acute toxicity study of *Ageratum conyzoides* in rats. To correlate the findings with gross and histopathological observations.

Figure 1. Identification and authentication of *Ageratum conyzoides* plant.

MATERIALS AND METHODS

The present investigation was performed in the Small Animal House, Veterinary College, Shivamogga. Thecurrent experiment was taken up to evaluate the phytochemical analysis and to conduct the safety assessment of the aerial parts of the plant *A. conyzoides* with the help of haematological, biochemical and histopathological tests. The experimental protocol was approved by the Institutional Animal Ethics Committee, Veterinary College, Shivamogga, with the approval No./VCS/IAEC/SA-61/2020-21; dated: 31.08.2021.

Collection of plant material and authentication

The tender aerial portions of plant were gathered in and around Soraba Taluk, Shivamogga District, Karnataka State, throughout the months of March and April 2021. Dr. K. G. Gopalakarishna Bhat, Rtd. Professor and Head, Department of Botany, Poornaprajna College, Udupi, verified the plant's botanical identity. The plant material was gathered, cleaned under running water, and then dried in the cool shade for approximately 20 days. The plant material was mechanically ground into a coarse consistency powder and sieved into a fine powder, both of which were then stored in airtight jars for later use (Figure 1).

Preparation of the extract

Preparation of plant extract for experimental purposes is an initial step and key in achieving quality research outcome. Before moving further with the desired biological testing, it includes the extraction and assessment of the quality and number of bioactive elements. Menstruum is another name for the extraction solvent used to extract herbal medicines. The kind of plant, the portion of the plant to be extracted, the chemical composition of the bioactive compounds, and the solvent's availability all impacted on the solvent choice (Abubakar and Haque, 2020).

Soaking

One thousand gram (1000 g) of the coarse powder was weighed using an electronic weighing balance and soaked in 5 liters of methanol (99% SDFCL), at a ratio of 1:5 (powder/solvent) in closed glass flasks at room temperature. The mixture was agitated using an electric orbital shaker (REMI® RS-12 plus) to enhance proper mixing of the solvent with the powder for the first 6 hour. Shaking and stirring of flasks done thrice a day for seven days (Odey *et al*., 2012)

Filtration and Concentration

After seven days, the contents were first filtered through muslin cloth and Buchner's funnel later with Whatman No.1 filter paper (Himedia, 24 cm). The filtrates were then separately concentrated in vacuum using Rotary Evaporator (DLAB® RE100-Pro, China) at 37 °C – 40 °C till solvent got evaporated and extract settled down. These were concentrated to complete dryness in the incubator (SLM®-INC-OS-250) at 50°C. The extracts were stored in a refrigerator in air-tight containers from where aliquots were used for the phytochemical analysis and pharmacological formulation preparation (Odey *et al*., 2012).

Calculation of yield

The per cent yield [dry weight of extract] calculated after solvent extraction using the formula given below.

$$\% \text{ yield} = \frac{\text{Final weight of extract}}{\text{Initial weight of the powder}} \times 100$$

Toxicity studies of methanol extract of *A. conyzoides*

Acute oral toxicity and Repeated dose 28-day oral toxicity studies of methanol extract of *A. conyzoides* were conducted in rats as per the broader outlines of OECD 423 and OECD 407 respectively.

Design of the experiment

Experimental animals

The Wistar albino rats with a body weight range of 150 ± 20 g were employed in the current investigation. The rats ranged in age from 6 to 7 weeks. The rats were purchased from Adita Biosys Private Limited in Tumkur under registration number 1868/PO/RcBt/S/16/CPCSEA. Date of Registration: February 23, 2016, effective till April 8, 2026. In accordance with regulations of the Committee for Control and Supervision of Experiments on Animals, all rats were housed in the Small Animal House at the Veterinary College in Shivamogga at a steady temperature of 23±3°C, relative humidity of 50-70%, and a 12-hour light/dark cycle. Prior to commencement of the actual studies, the rats underwent a one-week acclimatisation period. The Institutional Animal Ethical Committee of the Veterinary College, Shivamogga, as well as the CCSEA committee approved the study before it was permitted to be carried out.

Acute oral toxicity study

Acute oral toxicity study of MEAC was conducted in female Wistar albino rats as per the Organization for Economic Co-operation and Development (OECD) test guideline for testing of chemicals, Acute oral toxicity – Acute toxic class method (OECD 423).

Study procedure

Animal preparation

Adult healthy Wistar albino female rats in the age group of 7 to 8 weeks with the body weight of 160 ± 20 g were acclimatized to the laboratory conditions for 7 days prior to test and before assigning the animals to treatment groups.

Animal groups and number of animals

Acute oral toxicity study was conducted in nulliparous and non-pregnant female Wistar albino rats. The animals were divided into four groups of three animals each were used for the study

Selection and preparation of doses

As per OECD guidelines 423, three doses: 300, 2000 and 5000mg/kg were selected as low, medium and high dose respectively based on the previous pharmacological studies on methanolic, ethanolic, petroleum ether and ethyl acetate extract of *A. conyzoides* plant leaves (Diallo *et al*., 2010; Verma *et al*., 2013; Diallo *et al.*, 2014).

Administration of doses

The extract was dissolved in distilled water to make the solution of required quantity not exceeding 2 ml for the ease of administration. The doses of extract were administered to overnight fasted animals as a single dose by oral gavage. As per the OECD guidelines 423, the starting dose selected was 300 mg/kg as there was no mortality; Hence the treatment group in the study received dosage of 300 (low dose), 2000 (medium dose) and 5000 mg/kg (high dose) of methanol extract of *A. conyzoides* whereas the control group received the dosage of 1 ml/100g of distilled water as a vehicle. The design of the experiment is as follows;

Table 1. Experimental design for Acute oral toxicity study of MEAC.

Group	Dose [mg/kg, p.o.]	No. of female rats
Group I	Distilled water [10 ml/kg]	3
Group II	300	3
Group III	2000	3
Group IV	5000	3

Observation of animals

After the oral dosing of MEAC, food was withheld for further 3-4 h. All the animals were observed individually at least once during first 30 min after dosing, periodically during the first 24 h (with special attention during the first 24 h) and daily thereafter for a period of 14 days for the symptoms of toxicity and death. General clinical observations were made every day. All the animals were observed for changes in the level of activity, gait, posture, reactivity to handling or sensory stimuli, changes in skin and fur eyes, mucous membranes, behaviour pattern, salivation, diarrhea, lethargy, sleep, altered strength, health conditions, morbidity and mortality.

Repeated dose 28-day oral toxicity study

Repeated dose 28-day oral toxicity study for the MEAC was conducted in both male and female Wistar rats as per the broader outlines of Organization for Economic Co-operation and Development (OECD) guidelines for testing of chemicals, Repeated dose 28-day oral toxicity study in rodents (OECD-407).

Study procedure: Animal preparation

Adult healthy Wistar albino male and female rats in the age group of 7 to 8 weeks with the body weight of 160 ±20 g were acclimatized to the laboratory conditions for 7 days prior to test and before assigning the animals to treatment groups.

Selection and preparation of doses

As per OECD guidelines 407, three doses were selected based on previous pharmacological studies on methanolic, ethanolic extract of *A. conyzoides* plant leaves.(Antai *et al*., 2009; Diallo *et al*., 2010; Verma *et al*., 2013; Diallo *et al.*, 2014; Palmer *et al*., 2019).The change in the relative weight of the liver in the previous study which indicate that the extract might have toxicpotential on liver with increasing dose, the doses selected for this study were 500, 1000 and 1500 mg/kg as low, medium and high dose respectively (Diallo *et al*., 2010).

Animal groups and number of animals

Male and female rats acclimatized to laboratory conditions were assigned to control and treatment groups randomly. Each group consisting of 5 male and 5 female rats was used for the present study.

Administration of doses

For a total of twenty-eight days, oral gavage was used to administer the MEAC to each rat individually at various dose levels in a single dose every day at the identical time.

Observation of animals

Throughout the 28-day trial period, overall medical observations were conducted once each day while taking into consideration the time period for expected effects following dosing. The rats were examined twice a day, twice in the morning and again in the evening, for overall health status, mortality and morbidity. Variations in stride, posture, and responsiveness to handling, as well as the existence of clonic or tonic contractions and stereotyped behaviours, was also noted. Alterations in skin, fur, eyes, mucous membranes, secretions, excretions, and autonomic activity were also reported.

Body weight

Bodyweight of each animal in both acute and sub-acute oral toxicity studies was recorded weekly once till the completion of the study on day 14 and day 28 in acute and subacute toxicity study respectively.

Haematology

On day 14 in the acute toxicity study and day 28 in the sub- acute toxicity study blood was subjected for analysis of hematological parameters *viz* RBC, WBC, Hb and platelet count by using The Exigo® veterinary hematology analyzer, Sweden. Using micro hematocrit capillary tubes, the terminal blood sample was collected by retro orbital plexus puncture technique using diethyl ether as inhalant sedative.

Serum biochemistry

The serum biochemistry profile with respect to the following parameters was performed on day 14 in the acute toxicity study and day 28 in the sub-acute toxicity study using biochemical analyser (HY-SAC Vet Version: A/6 Semi-auto Chemistry Analyzer, Hycel® Handelsges Austria). Using micro hematocrit capillary tubes, the terminal blood sample was collected by retroorbital plexus puncture technique using diethyl ether as inhalant sedative.

Pathology

At the end of study period of 14 days and 28 days for acute and sub-acute oral toxicity study respectively, all the ailing rats in control and treated groups were humanely sacrificed using carbon dioxide chamber and subjected to detailed gross necropsy for the organs including examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents for the changes.

Collection of organs for histopathological study

In all the study groups of acute and sub-acute oral toxicity study, the rats were weighed individually and sacrificed humanely by using carbon dioxide chamber. Necropsy was conducted on each carcass to observe any gross pathological changes. The organs *viz*., liver, kidney, spleen, duodenum, lung and heart were separated from the adhering tissues and preserved in 10 % Neutral buffered formalin (NBF) for the period of 72 h for tissue fixation. Further the tissues were processed for histopathology using automatic tissue processor followed by routine paraffin embedding technique. Sections of 5 microns thickness were cut and stained with Haematoxylin and Eosin using the standard protocol (Luna, 1968).

Statistical analysis

Data was expressed as mean ± SEM. Differences were considered significant at***P<0.001, or **P < 0.01 or * P<0.05 when compared test groups v/s control group. For statistical analysis, one-way Analysis of Variance (ANOVA) with Dunnet's multiple comparisions test was performed using Graph Pad Prism 7.2.0 (435) Software.

RESULTS AND DISCUSSION

Food was not provided for additional 3-4 hours following oral administration of MAEC. Every rat was personally watched minimum once during the initial thirty minutes after dose, numerous times during the first 24 hours (with extra care during the first 4 hours), and then every day for the next 14 days to look for signs of toxicity and mortality. Every day, routine clinical evaluations were made.

All the animals were observed for any changes in activity, posture, gait, or sensory stimuli. The animals in both vehicle-treated control group and extract-treated groups were normal, did not display marked changes concerning behavior. There was no change in skin and hair coat, posture, and water consumption. There were some fluctuations observed in feed intake which was recorded accordingly. There were no signs of toxicity concerning the nervous system, respiratory system, and gastrointestinal system in all the experimental animals. The effect of MEAC aerial parts on body weights of the animals are shown in Table 5 and Figure 1. There was no significant change in body weight of control and treatment group animals. Serum biochemical parameters of rats of control group and methanolic extract of *A. conyzoides* aerial parts treated groups are shown in Table 7. There was significant ($p<0.05$) change observed in AST parameter of the 5000mg treated group and remaining all biochemical parameters there was no significant ($p<0.05$) change as compared with the control group. Haematological parameters of control and methanolic extract of *A. conyzoides* aerial parts treated groups are shown in Table 6. There was no significant ($p< 0.05$) change observed in all haematological parameters as compared with control group.

Table 2. Experimental design for Repeated dose 28-day oral toxicity study of MEAC.

Group	Dose [mg/kg, *p.o*]	No. of male rats	No. of female rats
Group I	Distilled water [1 ml]	5	5
Group II	500	5	5
Group III	1000	5	5
Group IV	1500	5	5

Table 3. Mortality pattern in rats in acute oral toxicity study of MEAC.

Groups	Treatment and dose (mg/kg, p.o.)	No. of female rats	Mortality observed	Mortality (%)
Group I	Distilled water (1 ml/kg)	3	0	0
Group II	300	3	0	0
Group III	2000	3	0	0
Group IV	5000	3	0	0

Table 4. Mortality and morbidity pattern of rats in repeated dose 28-day oral toxicity study of MEAC.

Groups	Treatment and dose	No. of female rats	No. of male rats	Mortality observed	Mortality [%]
Group I	Distilled water	5	5	0	0
Group II	500 mg/kg	5	5	0	0
Group III	1000 mg/kg	5	5	0	0
Group IV	1500 mg/kg	5	5	0	0

Table 5. Effect of methanolic extract of aerial partsof *A. conyzoides* on body weight of rats in acute toxicity study.

	Group			
Days	Group I	Group II	Group III	Group IV
0	160.00±2.89	166.67±1.67	166.67±1.67	165.00±2.89
7	171.67±3.33	176.67±1.67	178.33±3.33	171.67±1.67
14	185.00±2.89	188.33±3.33	188.33±4.41	178.33±1.67

Note: Data were analysed by two-way ANOVA followed by Dunnett's multiple comparisons test. Data were compared with the control group at different time intervals. Values are mean ±SEM, n=5, *p<0.05, **p<0.01, ***p<0.001.

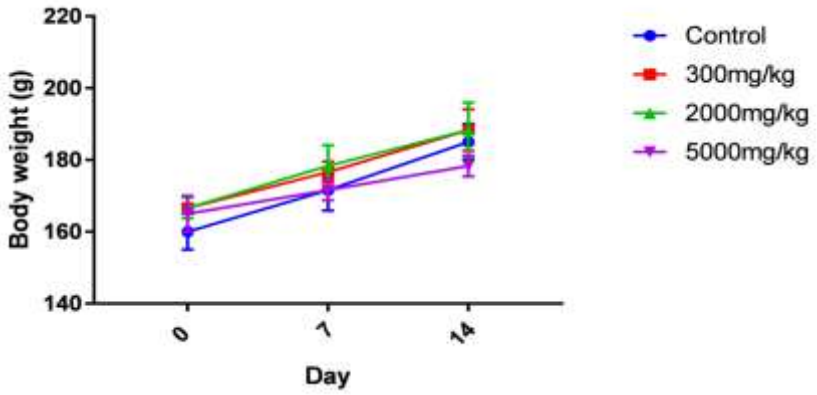


Figure 1. Effect of methanolic extract of aerial parts of *A. conyzoides* on body weight of rats in acute toxicity study.

Table 6. Haematological parameters in acute oral toxicity study of methanolic extract of aerial partsof *A. conyzoides.*

Parameter	Group			
	Group I	Group II	Group III	Group IV
WBC (10^3 /mm^3)	6.33±0.12	6.67±0.26	7.33±0.32	8.73±0.29
RBC (10^6 /mm^3)	8.18±0.15	8.26±0.26	8.31±0.24	7.98±0.50
Platelets (10^3/ml)	806.50±25.42	811.67±51.02	815.67±34.91	827.67±20.79
Hb (g %)	13.67±0.26	13.07±0.93	11.57±0.49	10.53±0.43

Note: Data were analyzed by one way ANOVA post hoc Dunnett's multiple comparisons test and compared with control group. Values are mean ± SEM, n=3.

Table 7. Serum biochemical parameters in acute oral toxicity study of methanolic extract of aerial parts of *A. conyzoides.*

Parameters	Group			
	Group I	Group II	Group III	Group IV
ALT (U/L)	39.97±3.01	40.81±0.95	41.63±0.88	40.32±0.92
AST (U/L)	118.65±3.55	114.67±3.19	119.75±0.61	136.69±2.32*
BUN (mg/dl)	30.53±2.00	27.77±1.66	33.34±1.15	31.70±1.09
Creatinine (mg/dl)	0.53±0.05	0.54±0.06	0.64±0.04	0.80±0.02
Calcium (mg/dl)	13.53±0.57	13.58±0.88	12.55±0.51	12.70±0.29
Phosphorous (mg/dl)	6.28±0.30	6.07±0.08	6.51±0.27	6.25±0.41

Note: Data were analyzed by one way ANOVA post hoc Dunnett's multiple comparisons test and compared with control group. Values are mean ±SEM, n=3. *p< 0.05.

Repeated dose 28-days oral toxicity study

Repeated dose 28-day oral toxicity study of methanolic extract of *A. conyzoides* aerial parts was conducted following broader outlines of OECD guideline-407 and the results obtained are presented below.

General observation

The behavioral patterns and clinical observations of animals were recorded once daily for a period of 28 days. There were no marked changes in the skin, fur, eyes and mucous membranes. The animals in both vehicle-treated and extract-treated groups did not display marked changes concerning to behaviours, breathing, food and water intake. All animals in both vehicle-treated and extract-treated groups were observed for morbidity and mortality.

Clinical signs

The animals were active and apparently normal in both vehicle-treated and extract-treatment groups with no significant changes in behavior. In treatment groups there were no apparent clinical signs like breathing difficulty, abnormal water consumption, postural abnormalities and mortality were observed in any of the tested groups in the repeated dose 28-day oral toxicity study (Table 8).

Body weight

The effect of methanolic extract of *A. conyzoides* aerial parts on the body weight of male and female rats is depicted in Figure 2 and Figure 3 respectively. The Mean ± SE values of body weight of male and female rats in different groups have been shown in Table 9 and Table 10 respectively. There was a significant change in gain in body weight of test animals in comparison with control groups on day 28th of the test. The treated groups of 1500 mg/kg animals exhibited significantly ($p<0.05$) less increment in body weight with time compared to the control group in both male and female rats.

Haematology

Haematological parameters of male and female rats are presented in Table 11 and Table 12, respectively. There were no significant changes noticed in RBC, haemoglobin and platelet count but WBC count in the Group III (1000 mg/kg) and Group IV (1500mg/kg) dose treated group showed significant ($p < 0.01$) increase when compared to Group I (Control).

Serum biochemistry

The serum biochemical parameters of repeated dose 28-day oral toxicity study are presented in Table 13 and Table 14 for male and female rats respectively. AST, BUN and creatinine values of Group III (1000 mg/kg) and Group IV (1500 mg/kg) in both male and female rats were significantly ($p < 0.05$; $p < 0.01$) increased when compared with control group. The other serum biochemical parameters *viz.*, ALT, calcium and phosphorus did not differ significantly.

Table 8. Mortality and morbidity pattern (n=10) in repeated dose28 days oral toxicity study of methanolic extract of aerial parts of *A. conyzoides.*

Groups	Group I	Group II	Group III	Group IV
Treatment and dose	Distilled water	500mg/kg	1000 mg/kg	1500 mg/kg
No. of female rats	5	5	5	5
No. of male rats	5	5	5	5
Feeding	N	R	R	R
Fur condition	NAD	NAD	NAD	NAD
Eye colour	NAD	NAD	NAD	NAD

Convulsion	No	No	No	No
Locomotion	NAD	NAD	NAD	NAD
Sedation	No	No	No	No
Mortality	0	0	0	0

Table 9.Body weight of male rats in repeated dose 28-days oral toxicity study of methanolic extract of aerial parts of *A. conyzoides.*

Days	Groups			
	Control	500mg/kg	1000mg/kg	1500mg/kg
0	179.00 ± 1.87	176.00 ± 2.17	169.00 ± 3.07	174.00 ± 1.87
7	190.00 ± 1.58	185.00 ± 3.16	179.00 ± 1.87	180.00 ± 1.58
14	201.00 ± 1.87	196.00 ± 4.0	188.00 ± 1.22	182.00 ± 1.81
21	215.00 ± 2.12	205.00 ± 3.16	196.00 ± 1.0	187.00 ± 2.57
28	230.00 ± 1.82	213.00 ± 3.00	208.00 ± 1.22	192.00 ± 3.12*

Note: Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test and compared with control group. Values are mean ±SEM (n=5)*p<0.05.

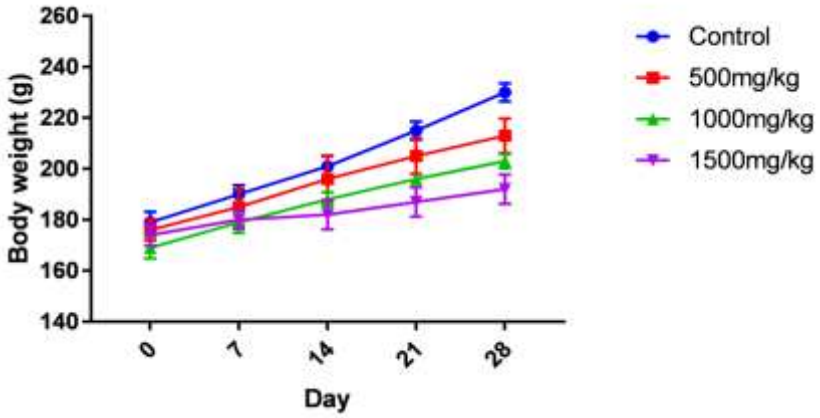


Figure 2. Body weight of male rats in repeated dose28-days oral toxicity study of methanolic extract of aerial parts of *A. conyzoides.*

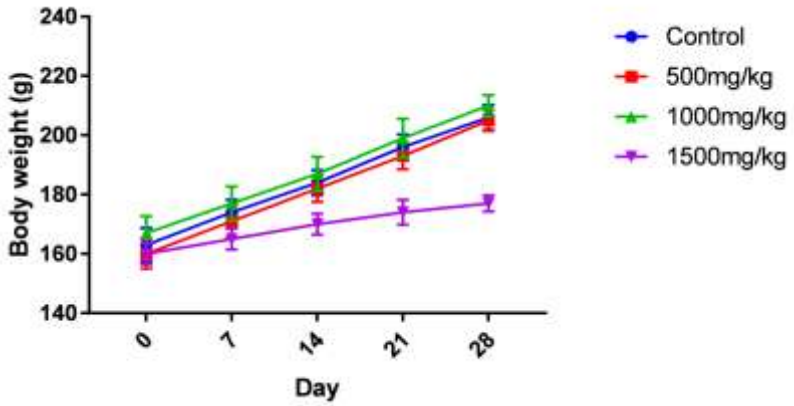


Figure 3. Bodyweight of female rats in repeated dose 28-days oral toxicity study of methanolic extract of aerial parts of *A. conyzoide.*

Table10. Bodyweight of female rats in repeated dose 28-days oral toxicity study of methanolic extract of aerial parts of *A. conyzoides.*

Days	Groups			
	Control	500mg/kg	1000mg/kg	1500mg/kg
0	163±2.55	160±2.24	167±3.15	160±2.45
7	174±1.47	171±2.45	177±1.94	165±2.15
14	184±2.80	182±2.0	187±2.55	170±1.58
21	196±1.87	193±3.18	199±2.92	174±1.85
28	206±1.54	205±2.58	210±1.58	177±1.22*

Note: Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test and compared with control group. Values are mean ±SEM, n=5 *p< 0.05.

Table 11. Haematological parameters of male rats in repeated dose 28-days oral toxicity study of methanolic extract of aerial parts of *A. conyzoides.*

Parameter	Group			
	Control	500mg/kg	1000mg/kg	1500mg/kg
WBC(10^3/mm^3)	7.18±0.42	8.30±0.31	10.16±0.68**	10.64±0.36**
RBC(10^6/mm^3)	7.94±0.45	7.93±0.25	8.82±0.16	8.27±0.25
Platelets(10^3/ml)	786.80±17.94	791.40±13.54	788.80±18.26	801.60±17.28
Hb (g/dl)	12.22±0.50	12.56±0.46	11.89±0.47	11.49±0.26

Note: Data were analyzed by oneway ANOVA followed by Dunnett's multiple comparisons test and compared with control group. Values are mean ± SEM, n=5. **p <0.01.

Table12. Haematological parameters of female rats in repeated dose28-day oral toxicity study of methanolic extract of aerial parts of *A. conyzoides.*

Parameter	Groupandtreatment			
	Control	500mg/kg	1000mg/kg	1500mg/kg
WBC(10^3/mm^3)	7.64±0.28	7.60±0.51	10.94±0.51**	11.76±0.37**
RBC(10^6/mm^3)	8.53±0.56	8.26±0.37	9.54±0.50	9.48±0.26
Platelets(10^3/ml)	810.60±18.69	810.80±9.86	805.40±14.54	811.20±7.32
Hb (g/dl)	13.38±0.32	11.82±0.36	11.20±0.16	10.14±0.26

Note: Data were analyzed by oneway ANOVA followed by Dunnett's multiple comparisons test and compared with control group. Values are mean ±SEM, n=5,**p <0.01.

Table 13. Serum biochemical parameters of male rats in repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *A. conyzoides.*

Parameter	Groups			
	Control	500mg/kg	1000mg/kg	1500mg/kg
ALT(U/L)	35.24±1.59	40.82±0.74	51.28±.093	60.82±2.80
AST(U/L)	116.19±1.41	121.50±1.1	137.89±1.49*	150.73±4.73*
BUN(mg/dl)	36.31±1.51	39.31±1.1	43.90±1.39**	46.97±1.79**
Creatinine (mg/dl)	0.28±0.03	0.46±0.03	0.87±0.04**	0.98±0.02**
Calcium (mg/dl)	13.11±0.19	13.43±0.22	13.55±0.39	14.05±0.50
Phosphorous(mg/dl)	6.09±0.20	5.26±0.28	5.56±0.21	5.14±0.29

Note: Values are mean ±SEM, n=5.Data were compared with control group. *p<0.05, **p<0.01.

Table 14. Serum biochemical parameters of female rats in repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *A. conyzoides.*

Parameter	Groups			
	Control	500mg/kg	1000mg/kg	1500mg/kg
ALT(U/L)	37.91±1.65	41.36±0.89	57.55±2.66	59.03±4.44
AST(U/L)	116.01±2.87	121.71±1.59	138.68±1.24*	154.15±3.12*
BUN(mg/dl)	37.06±1.05	39.05±1.16	48.02±0.89**	50.54±1.58**
Creatinine (mg/dl)	0.47±0.04	0.52±0.05	0.80±0.05**	0.89±0.05**
Calcium (mg/dl)	13.16±0.32	12.73±0.45	12.62±0.42	14.55±0.47
Phosphorous(mg/dl)	5.16±0.26	5.43±0.25	4.98±0.17	4.96±0.30

Note: Data were analyzed by one way ANOVA followed by Dunnett's multiple comparisons test and compared with control group. Values are mean ± SEM, n=5*p<0.05. **p<0.01.

Pathology of rats in Acute Oral and Repeated Dose 28-day Oral toxicity studies of methanolic extract of aerial parts of *A. conyzoides*

Acute toxicity study

Gross pathology

At necropsy organs *viz*., liver, kidney, heart, spleen and duodenum of control group rats were normal with no abnormalities. Enlargement and congestive changes were observed in kidney, liver and heart in groups treated with MEAC.

Histopathology

Group I (control group)

The histological appearance of the organs *viz*., heart, kidney and liver in the control group were found to be normal. Section of heart showed normal cardiomyofibres in myocardium (Plate 1). Section of liver lobules showed central vein surrounded by compactly arranged hepatocytes in branched cords and well-formed portal triads (Plate 2). Section of spleen showed well projected morphology of red pulp and white pulp with normal appearance of lymphoid cells (Plate 3) with well-formed germinal center in white pulp (Plate 4). Section of duodenum showed well defined villi with columnar epithelial cells, goblet cells and duodenal crypts (Plate 5). Section of kidney showed normal architecture of cortex with well-defined glomeruli, convoluted tubules with compact cuboidal renal epithelial cells (Plate 6).

Groups treated with methanolic extract of *A. conyzoides* aerial parts

Group II (300 mg/kg)

Section of liver revealed the vascular changes like central venous congestion and mild sinusoidal congestion (Plate 7). Section of kidney showed the vascular changes such as congestive and hemorrhagic lesions in medulla (Plate 8). Glomerular congestion, interstitial vascular congestion and mild degenerative changes were observed in renal tubules in the cortex (Plate 9). Section of spleen showed mild lymphoid proliferation around splenic arteriole in periarteriolar lymphatic sheath area in white pulp with mild congestion of red pulp (Plate 10). Section of heart revealed mild congestive changes of capillaries in myocardium (Plate 11). Section of duodenum indicated the desquamative changes and erosion of surface epithelium of villi with mild goblet cell hyperplasia (Plate 12).

Group III (2000 mg/kg)

Section of liver indicated the diffuse congestion in central and portal veins and sinusoidal congestion and hemorrhages in sinusoidal space of the hepatic lobules (Plate 13 and 14). Section of kidney showed vascular changes such as intertubular vascular congestion, interstitial congestion in medulla, glomerular congestion, mild degenerative changes in renal tubules with protein precipitates (Plate 15 and 16). Section of spleen exhibited the congestive changes in red pulp in diffused manner and reduction of width of lymphoid sheath in white pulp (Plate 17). Section of heart showed the myocardial vascular congestion, hemorrhages with mild degenerative changes of cardiomyofibres (Plate 18). Section of duodenum indicated goblet cell hyperplasia with necrotic and desquamative changes in villi (Plate 19). Loss of surface epithelium in villi up to crypts with accumulation of catarrh mixed necrotic debris in the remnants of lamina propria of eroded villi were observed (Plate 20).

Group IV (5000 mg/kg)

Section of liver tissue showed the diffuse sinusoidal congestion, portal vascular congestion, haemorrhagic lesions around central vein (Plate 21) and also in hepatic parenchyma (Plate 22). Section of kidney showed the vascular changes like interstitial congestion, glomerular congestion and multifocal hemorrhages (Plate 23) and focal haemorrhagic lesions in the intertubular areas along with tubular degeneration in the cortex (Plate 24).Section of spleen showed the enlargement of germinal centre with proliferation of lymphoid cells (Plate 25), trabecular vascular congestion and red pulp congestion (Plate 26). Section of heart showed myocardial capillary congestion and separation of myocardial fibres indicating edema (Plate 27). Section of duodenum showed the desquamative changes in villi with mild congestion and goblet cell hyperplasia of diffused nature (Plate 28).

Repeated Dose 28-day Oral toxicity study

Gross pathology

At necropsy kidney, liver, heart, spleen and intestine of control group rats were normal with no abnormalities. In groups treated with MEAC, vascular changes such as congestion and haemorrhages, degenerative changes such as cell swelling with enlargement of organs were observed.

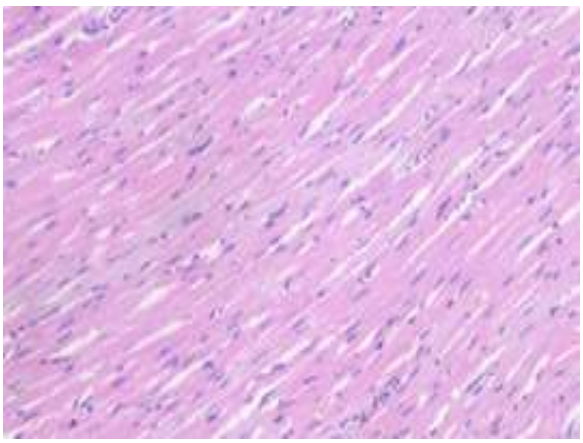


Plate 1

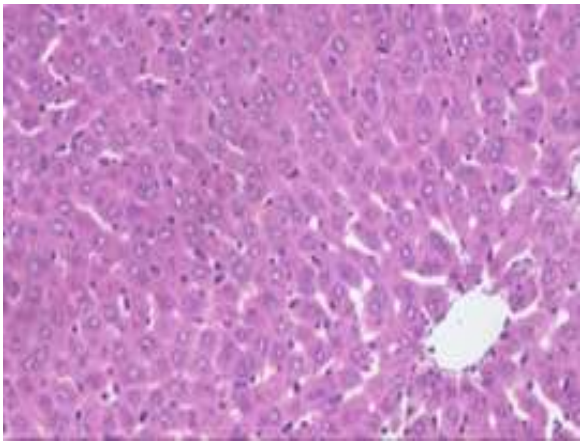
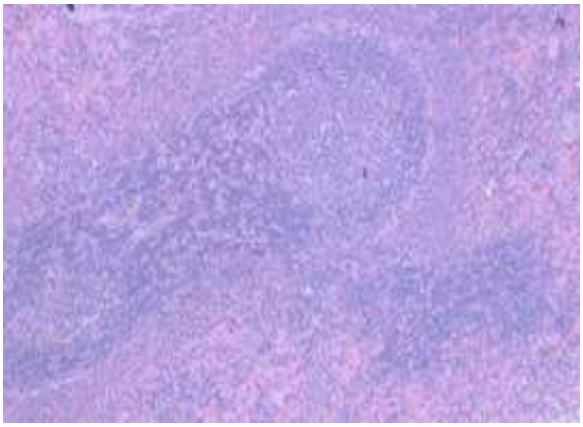


Plate 2



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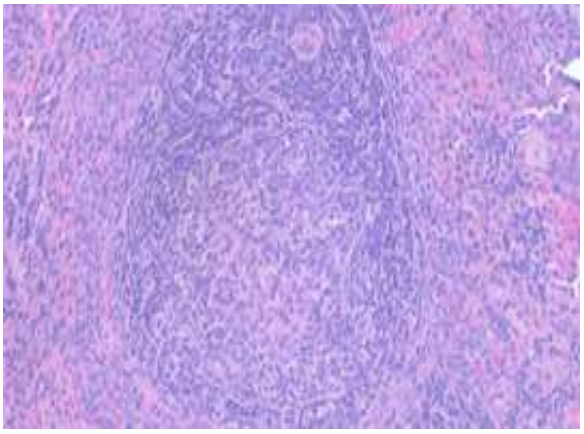

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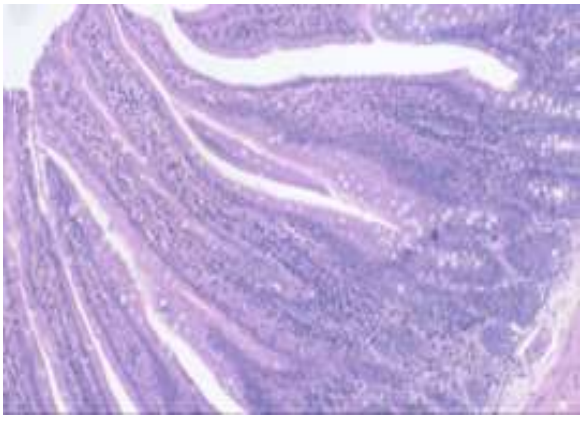

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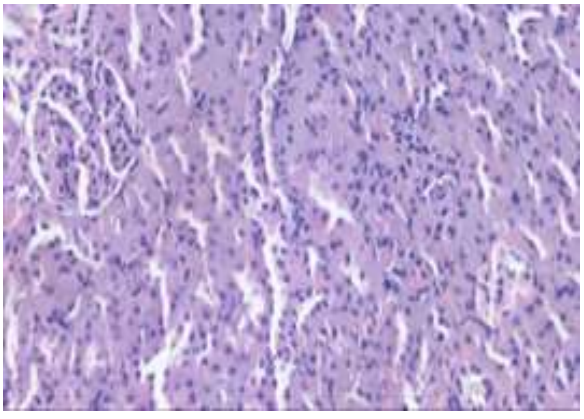
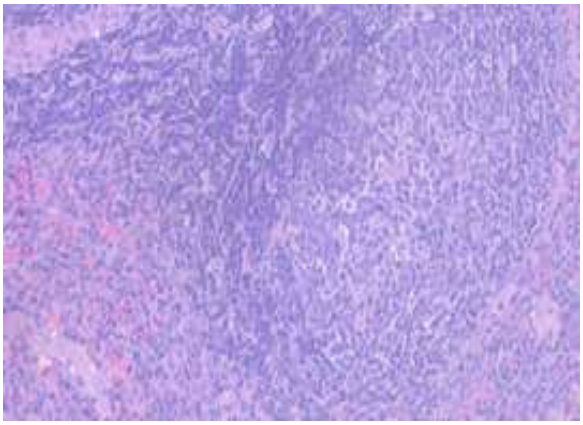


Plate 6

Acute oral toxicity study: Group I :(Control group)



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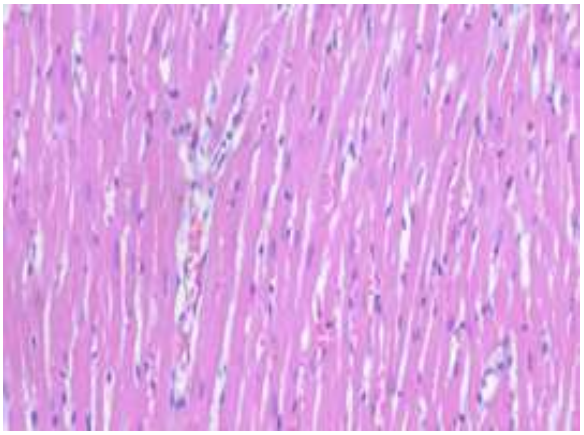
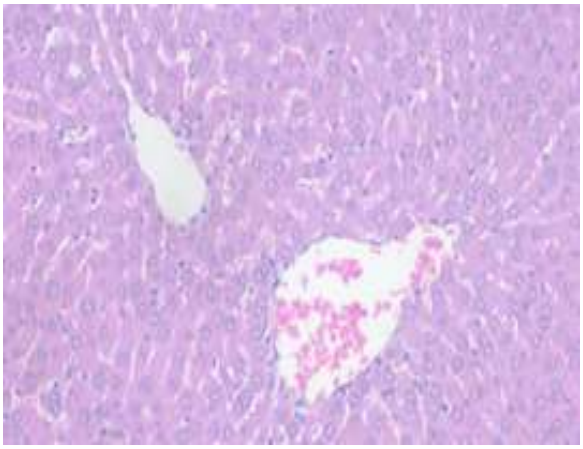


Plate 8



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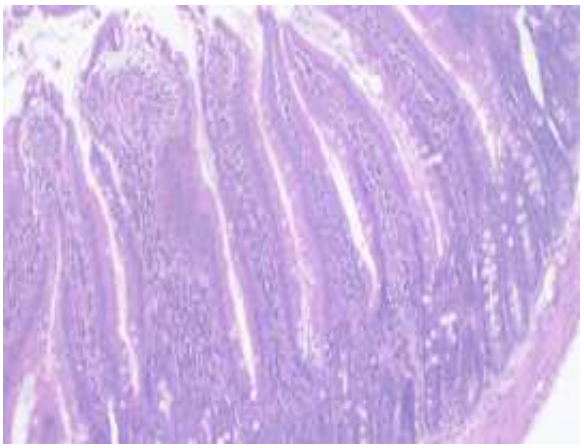

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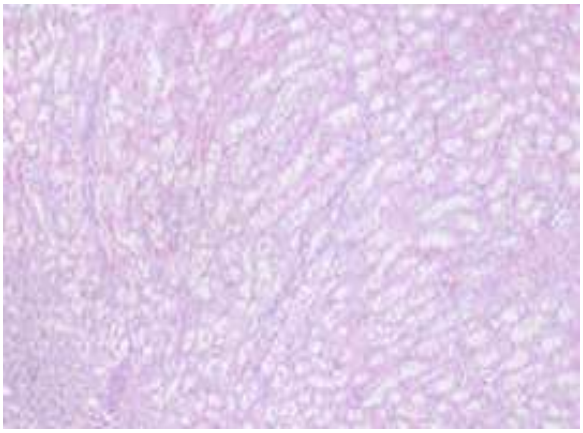

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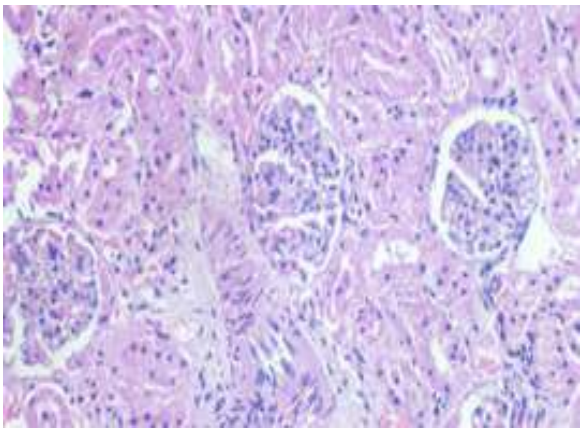


Plate 12

Group II: Acute oral toxicity study (300 mg/kg)

Histopathology, Group I (control)

The histological appearance of the organs namely heart, kidney, liver, duodenum and spleen in the control group were found to be normal.

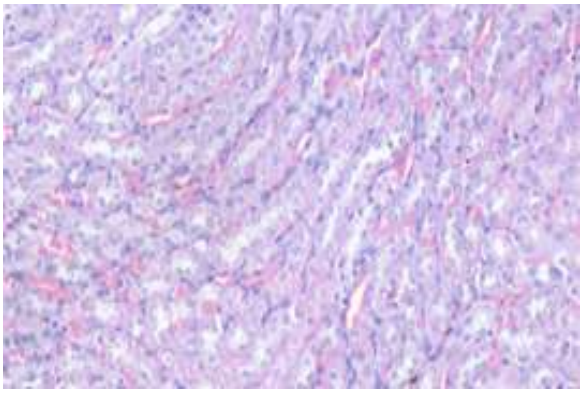
Groups treated with methanolic extract of *A. conyzoides* aerial parts

Group II (500 mg/kg)

Section of liver revealed the vascular changes such as mild sinusoidal, central venular congestion and focal hemorrhages in hepatic parenchyma (Plate 29) with mild degenerative changes in hepatocytes (Plate 30). Section of kidney showed mild congestive changes in glomerulus and interstitial vasculature with multifocal tubular degeneration in the cortex (Plate 31 and 32). Section of spleen showed the mild congestive changes and lymphoid cell hyperplasia in the germinal centre along with congestion of red pulp(Plate 33 and 34). Section of heart indicated myocardial capillary congestion and focal haemorrhagic lesions (Plate 35). Section of duodenum showed desquamation of surface epithelium along the tip of the villi, capillary congestion, goblet cell proliferation in duodenal crypts and necrotic debris in the entire lumen (Plate 36).

Group III (1000 mg/kg)

Section of liver showed diffuse sinusoidal and central vein congestion and parenchymatous haemorrhages (Plate 37), centrilobular congestion, sinusoidal congestion, diffuse cellular swelling and hydropic degeneration(Plate 38). Section of kidney showed interstitial vascular congestion and haemorrhagic changes in corticomedullary junction (Plate 39), focal degenerative changes in renal tubular epithelial cells with protein precipitates (Plate 40). Section of spleen showed congestive changes in the red pulp area along with lymphoid cell depletion in germinal centre (Plate 41) and splenic arteriolar congestion (Plate 42). Section of heart indicated the haemorrhagic changes, separation of myofibres with mild degenerative changes in myocardium (Plate 43). Section of duodenum showed the mild inflammatory changes, capillary congestion in lamina propria, desquamation of columnar epithelium on villi, presence of catarrh and mild goblet cell hyperplasia in crypts and villi (Plate 44).



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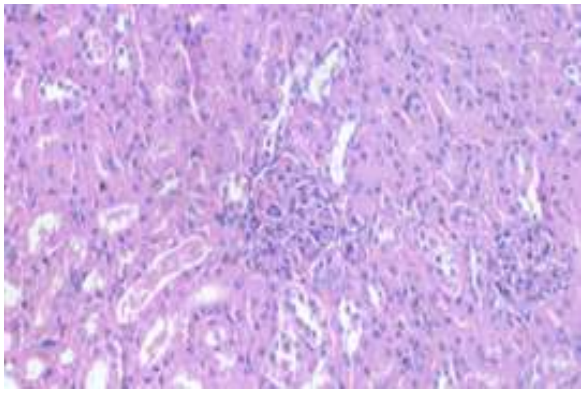

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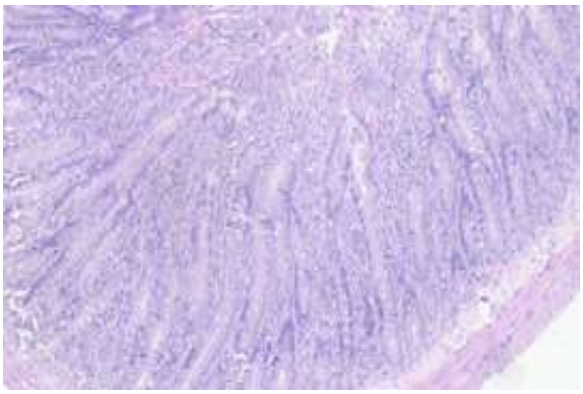

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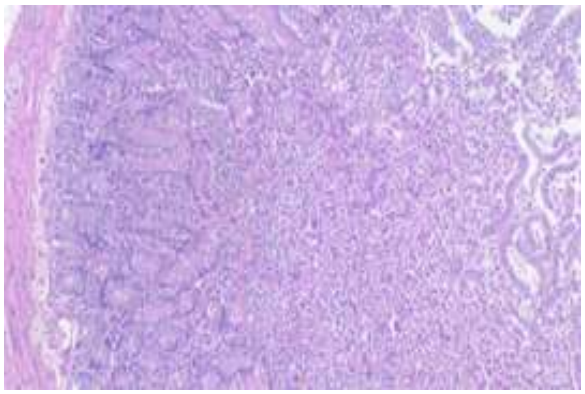

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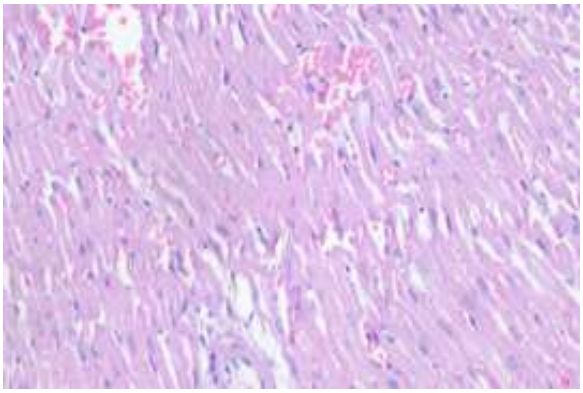

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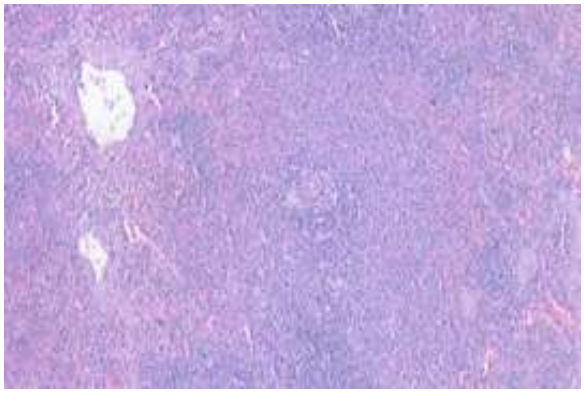

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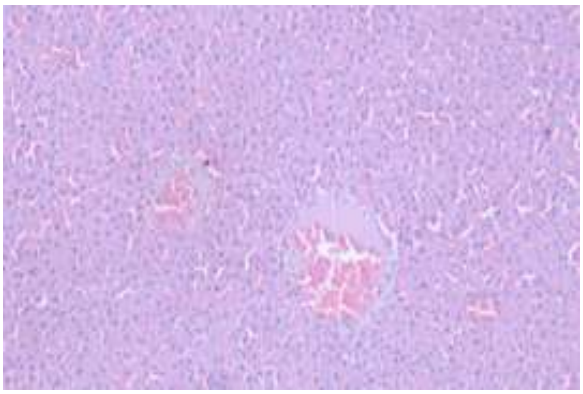

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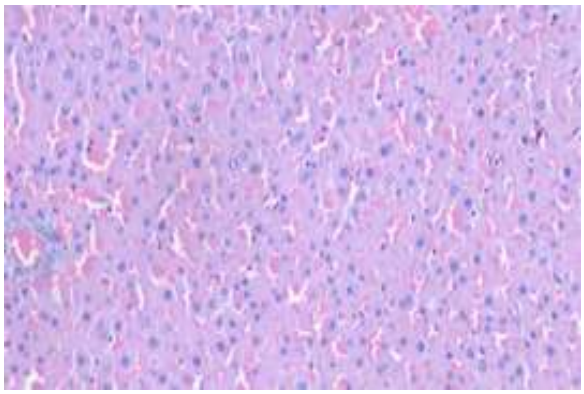


Plate 20

Group III: Acute oral toxicity study (2000 mg/kg)

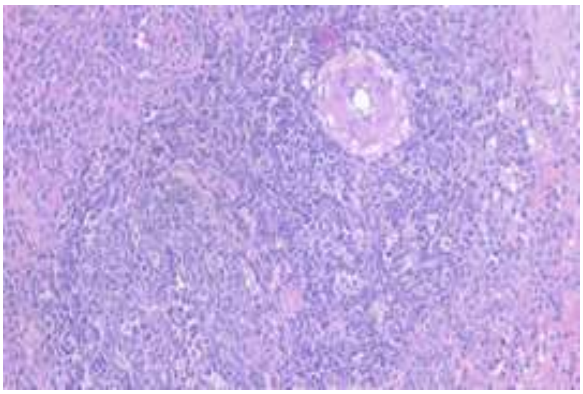
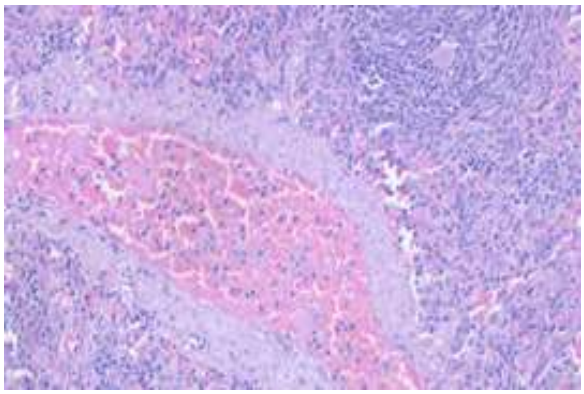


Plate 21



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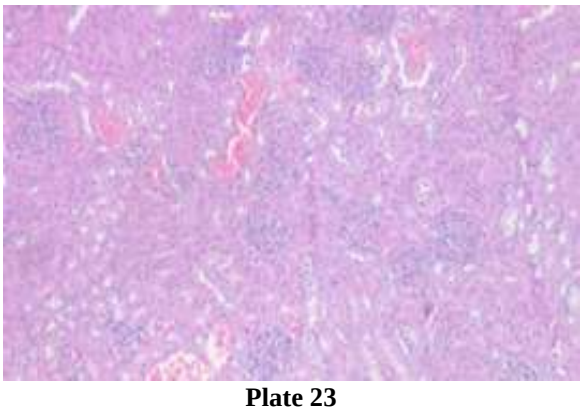

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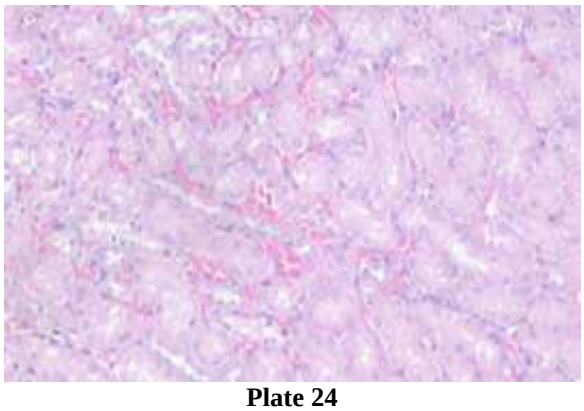

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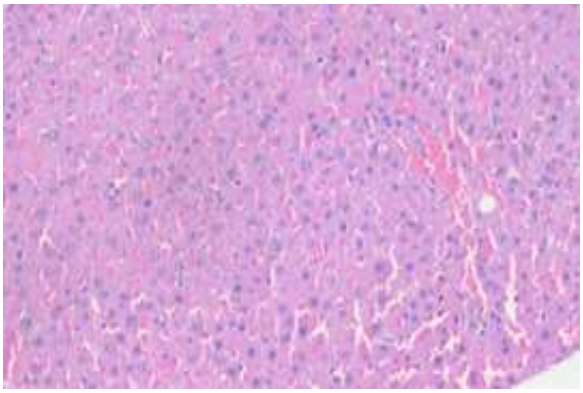

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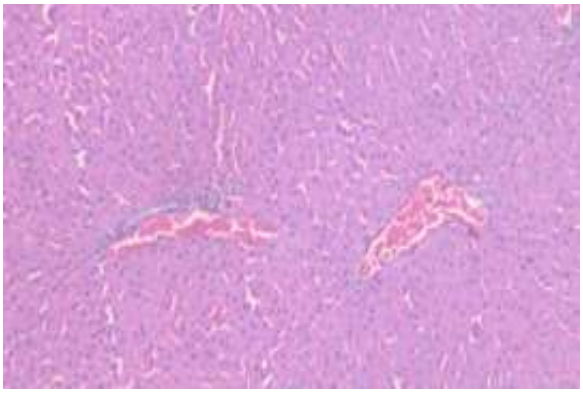

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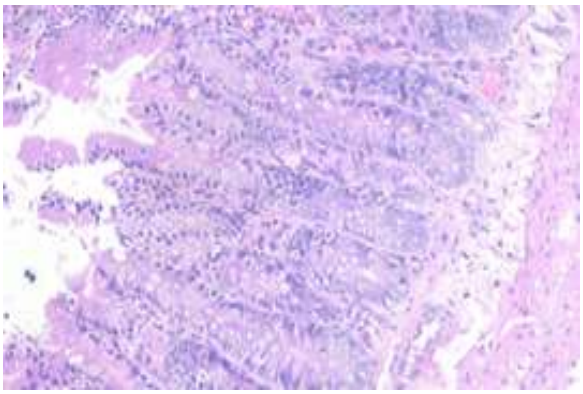

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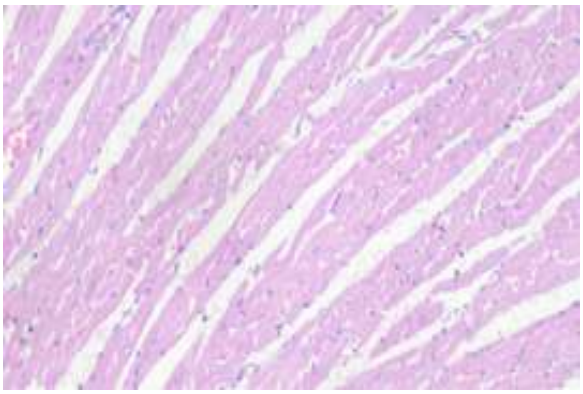


Plate 28

Group IV: Acute oral toxicity study (5000 mg/kg)

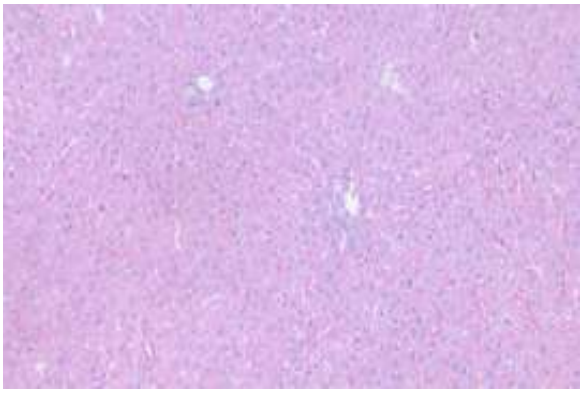


Plate 29

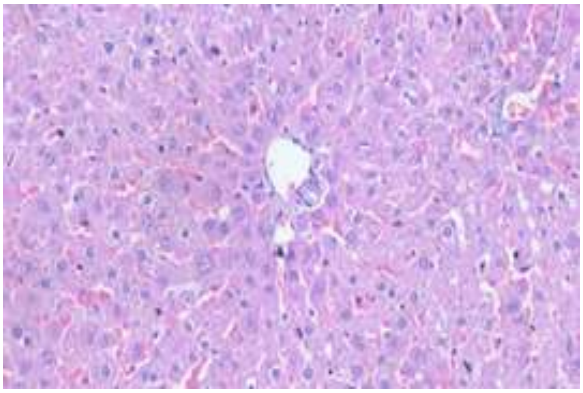
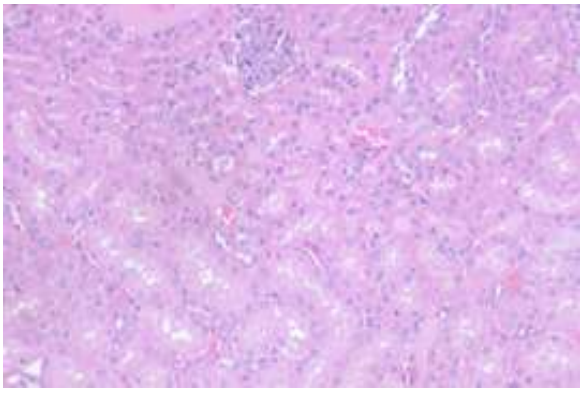


Plate 30



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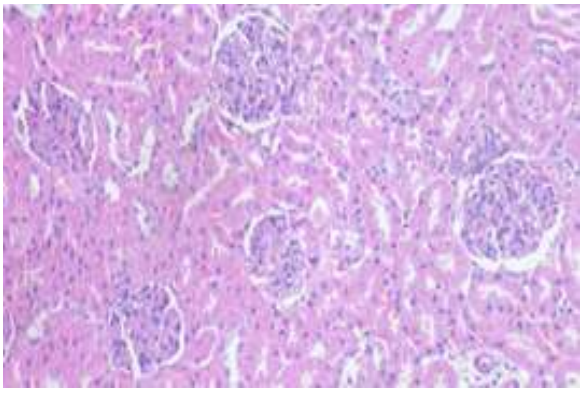

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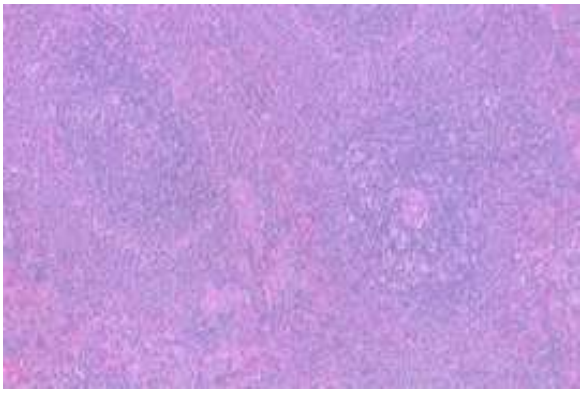
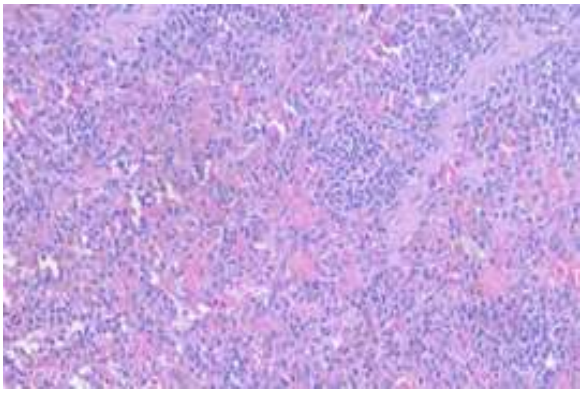


Plate 33



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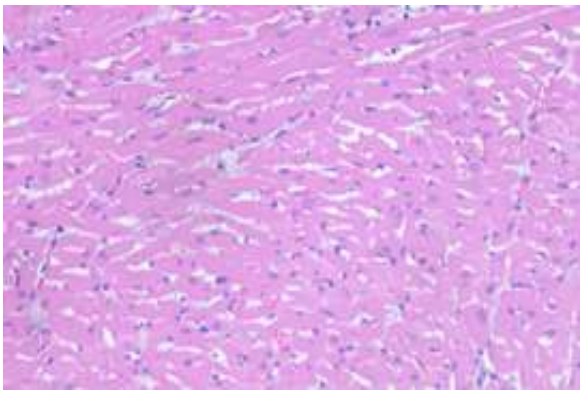

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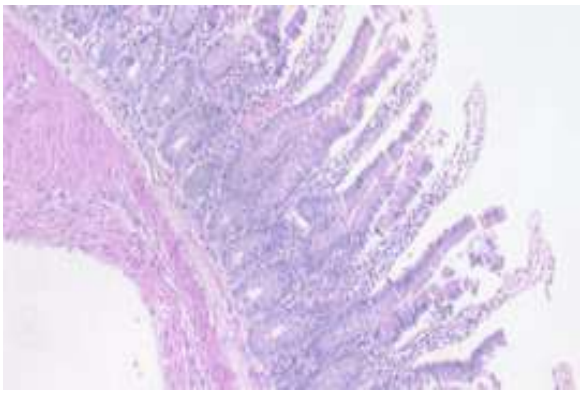
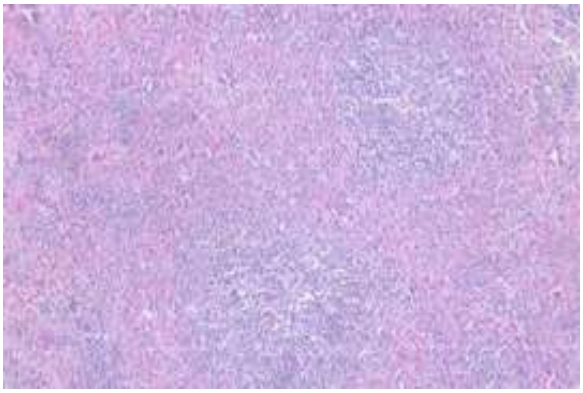


Plate 36

Group II: Repeated dose oral toxicity study (500 mg/kg)



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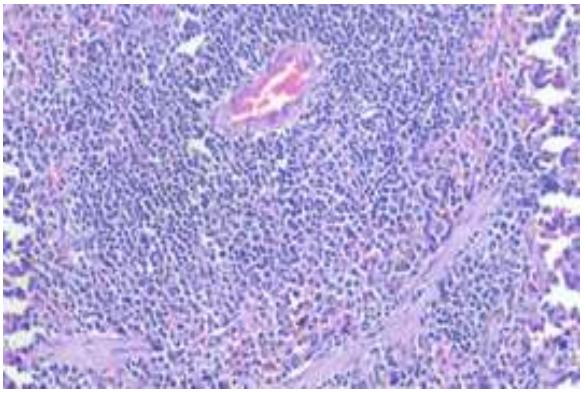

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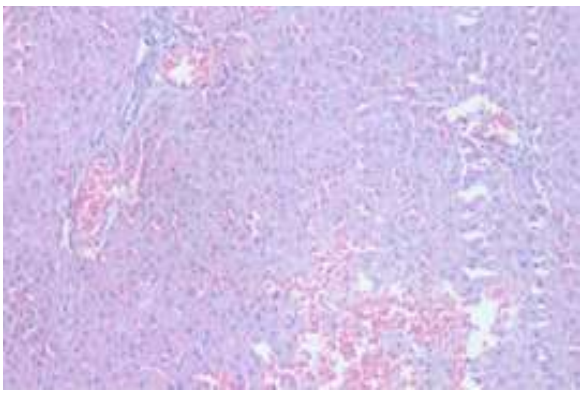


Plate 39

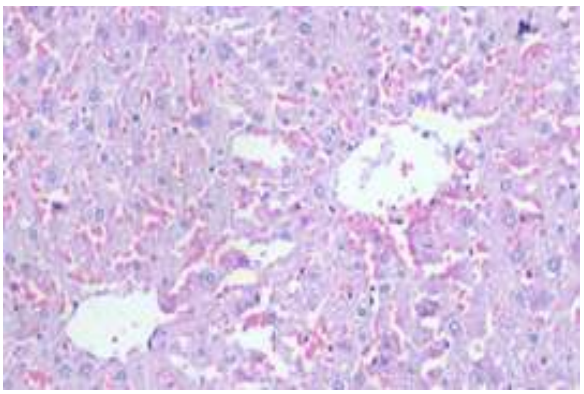
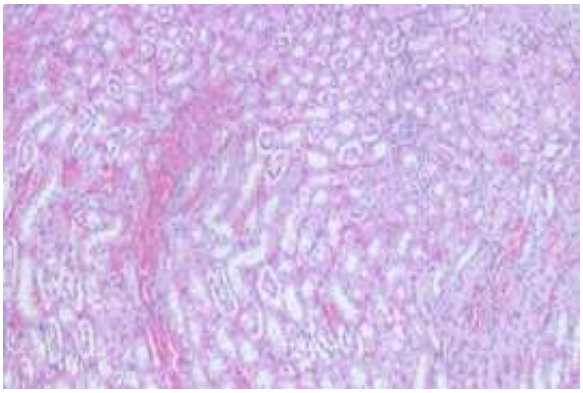


Plate 40



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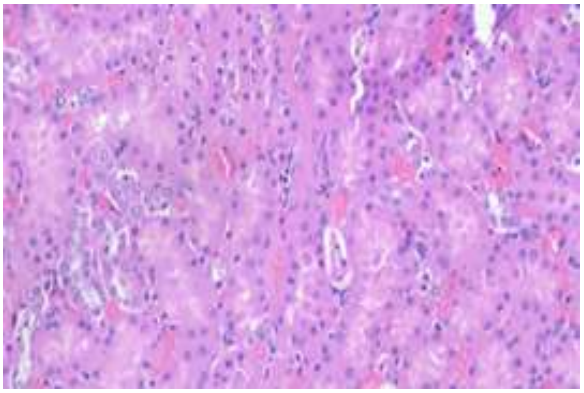

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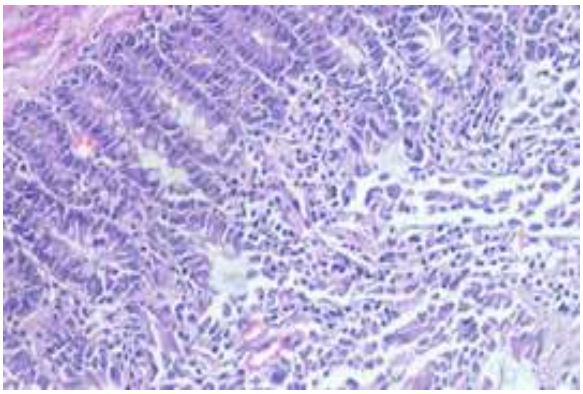

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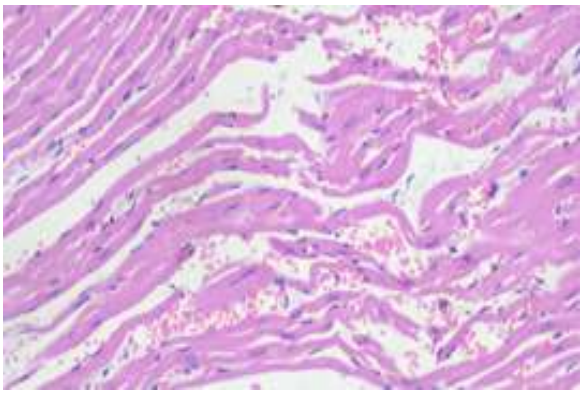

Plate 44

Group III: Repeated dose oral toxicity study (1000 mg/kg)

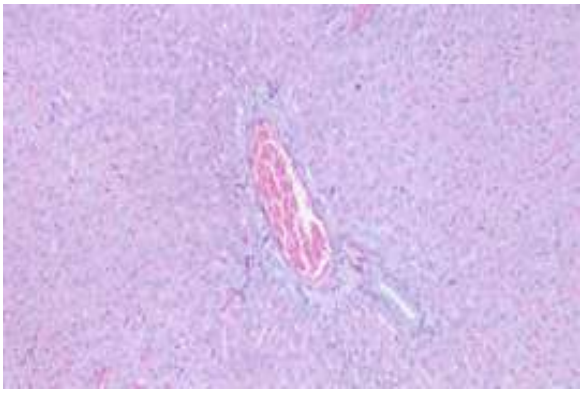

Plate 45

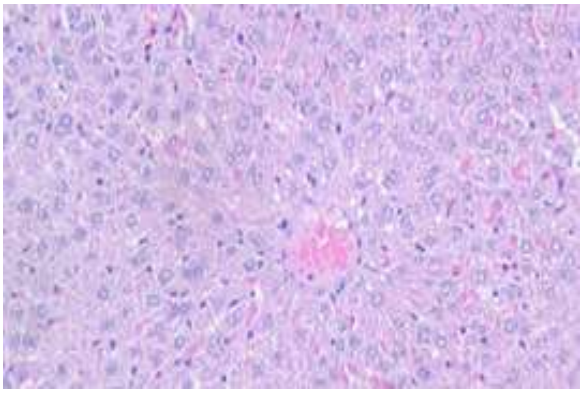

Plate 46

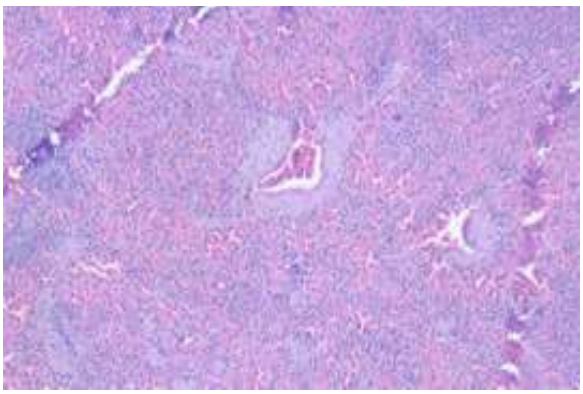

Plate 47

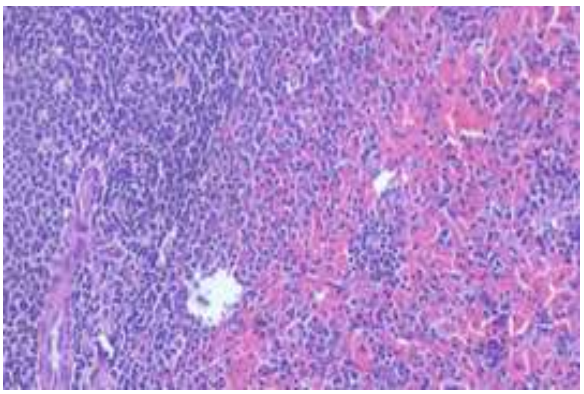

Plate 48

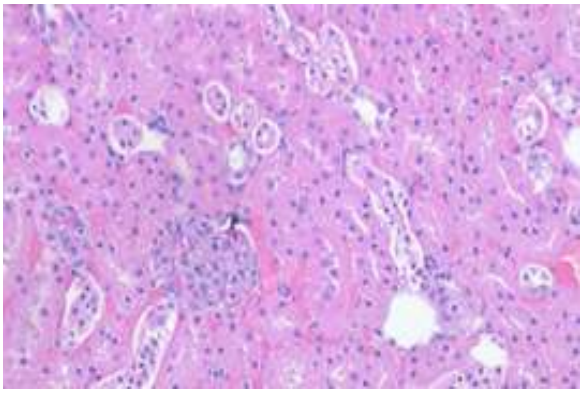

Plate 49

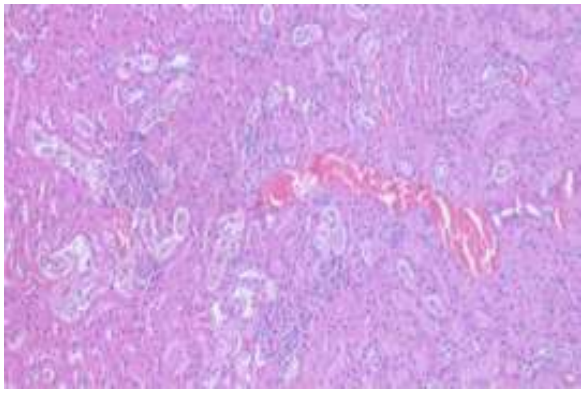

Plate 50

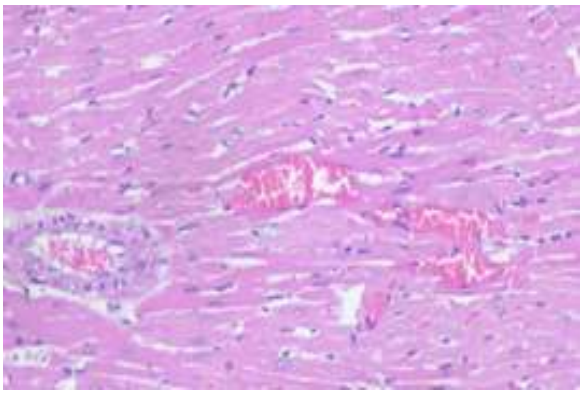

Plate 51

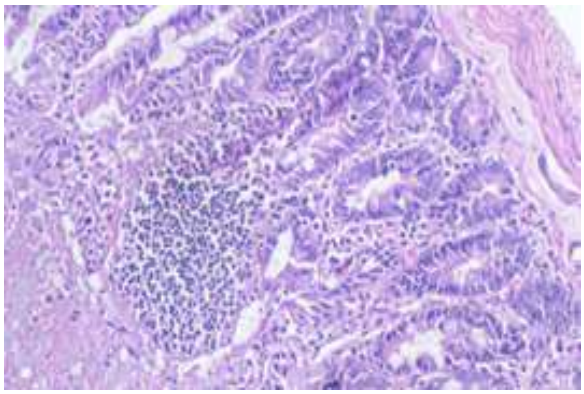

Plate 52

Group IV: Repeated dose oral toxicity study (1500 mg/kg)

Group IV (1500 mg/kg)

Section of livershowed the diffuse sinusoidal congestion, portal congestion with focal hepatitis and portal infiltration of inflammatory cells (Plate 45) and central venous congestion, granular degeneration of hepatocytes with acute cellular swelling and multifocal areas of fatty changes in hepatic parenchyma were observed (Plate 46). Section of kidney showed the vascular changes such asglomerular congestion, interstitial vascular congestion, hemorrhages, and degenerative changes in distal tubules with protein precipitates within the tubular lumen (Plate 47 and 48). Section of spleen showed the congested trabecular arteriole (Plate 49) and extensive congestion in red pulp with hemosiderosis characterized by deposition of golden brown pigments in splenic macrophages in red pulp (Plate 50). Section of heart showed the diffused congestion of myocardial capillaries along with mild degeneration of cardiomyofibres (Plate 51). Section of duodenum showed goblet cell hyperplasia in crypts, desquamation of villi up to the crypts with infiltrates of inflammatory cells in lamina propria of the eroded villi (Plate 52). The yield of methanolic extract was found to be 6.8 %. The pH of methanolic extract of *A. conyzoides* (MEAC) was 5.86. It was dark green in colour and the was dry and powdery in consistency with aromatic odour. In the present study the plant extraction was done using the methanol as solvent and the percent yield was 6.8. Methanol is a good solvent for most of the secondary metabolites of the plants and it is a more commonly used extraction technique. No universal extraction method is the ideal method and each extraction procedures is unique to the plants. Previously standardized methods can be used as a model method. The methanolic plant extraction process was used in this investigation, as it was also done by many others (Azwanida, 2015). Both the toxicity studies (Acute Oral toxicity study and repeated dose 28-day oral toxicity study) of methanolic extract of *A. conyzoides* aerial parts revealed no signs of toxicity and mortality at all the doses tested till completion of the study.

The results of the acute toxicity study indicate that the LD_{50} of the extract of *A. conyzoides* extract is more than 5000 mg/kg. In the acute toxicity study revealed that the plant was safe because, even at 5000 mg/kg hematological, serum biochemical and body weight comparison data showed no significant changes when compared to control animals, but mild histopathological changes such as circulatory disturbances in liver, kidney and heart were observed. The result of the present study is in accordance with the acute toxicity study of the ethanolic extract of *Ageratum conyzoides* leaves by Diallo *et al*., (2010) where they evaluated that no LD_{50} even at 5000mg/kg in rats upon oral route of administration and no mortality and morbidity observed in the study conducted by them, but they did not mention about the changes in the histopathological data of various organs of the animals

involved in the study. As per the guidelines of OECD 423, (2001) Acute Oral Toxicity – Acute Toxic Class Method the limit test is primarily used in situations where the investigator has information indicating that the test material is likely to be non-toxic or of low toxicity. Our results findings suggest that the extract at the limit dose tested is essentially non-toxic and safe in oral formulation. This result is in line with previous data from Moura *et al*., (2005) who reported that *A. conyzoides* LD_{50} in mice is more than 10,000 mg/kg. All the extract treated groups in acute toxicity study showed the normal increment in body weight with respect to time which was depicted in table 5 and fig. 3. There is no significance difference in increment of body weight gain among control and treatment groups.

The sub-acute repeated dose 28-day oral toxicity study of MEAC was carried out to determine the safety of the plant. All the extract treatment groups in the study showed reduced increment in body weight with respect to time which was statistically significant ($p< 0.01$) at 1000 mg/kg and ($p<0.05$) at 1500 mg/kg day 28 of the study when compared to control group. Change in body weight is an uncomplicated and sensitive indicator of the detrimental effects of drugs and chemicals (Bailey *et al*., 2004). In general, toxic nature of the drug could lead to abnormalities in body weight and a decrease in body weight could indicate a significant degree of toxicity, whereas reduced body weight gain represents only a mild form of intoxication (Michael *et al*., 2007). The results of present study are supported by the observations made in subacute toxicity study carried out by Diallo *et al*., (2010) inethanolic extract of *A. conyzoides* at the dose 500 and 1000 mg/kg with no significant difference in body weight in all experiment animals, although a slight decrease was observed in 1000mg/kg treatment group receiving ethanolic extract of *A. conyzoides*.

The haematological parameters of MEAC treated animals were normal and showed no significant increase in WBC, RBC and Platelets when compared with control group in the acute toxicity study. In subacute toxicity study the haematological parameters of 1000 mg/kg and 1500 mg/kg MEAC treated groups showed significant increase ($p<0.01$) in WBC There is no significant difference in RBC, Hb and platelet count among all treated groups when compared with parameters of control animals. The results are supported by the study conducted by Diallo *et al*., (2010) in the ethanolic extract of *A. conyzoides* leaves in rats. Results of haematological parameters indicated that there is non-significant increase ($p<0.05$) in the PCV, Hb, RBC, Platelet, MCH, MCHC, MCV, and MCHC in all treatment groups when compared to control. The increase in erythrocytes (RBC), leukocytes (WBC) may be due to overproduction of hematopoietic regulatory elements such as colony stimulating factors, erythropoietin and thrombopoietin by the stromal cells and macrophages in the bone marrow thus providing the local environment for hematopoiesis (Rhiouani *et al*., 2008).

It was evident from the observed considerable rise in WBC values that a rise in WBC is a typical response of rats to xenobiotics which impact their regular biological functions. The leukocytosis seen in the current study suggests that the immune system is being stimulated, defending the rats against infections that could have been brought on by toxins as well as secondary infections that could have developed as a result of the poor wellness of the rats. Leukocytosis which may be directly proportional to the severity of the causative stress condition, may be attributed to an increase in leukocyte mobilization (Celik and Suzek, 2008). In the acute toxicity study, only AST is increased significantly ($p < 0.05$) in 5000mg/kg treated group and all other serum biochemical parameters were normal in all the extract treatment groups when compared with control group. In subacute toxicity study, AST is increased significantly ($p<0.05$) in 1000mg/kg and 1500mg/kg MEAC treated groups but BUN and creatinine were also increased significantly ($p<0.01$) in 1000mg and 1500mg/kg treated groups. All other parameters were normal when compared with control animals.

The results are supported by the study conducted by Diallo *et al*., (2010) in the ethanolic extract of *A. conyzoides* leaves in rats. Results of serum biochemical parameters indicated a non-significant increase ($p<0.05$) in the AST, ALT, Creatinine, Total proteins and Alkaline phosphatase all treatment groups when compared to control but there is significant decrease ($p<0.05$) in Urea level in 1000mg/kg treated group. Serum Aspartate transaminase, Alanine transaminase, Alkaline phosphatase and bilirubin are the most sensitive markers employed in the diagnosis of hepatic ailments. These enzymes will leak into the bloodstream and thus increased concentration is detected in peripheral blood in hepatic dysfunction. Thereby the assessment of extent of liver injury can be estimated by recording the circulating levels of these cytoplasmic enzymes (Vijayakumar *et al*., 1997). Serum biochemical parameters like AST, ALT, urea and creatinine evaluation is still considered as reliable indices of liver and kidney health even though their sensitivity and specificity are limited (Campion *et al*., 2013). Histology of organs in control group in both the toxicity studies was found to be normal. Histopathology of organs in extract treated groups in both acute and repeated dose 28-day toxicity study revealed microscopic changes in organs *viz.*, liver, kidney, heart, spleen and duodenum with progressive vascular, degenerative and inflammatory changes. The severities of the microscopic lesions were dose dependent.

Histopathology of the liver and kidneys of animals in the present subacute toxicity study revealed congestive, edematous, hemorrhagic and degenerative changes whose intensity increased with dose among the treated group in comparison with the control group. The changes in the liver may be due to the presence of several alkaloids, including 1.2-desifropirrolizidinic and licopsamine which can have hepatotoxic activity indicate that the extract might have toxic potential on liver with increasing dose (Moreira *et al*., 2018).

The congestive changes observed were indicative of mild acute toxicosis affecting in a generalized manner in visceral organs, which can be attributed to continuous

exposure of the animals to the herbal preparation. Desquamation of duodenal epithelium indicates affection of GIT upon continuous feeding of the herbal preparation indicating non-tolerance despite an increase in body weight. Histopathological findings from the present study are in accordance with the study conducted by Adebayo *et al*., (2010) where they investigated the toxicological effects of precocene II which was isolated and purified from *A. conyzoides* plant parts studied in Sprague Dawley rats. Precocene II was isolated from the petroleum ether fraction of hydroalcoholic extract of *A. conyzoides*. The histopathological examination of liver, kidney and spleen excised from these rats had showed some vascular and degenerative changes.

CONCLUSION

The present study was conducted to evaluate pharmacological and toxicological properties of the *A. conyzoides* plant extract. The acute oral and repeated dose 28-day oral toxicity of methanol extract was evaluated in Wistar albino rats. The physical nature of the methnol extract of *A. conyzoides* aerial parts (MEAC) revealed the acidic pH, dry and powdery consistency after extracting with the solvent methanol. Acute oral toxicity study and repeated dose 28-day oral toxicity study was conducted in Wistar albino rats. The maximum tolerable dose of MEACin female rats was found to be more than 5000 mg/kg. The general condition of the animals did not change and all the animals remained in normal health condition throughout the experiment. Haematology (WBC) and serum biochemical parameters (AST, BUN and creatinine) of extract treated rats showed significant differences from control groups in subacute toxicity study which might be attributed to presence of various phytochemicals (saponins, alkaloids, flavonoids, phenolic compounds, phytosterols and triterpenoids) in the extract. Toxicity studies revealed no mortality but organ specific histopathological changes were evident which might be due to presence of secondary metabolites like alkaloids, flavonoids, phenolic compounds and triterpenoids in the extract. These may be correlated with significant raise in AST, BUN and creatinine levels in serum which could be attributed to the phytoconstituents present in the extract. Further, the histopathological changes in organs *viz*., liver, kidney and heart in the MEAC treated rats showed dose dependent and organ specific circulatory, degenerative and inflammatory changes were noted. Very mild to moderate vascular changes such as congestion and haemorrhages, degenerative changes with granular changes and infiltration of inflammatory cells were suggestive of effect of phytochemicals present in the extract. The histopathological changes were dose dependent and the changes were very mild to moderate and are not the major signs to conclude the extract is toxic in acute stage, in repeated dose toxicity study showed the alarming signs where the animals fed solely on *A. conyzoides* suffered from toxicity. In future studies, long term toxicity studies are needed to ensure the toxic potency of the same plant. It can be concluded that, further research can be done to explore the role of individual phytochemical constituents responsible for anti-inflammatory, analgesic activity and toxic effects exerted by the extract in rats.

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REFERENCES

Abubakar, A.R. and Haque, M., (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy & Bioallied Sciences*, *12*(1), 1-10.

Adebayo, A. H., Zeng, G. Z., Zhang, Y. M., Ji, C. J., Akindahunsi, A. A., & Tan, N. H. (2010). Toxicological evaluation of precocene II isolated from Ageratum conyzoides L. (Asteraceae) in Sprague Dawley rats. *African Journal of Biotechnology*, 9(20), 10-22.

Antai, A. B., Eyong, E. U., Eteng, M. U., Itam, E. H., Eko, M. E., & Ita, S. O. (2009). Serum protein and enzyme levels in rats following administration of ethanolic leaf extract of Ageratum conyzoides (goat weed). *Nigerian Journal of Physiological Sciences*, *24*(2), 117-120.

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromat Plants*, *4*(196), 2167-2412.

Bailey, S. A., Zidell, R. H., & Perry, R. W. (2004). Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint. *Toxicologic Pathology*, 32(4), 448-466.

Burkill, H. M., Dalziel, J. M., & Hutchinson, J. (1985). Royal Botanic Gardens. The useful plants of West tropical Africa. Kew: Royal Botanic Gardens; 1985, 960-976.

Campion, S., Aubrecht, J., Boekelheide, K., Brewster, D. W., Vaidya, V. S., Anderson, L., & Goodsaid, F. (2013). The current status of biomarkers for predicting toxicity. *Expert opinion on drug Metabolism & Toxicology*, 9(11), 1391-1408.

Celik, I., & Suzek, H. (2008). The hematological effects of methyl parathion in rats. *Journal of Hazardous Materials*, *153*(3), 1117-1121.

Diallo, A., Eklu-Gadegkeku, K., Agbono, A., Aklikokou, K., Creppy, E. E., & Gbeassor, M. (2010). Acute and sub-chronic (28-day) oral toxicity studies of hydroalcohol leaf extract *of Ageratum conyzoides* L (Asteraceae). *Tropical Journal of Pharmaceutical Research*, *9*(5), 463-467.

Diallo, A., Eklu-Gadegbeku, K., Amegbor, K., Agbonon, A., Aklikokou, K., Creppy, E., & Gbeassor, M. (2014). In vivo and in vitro toxicological evaluation of the hydroalcoholic leaf extract of Ageratum conyzoides L.(Asteraceae). *Journal of Ethnopharmacology*, *155*(2), 1214-1218.

Luna, L.G., (1968). Manual of Histological Staining Methods of the Armed Forces Institute of Pathology. Edn. 3rd., McGraw Hill Book Co., New York., *pp* 1-46.

Michael, B., Yano, B., Sellers, R. S., Perry, R., Morton, D., Roome, N., & Schafer, K. (2007). Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. *Toxicologic Pathology*, 35(5), 742-750.

Moreira, R., Pereira, D. M., Valentão, P., & Andrade, P. B. (2018). Pyrrolizidine alkaloids: chemistry, pharmacology, toxicology and food safety. *International Journal of Molecular Sciences*, *19*(6), 1668.

Moura, A. C. A., Silva, E. L. F., Fraga, M. C. A., Wanderley, A. G., Afiatpour, P., & Maia, M. B. S. (2005). Antiinflammatory and chronic toxicity study of the leaves of Ageratum conyzoides L. in rats. *Phytomedicine*, *12*(1-2), 138-142.

Odey, M. O., Iwara, I. A., Udiba, U. U., Johnson, J. T., Inekwe, U. V., Asenye, M. E., & Victor, O. (2012). Preparation of plant extracts from indigenous medicinal plants. *International Journal of Science and Technology*, *1*(12), 688-692.

OECD 407, (1995). Organization for Economic Co-operation and Development [OECD] guideline for the testing of chemicals, repeated dose 28-day oral toxicity study in rodents.

OECD, 423, (2001). Organization for Economic Co-operation and Development [OECD] guideline for the testing of chemicals, Acute oral toxicity- Acute toxic class method.

OECD, (2000), Organization for Economic Co-operation and Development [OECD] guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation, 2000.

Palmer, P. A., Bryson, J. A., Clewell, A. E., Endres, J. R., Hirka, G., Vértesi, A., & Szakonyiné, I. P. (2019). A comprehensive toxicological safety assessment of an extract of Ageratum conyzoides. *Regulatory Toxicology and Pharmacology*, *103*, 140-149.

Pandey, M. M., Rastogi, S., & Rawat, A. K. S. (2013). Indian traditional ayurvedic system of medicine and nutritional supplementation. *Evidence-Based Complementary and Alternative Medicine*, *2013*.

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy Reviews*, 6(11), 1-6.

Rahman, A., Akter, N., Rashid, H., Ahmed, N. U., Uddin, N., & Islam, S. (2012). Analgesic and anti-inflammatory effect of whole Ageratum conyzoides and *Emilia sonchifolia* alcoholic extracts in animal models. *African Journal of Pharmacy and Pharmacology*, 6(20), 1469-1476.

Rhiouani, H., El-Hilaly, J., Israili, Z. H., & Lyoussi, B. (2008). Acute and sub-chronic toxicity of an aqueous extract of the leaves of Herniaria glabra in rodents. *Journal of Ethnopharmacology*, *118*(3), 378-386.

Shinwari, Z. K., & Gilani, S. S. (2003). Sustainable harvest of medicinal plants at Bulashbar Nullah, Astore (northern Pakistan). *Journal of Ethnopharmacology*, 84(2-3), 289-298.

Singh, A., Singh, S., Krishna, G., & Pandey, A. K. (2014). Traditional knowledge and ethnopharmacological perspectives of herbal medicine in India: a review. *Journal of Medicinal Plants*, *2*(1), 20-30.

Srivastava, K. A., 2018. Significance of medicinal plants in human life. *Synthesis of Medicinal Agents from Plants*. Publication-Elsevier Ltd. Chapter -1: 1-24. http://dx.doi.org/10.1016/B978-0-08-102071-5.00001-5.

Verma, P. K., Sultana, M., Raina, R., Prawez, S., Pandita, S., Jamwal, N., & Mir, A. H. (2013). Hepatoprotective Effects of Ageratum conyzoides L. on biochemical indices induced by acetaminophen toxicity in Wistar rats. *Journal of Applied Pharmaceutical Science*, *3*(4), 23-27.

Vijayakumar, G., Srinivasan, S. R., & Dhanapalan, P. (1997). Preliminary study on the effect of indigenous plant Sesbania grandiflora in carbon tetrachloride induced hepatitis in calves. *Indian Journal of Veterinary Medicine*, *17*, 17-21.

WHO, (1993). World Health Organization. Research Guideline for Evaluating the Safety and Efficacy of Herbal Medicines. WHO, Manila, Philippines, pp 2.