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ACUTE AND 28-DAY REPEATED DOSE TOXICITY OF *AVERRHOA CARAMBOLA* LEAVES ETHANOLIC EXTRACT IN RODENTS

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Keywords:

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ABSTRACT: *Averrhoa carambola* -L. (Oxalidaceae) has great importance in the Indian traditional system of medicine for controlling diabetes and piles: the combined extracts of leaves as well as fruits of *A. carambola* are used for the treatment of jaundice, gastric problems and other chronic conditions as well as in food supplements. The objective underlying this experiment is to determine the safety profile of the plant used on a daily basis as well as for prolonged exposure to subjects. In this study, we analyzed the influence of ethanol extract of *Averrhoa carambola* at a dose level of 2000 mg kg-^{1} bw on the acute and with a graded dose levels from 250, 500, 1000 and 2000 mg kg-^{1} bw for 28-day repeated-dose toxicity test in Wistar rats. The analysis revealed that the repeated oral dose of *Averrhoa carambola* L. at a dose level upto 2000 mg kg-^{1} bw for 28 days did not induce any biochemical, hematological, morphological, histopathological signs of toxicity. High dose of *Averrhoa carambola* L. extract did not inhibit the body weight gain, while the food consumption decreased slightly for the nutritional effects of it in both male and female Wistar rats. In the histopathological findings, no toxic signs were observed on any of the tissues and organs examined and thus determining its safety profile on prolonged exposure.

INTRODUCTION: The conception of Indian traditional system of medicine had served from the prehistoric ages as the promising sources of therapeutics for the mankind. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world [1].

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Now a day, it has been seen that slowly and steadily, the modern synthetically developed drugs are replacing the ones obtained from natural source because of their well defined and systematic scientific approaches owing to its safety and curative concern.

It has been seen that natural products and herbal formulations under the roof of modern medicines are administered in the majority of the disease conditions without emphasizing on proper dose monitoring, regulatory enforcement and toxicological effect that might result from such an extended use. So setting up of a regulatory toxicological profile is very essential for natural products. As per the Indian Drugs and Cosmetics Act, currently there are no separate categories of herbal drugs or allopathic medicines [2].

Averrhoa carambola L. (Oxalidaceae) has been reported to have potential therapeutic properties in the Indian traditional system of medicine. The aqueous bark extract is used for controlling diabetes and treatment of piles by Meitei-Pangals of Manipur [3]. The combined extracts of leaves and fruits of *A. carambola* is used by the Tai-Khamyangs and Dimasa tribe for the treatment of jaundice, gastric problems and chronic conditions like olguria, boils, pyodermas, postpartum edema and traumatic injuries [4, 5].

Leaves of *Averrhoa carambola* contains β-sitosterol, apigenin-6-*C*-β-L-fucopyranoside, apigenin6-*C*-(2"-*O*-α-L-rhamnopyranosyl)-β-L-fucopyranoside, Apigenin6-*C*-(2"*O*-α -L-rhamnopyranosyl)-β -D-glucopyranoside [6]. Because of the utility of *Averrhoa carambola* against various diseases/disorders, the present investigation was considered so as to accumulate enough evidences about the regulatory toxicological profile of leaves of *Averrhoa carambola*.

MATERIALS AND METHODS:

Plant material and extraction:

Leaves of *Averrhoa carambola* were munificently collected from Cachar district of Southern Assam, India. Macroscopic and microscopic examinations as well as thin-layer chromatographic technique were used to confirm the authenticity of the plant material and a voucher specimen (CIL/H/AC/SM-001ANG/2013) has been deposited in our laboratory for future reference. The leaf samples were shade dried and milled followed by pulverization at 24 ± 2º C. 5.5 kg of the pulverized plant material was added to 10 L of 95% ethanol at room temperature for 5 days and was shaken occasionally. The ethanol extract of *A.carambola* leaves (EEAC) was concentrated to dryness in a rotary evaporator which was followed by lyophilization, yielding an approximate weight of 610 g of dry residue (w w^{-1} yield: 12.2%). EEAC was kept at -20ºC for further analysis.

Animals:

Rat approximately (215 ± 3) g body weight of both the sexes were taken for consideration. The rat were grouped and housed in poly acrylic cages (38 x 23 x 10 cm) with not more than 6 animal per cage and maintained under standard laboratory condition (temperature:- 25 ± 2°C and dark/light cycle 14/10 h) and relative humidity of 55 ± 5%. They were allowed free excess to standard dry pellet diet (Hindustan lever, Kolkata, India) and water *ad libitum.* The rat were acclimatized to laboratory condition for 7 days before starting the experiment. The experimental protocol was strictly designed in accordance to the "Guide for the care and use of laboratory animals" [7] and related ethical regulations set by the Institutional Animal Ethical Committee of Assam University, Silchar.

Acute oral toxicity study:

The experiment was done in accordance with the guide and ethics set by the OECD guidelines-423 and OECD guidelines-425 [8, 9]. All the animals were fasted overnight before the commencement of the experiment. EEAC at a dose level of 2000 mg kg^{-1} body weight in the form of suspension with carboxy methyl cellulose and 10 mL $kg^{-1}$of the sample was fed by gavage thrice daily. The time interval of EEAC administration was set to 8 h so as to mimic the dose frequency of humans. Animals were observed individually once first 30 mins after dosing and periodically during first 24 h, and daily thereafter for a total of 14 days. On day 15, rats were euthanized, gross observations were recorded and necropsies were performed.

Repeated Dose 28-Day Oral Toxicity Study:

The sub-chronic oral toxicity was performed in accordance to the instructions laid down by OECD test guidelines-407 with slight modifications. EEAC was made to suspend with carboxy methyl cellulose and was then administered to different groups of rat at the dose levels of 250, 500, 1000 and 2000 mg kg^{-1} respectively by gavage of 10 mL kg^{-1} for 28 days. The normal control group received the vehicle only. The animals were monitored for signs of toxicity and mortality throughout the experimental period of 28 days. The terminal weight of each animal was recorded at a weekly interval throughout the due course of experiment.

Food and water consumption were also monitored twice a week upto 28 days. On day 29, the animals were sacrificed by decapitation under anesthesia with sodium pentobarbital administration

intraperitoneally (40 mg kg^{-1}). Blood samples were collected on day 0,7,14 and on 28th day from retro-orbital venous plexus into two tubes. Tube 1 containing EDTA, was processed instantly for the assessment of haematological parameters. Tube 2 happened to be without additives was centrifuged at 3000× g at 4°C for 10 min so as to attain serum which was then stored at −20°C for future analysis. Organ samples were fixed in 10% formalin for histopathological examination[10].

Mortality and Clinical Signs:
Mortality and clinical signs of animals and any adverse reactions such as diarrhea, weight loss, immobility and other visual changes in the behavior were observed.

Food, water consumption and body weight:
Food, water consumption and body weight were measured every third day in acute and every week in repeated dose toxicity studies. For this food particles were removed from the cage and a known quantity of the feed was added in the cage. After 24h, remaining feed was collected and weighed. Water of known quantity was given in bottles and remaining water was collected after 24hrs and measured.

Blood analyses:
Hematological parameters were recorded using a fully automatic hematological analyzer. The parameters included are white blood cell count (WBC), total red blood cell (RBC) count, hemoglobin (Hb) concentration, differential leukocyte percentage, mean corpuscular volume (MCV), hematocrit (Hct), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and platelet count (PLT), Prothrombin time (PT) and activated partial thromboplastin time (APTT). For biochemical analysis, the following parameters were recorded using an auto analyzer using kit (Siemens): aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Total bilirubin, Direct bilirubin, Total Protein, Urea, Uric acid, Creatinine, Glucose, Triglyceride, Cholesterol, HDL, LDL, VLDL.

Gross Necropsy and Histopathology Findings:
At the end of repeated dose 28-day oral toxicity study, all the animals were subjected to necropsy or may be earlier in case of death. Necropsy was performed as to analyze and ascertain the macroscopic external features of brain, lungs, kidney and liver. These organs were removed and weighed individually and their weights were expressed in both absolute and relative terms i.e 'g' and 'g 100 g^{-1}' of body weight respectively. The organs were preserved in 10% formalin (lungs were inflated with intratracheal perfusion of 10% formalin) and fixed for 3 days, dehydrated, embedded in paraffin, sectioned at 5µm and subsequently stained with hematoxylin/eosin. Slides of organs taken from all animals in the vehicle control and treated groups were examined microscopically and photographed with a (Olympus) microscope at 200x and 400x magnification.

Statistical analysis:
The data were expressed as mean ± S.E.M of six animals in each group and were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet test and results were considered significant when $P \leq 0.05$.

RESULTS:
Acute oral toxicity study:
Both the sexes of EEAC treated animals did not show any sign of mortality during the course of experimental studies. Neither the treated animals showed any clinical signs of abnormalities during their gross examination of skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic, central nervous systems and behavioral pattern. Even no signs of tremors, convulsions, salivation, diarrhea and coma were recorded after the administration EEAC which could indicate towards the evidences of substance related toxicity. Even after the scarification of animals on the 14th day and 28th day in certain cases, pathophysiological observations revealed no visible lesions in any animals. Thus, no validative indications could be gathered to ascertain the acute oral toxicity of EEAC in Wistar rat. So the oral LD_{50} values for both female and male rat must be greater than 2000 mg kg^{-1} bw.

Repeated Dose 28-Day Oral Toxicity Study:

EEAC treated animals did not show any sign of mortality during the repeated dose 28-day oral toxicity study.

Mortality and Clinical Observation:

There was no mortality observed in the EEAC treated animals during the acute and repeated dose toxicity studies. No abnormality was observed in the ophthalmic, autonomic, central nervous system, somato-motor activity and behavior pattern that were considered to be biologically significant. A summary of the mortality and clinical observation were shown in **Table 1**.

TABLE 1: PRELIMINARY OBSERVATION AND SCREENING EFFECT OF EEAC UPTO 48HRS AFTER GAVAGE ADMINISTRATION

	30 Minutes	1 Hour	2 Hour	6 Hour	24 Hour	48 Hour
Stimulation (CNS)						
Hyperactivity	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-
Twitching	-	-	-	-	-	-
Rigidity	-	-	-	-	-	-
Irritability	-	-	-	-	-	-
Jumping Clonic	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-
Tonic Convulsions	-	-	-	-	-	-
Ptosis	-	-	-	-	-	-
Depression (CNS)						
Sedation	-	-	-	-	-	-
Loss of Pinna	-	-	-	-	-	-
Reflux Catatonia	-	-	-	-	-	-
Loss of Muscle tone	-	-	-	-	-	-

Remarks: = Normal; + Mild or Moderate effect; ++ Marked effect; - No effect

Food, water consumption and body weight:

No significant change of terminal bodyweight was recorded between the normal control and the treatment groups of both male and female animals **(Figure 1)**. A non-significant body weight gain of female rat at 500 mg kg^{-1} day^{-1} in 3rd week was observed but was considered to have no toxicological significance because it was an isolated finding and not dose related. An average overall (Test Days 1-28) food **(Figure 2)** and water consumption **(Figure 3)** data indicated that there were no statistically significant differences among treated groups when compared with the controls.

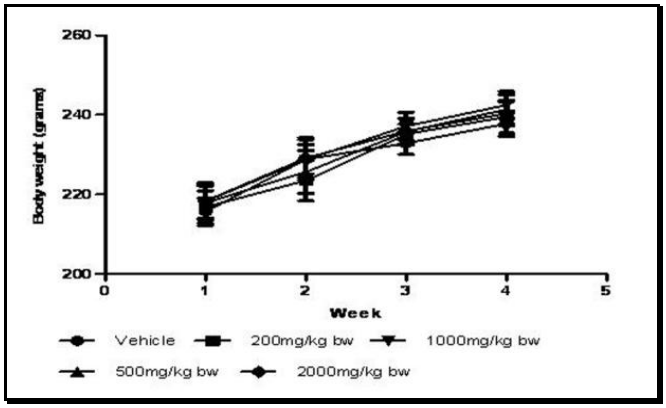

FIG. 1: GROUPS MEAN BODY WEIGHTS OF RATS GIVEN DAILY DOSES OF EEAC BY GAVAGE FOR 28 DAYS.

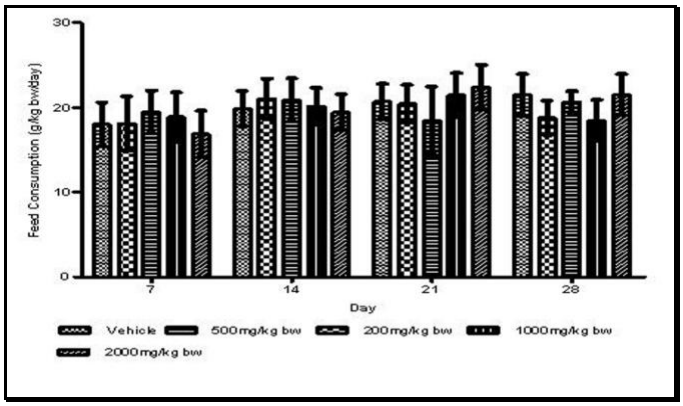

FIG. 2: GROUPS MEAN FEED CONSUMPTION OF RATS GIVEN DAILY DOSES OF EEAC BY GAVAGE FOR 28 DAYS.

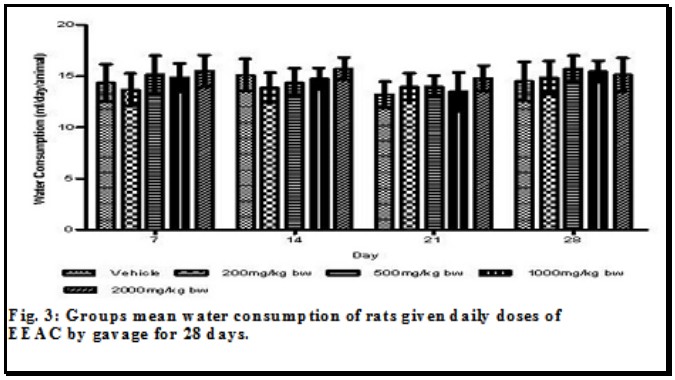

Fig. 3: Groups mean water consumption of rats given daily doses of EEAC by gavage for 28 days.

FIG. 3: GROUPS MEAN WATER CONSUMPTION OF RATS GIVEN DAILY DOSES OF EEAC BY GAVAGE FOR 28 DAYS.

Blood analyses:

EEAC did not induce any drastic changes in the hematological parameters. Even at a dose level of 250 mg kg^{-1} bw and 500 mg kg^{-1} bw, no considerable significant changes were observed in different hematological parameters between the normal control and treated groups of animals. Non-significant increase in Lymphocyte, hemoglobin (Hb) and MCV were observed whereas Hct, MCHC, Reticulocyte, PT were slightly reduced in case of animals treated with the EEAC at a dose level of 2000mg kg-1 bw when compared to that of the normal control animals **(Table 2)**.

TABLE 2: EFFECT OF REPEATED DOSE 28-DAY ORAL TOXICITY STUDY ON HEMATOLOGICAL PARAMETERS OF RAT TREATED WITH EEAC

	Control Group	Group 1 (250 mg/kg)	Group 2 (500 mg/kg)	Group 3 (1000 mg/kg)	Group 4 (2000mg/kg)
WBC (K)	6.072 ±0.0054	7.247±0.012	6.938±0.011	7.857±0.012	8.017±0.018
Neutrophil (%)	19.362±0.010	19.763±0.0095	20.558±0.010	22.86±0.009	24.548±0.012
Lymphocyte (%)	77.658±0.009	79.373±0.013	83.458±0.008	83.952±0.01	87.362±0.015
Monocyte (%)	4.863±0.017	4.233±0.018	4.36±0.013	4.615±0.017	4.847±0.012
Eosinophil (%)	1.682±0.015	1.723±0.009	1.823±0.07	1.852±0.016	1.955±0.009
Basophil (%)	0.548±0.011	0.578±0.010	0.638±0.009	0.761±0.009	0.853±0.011
RBC (M)	9.85±0.01	9.268±0.011	9.513±0.012	9.773±0.006	9.945±0.008
Hb (g/dl)	16.77±0.015	17.915±0.009	19.38±0.01	20.25±0.011	24.67±0.022
Hct (%)	49.33±0.013	52.560.012	47.35±0.013	45.39±0.018	45.25±0.013
MCV (fl)	60.69±0.025	63.82±0.025	64.61±0.021	67.90±0.028	71.38±0.021
MCH (pg)	12.85±0.025	13.00±0.029	13.31±0.021	13.63±0.023	13.94±0.022
MCHC (g/dL)	41.40±0.029	41.62±0.023	41.93±0.038	41.69±0.028	40.80±0.030
Reticulocyte (%)	3.52±0.033	2.90±0.028	2.74±0.025	2.57±0.026	2.51±0.047
PLT (K)	1606±1.59	1584.5±1.74	1633.8±1.24	1694.8±1.44	1733.2±1.30
PT (sec)	1674±1.31	1632.2±1.99	1583.5±1.40	1528.3±1.87	1515.8±1.19
APTT (sec)	22.24±0.012	20.75±0.015	22.62±0.026	22.73±0.015	23.61±0.15

WBC: White Blood Cell, RBC: Red Blood Cell, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, PLT: Platelet. PT: Prothrombin Time, APTT: Activated Partial Thromboplastin Time.

TABLE 3: EFFECT OF REPEATED DOSE 28-DAY ORAL TOXICITY STUDY ON BIOCHEMICAL PARAMETERS OF RAT TREATED WITH EEAC

	Different Doses of EEAC (mg kg^{-1} per day)				
	Control	**250 mg kg^{-1}**	**500mg kg^{-1}**	**1000mg kg^{-1}**	**2000 mg kg^{-1}**
[a]**Glucose**	118.68±0.326	118.96±0.501	120.35±0.361	119.94±0.56	120.33±0.274
[a]**Triglyceride**	56.830±0.230	56.787±0.320	58.23±0.409	56.04±0.23	57.28±0.196
[a]**Cholesterol**	74.090±0.313	74.932±0.389	75.39±0.268	75.75±0.34	76.03±0.380
[a]**HDL**	29.313±0.320	30.187±0.165	31.22±0.207	31.80±0.14	30.92±0.229
[a]**LDL**	34.003±0.198	34.245±0.268	34.89±0.278	35.71±0.3	35.56±0.163
[a]**VLDL**	11.962±0.184	12.255±0.210	12.30±0.139	12.30±0.059	13.13±0.168
[a]**Urea**	20.935±0.201	21.035±0.316	21.43±0.110	21.57±0.21	21.58±0.212
[a]**Uric acid**	1.019±0.0031	1.027±0.0019	1.03±0.001	1.03±0.003	01.03±0.001
[a]**Creatinine**	0.715±0.002	0.725±0.0016	0.73±0.016	0.72±0.001	0.72±0.001
[b]**AST**	87.53±0.074	87.932±0.088	89.57±0.266	89.58±0.26	89.95±0.245
[b]**ALT**	65.89±0.172	65.983±0.197	66.43±0.142	66.89±0.19	66.89±0.129
[b]**ALP**	33.628±0.11	33.875±0.194	33.99±0.071	34.22±0.063	34.24±0.069
[a]**Total bilirubin**	0.125±0.0015	0.128±0.0009	0.12±0.001	0.13±0.008	0.13±0.008
[a]**Direct bilirubin**	0.081±0.0004	0.086±0.0011	0.09±0.006	0.09±0.009	0.10±0.004
[c]**Total Protein**	5.162±0.009	5.212±0.010	5.36±0.099	5.57±0.17	6.12±0.031
	5.162±0.009	5.212±0.010	5.36±0.099	5.57±0.17	6.12±0.031

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase. (a- mg/dl, b- U/L, c- g/dl)

No considerable changes in biochemical profile were observed with the animals treated with EEAC at different doses upto a dose level of 2000 mg kg^{-1} bw. Urea, uric acid, creatinine, Cholesterol, VLDL, AST, ALT, ALP, Direct bilirubin and Total Protein of animals treated with EEAC upto 2000 mg kg^{-1} bw was found quite comparable with that of the vehicle-treated and EEAC treated animals at any dose at the end of 28 days treatment **(Table 3)**.

Gross Necropsy and Histopathology Findings:
Gross necropsy findings after sacrifice for all groups were generally unremarkable. Photomicrograph of brain, lungs, kidney & liver in control and treated groups is shown in **Figure 4(A-L)**. Histological findings of brain shows no changes in neuropathology or histological changes viz., shrunken neurons with eosinophilic cytoplasm, stromal oedema etc and even no changes in the astrocyes arrangement can be seen in all the groups of animals treated with EEAC **(Figure: 4 A-C)**. However, at dose of 2000 mg kg^{-1} bw, mild glial cell hyperplasia is seen in the brain which is not observed in case of rat treated with 1000 mg kg^{-1} bw.

No histoarchitechtual changes were recorded in lungs **(Figure 4. D-F)** Furthermore microscopically observed photomicrograph reveals that the kidneys in all the groups **(Figure 4. G-I)** had no tubular atrophy, basal labyrinth expansion, vacuolization or cellular swelling in the cortex. No intraglomerular congestion with inflammatory infiltration were observed. Histopathology of liver showed no marked centrolobular sinusoidal and central vein congestion **(Figure: 4 J-L)**.

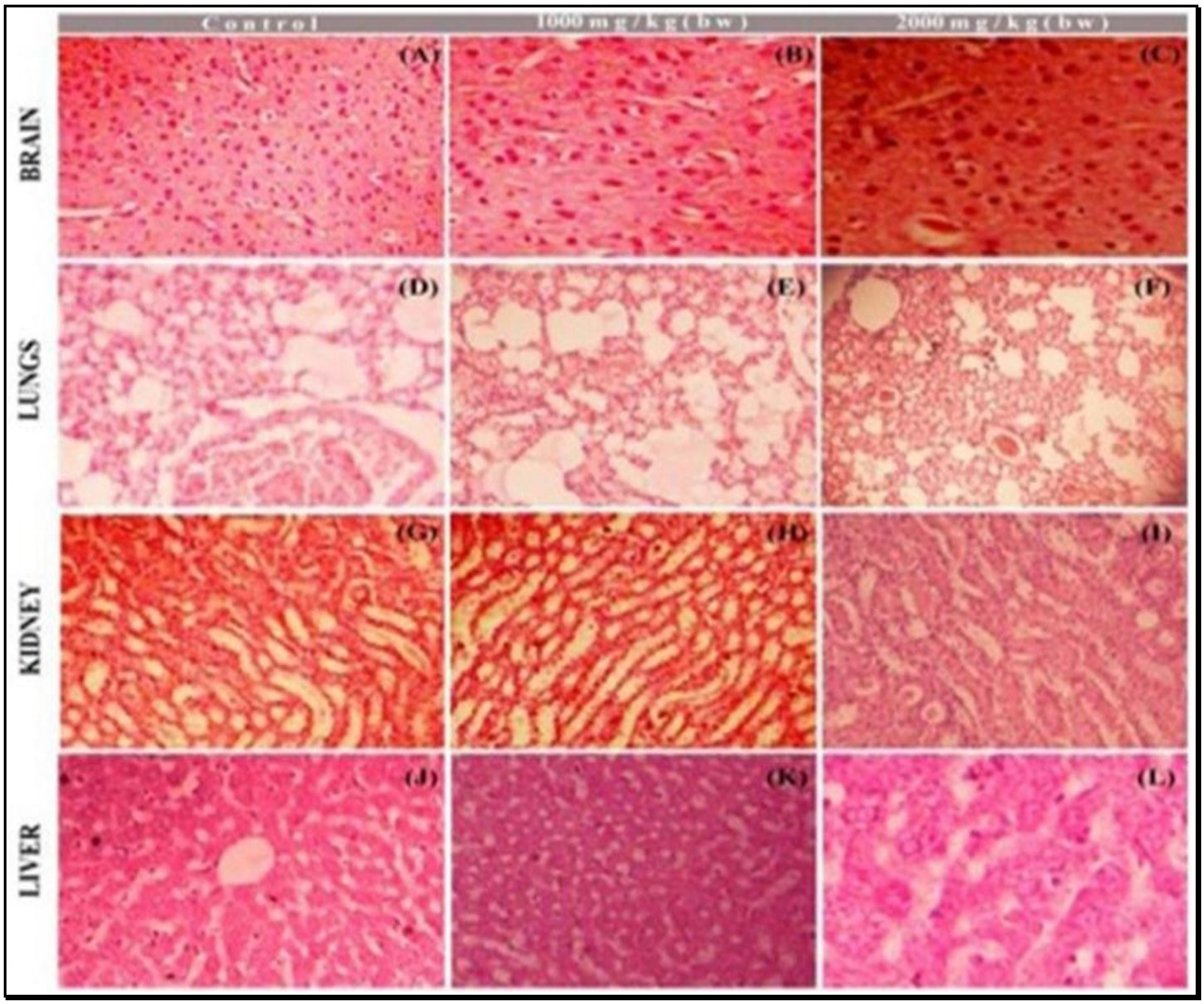

FIG. 4: PHOTOMICROGRAPHS OF THE SECTIONS OF THE BRAIN, LUNGS, KIDNEY AND LIVER OF WISTAR RATS TREATED WITH VEHICLE (CONTROL) AND EEAC (mg/kg bw AND 2000 mg/kg bw))

Absolute Organ weight and Relative organ weight:

The absolute as well as relative tissue weights were not at all altered by EEAC treatments at different dose levels upto 2000 mg kg^{-1} bw. Liver, Kidney, Lungs, Brain, Stomach, Heart, Spleen and Esophagus showed no significant change in the organ weight/body weight ratios and were comparable to controls **(Table 4)**.

TABLE 4: EFFECT OF REPEATED DOSE 28-DAY ORAL TOXICITY STUDY ON ORGAN WEIGHTS OF WISTAR RAT TREATED WITH EEAC

	Organ Weight (g), EEAC treatment mg kg^{-1} per day					Relative Organ Weight (g), EEAC treatment mg kg^{-1} per day				
Organs	Vehicle	250	500	1000	2000	Vehicle	250	500	1000	2000
Liver	6.33±0.014	7.45±0.013	8.08±0.210	8.05±0.24	8.03±0.04	2.66±0.04	3.05±0.05	3.34±0.089	3.26±0.12	3.33±0.033
Kidney	0.855±0.023	0.71±0.030	0.788±0.013	0.932±0.021	0.901±0.022	0.35±0.12	0.29±0.018	0.32±0.017	0.38±0.013	0.37±0.008
Lungs	1.304±0.015	1.303±0.051	1.300±0.008	1.315±0.019	1.298±0.007	0.54±0.016	0.54±0.011	0.53±0.011	0.54±0.008	0.53±0.006
Brain	1.123±0.009	1.124±0.032	1.119±0.002	1.102±0.038	1.114±0.042	0.47±0.026	0.46±0.019	0.45±0.008	0.45±0.018	0.46±0.004
Stomach	1.386±0.062	1.387±0.081	1.386±0.031	1.286±0.003	1.271±0.014	0.57±0.097	0.57±0.029	0.57±0.009	0.52±0.014	0.52±0.005
Heart	0.853±0.091	0.847±0.011	0.824±0.024	0.759±0.036	0.858±0.022	0.35±0.035	0.34±0.068	0.33±0.014	0.31±0.017	0.35±0.003
Spleen	0.445±0.022	0.442±0.020	0.432±0.064	0.416±0.005	0.446±0.091	0.18±0.072	0.18±0.043	0.17±0.002	0.17±0.002	0.18±0.011
Oesophagus	0.092±0.018	0.091±0.034	0.075±0.071	0.060±0.002	0.068±0.013	0.03±0.011	0.03±0.005	0.03±0.017	0.02±0.009	0.02±0.001

DISCUSSION: Earlier studies did not found any evidences of acute toxicity of carambola leaves in mouse, rats and dogs suggesting toxicity to carambola leaves are absent [11] that correlates with the results of the acute oral toxicity studies which revealed that EEAC via oral route of administration upto a dose level of 2000 mg kg^{-1} bw do not produce any sign of toxicity or mortality in rat, thus indicating the LD_{50} value above 2000 mg kg^{-1} bw via oral route of drug administration. Repeated oral dose toxicity studies were also conducted so as to estimate the undesirable effects of the test substance after its prolonged use and were also done to assemble enough evidences about the possible health hazards in terms of cumulative effects which are credible to ensue from repetitive exposure over a limited period of time[12-20].

The repeated dose treatment with EEAC revealed that repeated exposure to different dose level upto 2000 mg kg^{-1} bw during the period of 28 days did not produce any clinical sings of toxicity or death. A change in body weight, food and water consumption could be an indicative factor so as to assess the limit of adverse effects, but there were no significant change found in animal behavior, food and water consumption and also in body weight with EEAC treated groups of animals upto a dose level of 2000 mg kg^{-1} bw. The effects of EEAC on hematological, biochemical and histological parameters were also considered in this study since their analysis is applicable to risk assessment of alterations in a living system. But out of all the other parameters evaluated, only glucose, cholesterol, VLDL, Lymphocyte, Hb and MCV showed just a slight augmentation at a dose level of 2000 mg kg^{-1} bw in case of repeated oral dose toxicity studies with EEAC[21-25].

Liver and kidney are known to be very sensitive organs whose integrity and functions are liable to be effected by number of factors and thus assessment of probable hepatocellular damage are made by estimating transaminases i.e. ALT/SGPT and AST/SGOT, ALP, Total bilirubin, Direct bilirubin, Total Protein which indicated no such major biochemical changes and even in case of plasma urea, creatinine and uric acid levels, EEAC did not produce any major alteration and thus revealed that EEAC did not have any negative effect toward the liver and kidneys .

Even the observations recorded while monitoring all the hematological profiles suggests that hemato-toxicological conditions will not prevail during the clinical use of EEAC since all the hematological parameters of the treated groups are within the normal range. Furthermore, no significant difference was observed in the weight and structure of organs between the control and the treated groups with different dose levels upto a highest dose level of 2000mg kg^{-1} bw **(Table: 4)**. Moreover, repeated oral dose indicated that EEAC did not induce any detrimental alterations as well as any morphological changes in the organs even

though the doses subjected in this experiment are a number of times higher.

CONCLUSION: As the repeated oral dose administration of EEAC at a dose level of 2000mg kg^{-1} bw for 28 days did not induce any biochemical, hematological, morphological, histopathological signs of toxicity, thus substantiate the claim of safety of which may not be same in case of humans since animal experimental data cannot be always extrapolated in humans. These data suggests that adverse effects in human at lower levels of daily exposure would not be expected.

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