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Research Article

ACUTE AND SUB-ACUTE DERMAL TOXICITY STUDIES OF *MORINDA CITRIFOLIA* L. FRUIT EXTRACT IN SPRAGUE DAWLEY RATS

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ABSTRACT

Objective: *Morinda citrifolia* is one of the most significant plants that are used in traditional medicine. However, details of the dermal toxicity of *M. citrifolia* are still undiscovered. The objective of this study is to investigate the *in vivo* acute and subacute dermal toxicity of ethanolic extract of *M. citrifolia* fruit extract at doses 2000 and 5000 mg/kg in acute and 500, 1000 and 2000 mg/kg body weight in rats.

Methods: In acute experiment, a total of 40 female rats were divided into eight groups, each group had five rats, and a total of 66 male rats were divided into 11 groups of six rats in sub-acute experiment.

Results: The extract at a single dose of 2000 and 5000 mg/kg of body weight did not produce treatment-related signs of toxicity or mortality in any of the animals tested during the 14-day observation period. In the repeated dose 28-day study, the application of 500, 1000 and 2000 mg/kg of body weight/day of fruit extract revealed no significant change ($p>0.05$) in bodyweight, hematological and biochemical parameters compared with the control group. Similarly, grosspathology and histopathology examinations of liver, spleen, kidneys, and skin did not reveal any morphological alteration.

Conclusion: Overall, the results recommend that the close application of *M. citrifolia* fruit extract did not deliver any critically dangerous impact in rats. Subsequently, the concentrate can be employed for pharmaceutical plans.

Keywords: *Morinda citrifolia*, Acute dermal toxicity, Fruit extract, Sub-acute dermal toxicity

INTRODUCTION

The expression of toxicity can be considered the state of being poisonous, which is a sign of unfriendly impacts, coming about because of the association among toxicants and living cells [1]. These interactions might vary relative to cell membrane and the biochemical properties of the toxicants, since it might take place either in the extracellular matrix and beneath tissues or on the surface of the cell, and within the cell body [1]. Moreover, before binding the toxicants to the vital organs, for instance, kidneys and liver or spleen, the toxic effects might have occurred. Therefore, evaluating the toxic characteristics of a given substance is considered important for the protection of public health, since the exposure to the chemical is hazardous and can lead to adverse influences on the human body. From a pragmatic angle, the assessment normally incorporates intense, sub-intense, ceaseless, sub-incessant, cancer-causing and conceptive impacts [1]. The issue of medicinal plants has globally garnered attention to a large degree by several health care systems. In addition, the knowledge of related dose toxicity of medicinal plants is relatively unknown. On the other hand, it must be noted that conventional utilization of any plant for restorative purposes, in no way, shape or form, guarantees the security of said plant(s). *Morinda citrifolia* L. (Noni) is an important herb of tropical regions of the world [2]. It has been used for over 2000 years in Polynesia [2]. Noni is known by various names such as *mengkudu* in Malaysia, *nhau* in Southeast Asia, and *nonu* in Samoa and Tonga. Likewise, it is also known as *nonon* in Raratonga and Tahiti, and *Noni* in the Marquesas Islands and Hawaii [2-6].

Around 160 phytochemical mixes have been now recognized in the Noni plant, while the significant micronutrients are phenolic mixes, natural acids, and alkaloids [7]. The fruit of *M. citrifolia* is in high demand in alternative medicine for different kind of illnesses such as arthritis, diabetes, high blood pressure, muscle aches, menstrual irregularities, headache, heart disease, AIDS, cancers, gastric ulcer, sprains, mental depression, senility, poor digestion, arteriosclerosis, blood vessel problems and drug addiction [8]. It is reported to have anti-bacterial, anti-cancer, anti-viral, anti-oxidant, anti-fungal, anti-inflammatory, and immune-stimulatory effects, as well as strong cancer preventive effect [9,10]. In addition, to increase confidence in their safe use in humans, especially in the development of pharmaceuticals, studies of acute and sub-acute data on medical plants or preparation must to be acquired [11]. In accordance with the Organization of Economic Cooperation and Development (OECD) guidelines, toxicological studies are considered to be vital in animals, such as rat, mice, rabbits, monkeys, guinea pigs, dogs, etc., under different drug conditions to establish the safety and efficiency of a new drug. In this study, the estimation of the toxic effects of ethanolic fruit extract of *M. citrifolia* in Spargue Dawley's rats (male and female) has been achieved at dosage of 2000 and 5000 mg/kg bodyweight for a period of 14 days (acute study) and dosage of 500, 1000 and 2000 mg/kg bodyweight for a period of 28 days (sub-acute study) using OECD 402 and 410.

METHODS

Animals

An 8-week-old male and female Sprague-Dawley rats with body weight range between 200 and 250 g were purchased from a local supplier. On arrival, the rats were weighed and assigned randomly in polypropylene plastic cages, where one rat was placed in each cage with wood chips for bedding and housed in an animal room with controlled conditions

involving these parameters; temperature (22±2°C), humidity (55±10%) and lighting (12 hrs light/dark) in the animal house at the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor.

Preparation of *M. citrifolia* fruit extract

M. citrifolia L. (Rubiaceae) ripe fruits were obtained from Bukit Ridan, Pahang, Malaysia. The ripe fruits were sliced into small pieces and dried in an oven at a temperature between 55°C and 60°C. The dried fruits were ground into powder form and kept in a refrigerator at 4°C. The fruit powder was extracted using 97% ethanol via a standard method. The fruit extract was then mixed with white soft paraffin (10%) as shown in Table 1 before it was applied on the skin of the rats.

Skin preparation for dermal toxicity study

Skin at the dorsal thoracic area of the rats was clipped under general ketamine (50 mg/kg) and xylazine (5 mg/kg) anesthesia using an electric clipper (OSTER model, 45 Watts, Head No. 40, Blade size 1/10 mm), followed by manual shaving using razor blade. Based on OECD guidelines 402 and 410, not less than 10% of the body surface area should be clear for the application of the test substance. The dorsum area was applied with the fruit extract (Fig. 1).

Acute dermal toxicity study

An intense dermal toxicity test was made in accordance with the guidelines no 402 given by the OECD for the chemical testing. Healthy young adult female rats (6-8 weeks) were acclimatized to the laboratory condition for 2 weeks. Before the test, the rats were selected randomly and assigned to the treatment and control groups. 24 hrs before the test, fur was removed from the dorsal area of the trunk of the test animals by clipping. Repeat clipping or shaving was carried out at approximately weekly intervals. About 10% of the body surface area was cleared for the application of the test substance. Each group contain five female rats (n=5), the rats were nulliparous and non-pregnant. Based on OECD guideline 402, a limited test at one dose level was 2000 mg/kg bodyweight. As a result, treatment groups in this study received the dosage of 2000 mg/kg, which was the lower dose and 5000 mg/kg, which was the higher dose of ethanolic fruit extract of *M. citrifolia*, whereas the positive control group received the dosage of 5000 mg/kg of white soft paraffin 10% as a vehicle. These doses were applied locally only once at the first day of the study. Rats were monitored for the duration of 24 hrs, with special attention given to the first 6 hrs and once daily further for a period of 14 days. The rats were weighed and visual observations for mortality, behavioral pattern (salivation, tremors, convulsions, diarrhea, lethargy, sleep and coma), changes in physical appearance, injury, pain, and signs of illness were conducted once daily during the period, as well as any changes in fur, eyes and mucous membranes and also respiratory, circulatory, autonomic, central nervous system, and behavior patterns.

At the end of the experiment, the rats were humanely sacrificed by complete exsanguinations under general anesthesia with a mixture of 75 mg/kg ketamine and 10 mg/kg xylazine. Blood samples were withdrawn from the posterior vena cava from each rat and collected into non-heparinized and ethylene diamine tetra acetic acid (EDTA)-containing tubes for biochemical and hematological analyses, respectively. The organs were excised, weighed, and examined macroscopically. The relative organ weight was calculated. Principal vital organs, liver and kidneys, as well as skin, were preserved in a fixation medium of 10% solution of buffered formalin for histopathological study. After sacrificing the rats, parts of the liver, kidney, and skin tissues were collected for histological studies. The tissues were washed in normal saline and fixed immediately in 10% formalin for a period of at least 24 hrs, dehydrated with alcohol, embedded in paraffin, cut into 4-5 µm thick sections and stained with hematoxylin-eosin (H and E) dye for photo microscopic observation.

Table 1: Preparation of *M. citrifolia* fruit extract mixed with white soft paraffin (10%)

Dosage	Mixing ratio of extract*	Mixing ratio of paraffin*
5000 mg/kg	5	1
2000 mg/kg	2	1
1000 mg/kg	1	1
500 mg/kg	0.5	1

*White soft paraffin (10%)=Paraffin powder (20 g)+distal water 70°C (200 ml), Homogenized (5 minutes), *Extract of *M. citrifolia* fruit=[Amount of fruit extract for 1 day=Dosage (g)/1000×bodyweight (g)], *M. citrifolia: Morinda citrifolia*

Sub-acute dermal toxicity study

Subacute dermal toxicity test was performed according to the OECD guideline 410 for testing of chemicals. The test substance is applied daily to the skin in graduated doses to several groups of experimental animals, one dose per group, for a period of 28 days. During the period of application of treatment, the animals are observed daily to detect signs of toxicity. Animals, which died during the tests, were necropsied and at the conclusion of the tests the surviving animals were sacrificed and necropsied. Healthy young adult male rats (6-8 weeks) were acclimatized to the laboratory condition for 2 weeks. Before the test, rats were randomized and assigned to the treatment and control groups. Shortly (24 hrs) before testing, fur was clipped from the dorsal area of the trunk of the test animals. Repeat clipping or shaving was usually at approximately weekly intervals. About 10 % of the body surface area was cleared for the application of the test substance. Each group contain six healthy male rats (n=6). Based on OECD guidelines 410, at least three dose levels of the extract, as well as a vehicle and control groups, was used in the study. As a result, treatment groups received the dosage of 500 mg/kg, 1000 mg/kg and 2000 mg/kg of ethanolic fruit extract of *M. citrifolia*, whereas the positive control group received the dosage of 2000 mg/kg of white soft paraffin 10% as a vehicle.

Hematological analysis

Blood samples were withdrawn from the posterior vena cava of each rat and collected in EDTA vacuumed blood collection tubes and were gently mixed immediately to mix the blood with EDTA-anticoagulant material inside the tubes for automatic and manual hematology analyses. The blood samples were analyzed using an automatic hematology analyzer (Cell Dyn, 3700, Abbot, USA) for the total number of white blood cell (WBC), red blood cell (RBC) and hemoglobin concentration. Capillary microhematocrit tube was filled to about three-fourths of their length with the EDTA blood by capillary action. The dry end of microhematocrit tubes was sealed by melting with a heat then placed in a micro-centrifuge machine (Hettich Hematokrit 210, Germany) and centrifuged at 10,000 rpm for 5 minutes to separate the RBCs from plasma. The plasma was at the top and the RBCs were at the bottom of the microhematocrit tube. The centrifuged microhematocrit tubes were used for determination of packed cell volume (PCV), icteric index and plasma protein concentration. For the PCV, the microhematocrit tube was placed in holder slots of the microhematocrit tube reader (Hawksley), where the base of RBC was intersected with baseline of reader and the top of plasma was intersected with the top line of the reader by moving the holder left or right, before the middle line of the reader was adjusted to intersect with the top of the RBCs and the measuring ruler. The PCV result was obtained from the middle line and the measuring ruler point (e.g.,: 24% is equal to 0.24 L). The icteric index result was obtained by comparing the plasma color in the microhematocrit tube with the icteric index standard board color degree. Although the blood plasma protein concentration was obtained by dropping the plasma on the refracto meter glass (Atago T2-NE, Japan) to obtain the result from the measuring ruler and the plasma unit was read in protein in gram divided by plasma in liter (e.g.: 6.2 % is equal to 62 g/L).

Biochemical analysis

Blood samples were withdrawn from posterior vena cava from each rat and collected in serum vacuumed blood collection tubes. The blood

samples were centrifuged (Hettichzent- EBA20, Germany) at 5000 rpm for 5 minutes and then the serum were collected in microcentrifuge tubes and stored at −20°C. The serum samples were analyzed using an automatic biochemistry analyzer (TRX 7070, Biorex, Germany) to determine the levels of alanine transaminase, aspartate transaminase, lactate dehydrogenase, albumin, total protein, creatinine, uric acid, urea and bilirubin. Globulin levels were calculated via this formula: total protein-albumin.

Histopathological assessment

Skin, liver, and kidney samples were collected and fixed in 10% formalin for 48 hrs. After fixation, the samples were sliced to 0.5 cm thickness and placed in plastic cassettes for dehydration using an automated processor (Leica ASP300, Germany), before embedded in paraffin (Leica EG1160, Germany) using the routine paraffin embedding method. The tissue samples were then trimmed and sectioned at 4 μm thicknesses (Leica RM2155, Germany) then the tissue sections were mounted on glass slides using a hot plate (Leica HI1220, Germany). Subsequently, the tissue sections were deparaffinized by two changes of xylene for 2 minutes each and rehydrated by three changes of different ethanol dilutions (100%, 90% and 70%) for 2 minutes each, respectively. The tissue sections were then further rinsed in tap water and stained with Harris's H and E stain. Digital photomicrographs were captured at representative locations using a digital camera mounted to a Nikon Eclipse FX-35DX microscope. The slides were observed using a light microscope at ×40, ×100, ×200, ×400 and ×1000 magnifications.

Peripheral blood smear

Peripheral blood smear was conducted by dropping a small blood drop on a glass slide, and then the drop was drawn by a cover slip on the slide surface and left to dry for 20 minutes at room temperature. The blood smear was stained using standard Wright's stain method. The blood smear slide was examined using a light microscope at ×100, ×200, ×400 and ×1000 magnifications. The manual WBC differential count was conducted by counting one hundred WBCs on the blood smears. The numbers of each WBC type in these one hundred cells were converted to the percentage and multiplied by the automated total WBC count to obtain the absolute WBC differential count ($\times 10^9$/L) for each cell type.

Necropsy and gross pathology

All rats were humanely scarified by complete exsanguinations under general anesthesia with a mixture of 75 mg/kg Ketamine and 10 mg/kg xylazine. Complete gross examination was conducted to detect any gross changes, especially skin necrosis. The liver and kidney were blotted dry and weighed immediately after necropsy.

Statistical analysis

The data obtained were statistically analyzed by using Statistical Package for Social Science (SPSS) software version 20. The values were expressed as mean ± standard deviation for different parameters. Repeated measurements of Analysis of Variance tests were done to compare the differences of data between and within the groups. *Post-hoc* analysis using Duncan test was used to determine the level of statistical significance, which was set at $p<0.05$.

Table 2: Mortality rate of rats after applied with topical ethanolic fruit extract of *M. citrifolia* at 2000 mg/kg and 5000 mg/kg body weight, once, for the acute dermal toxicity study

Group	Mortality rate*(%)
G1	0
G2	0
G3	0
G4	0

*Mortality rate is number of dead rats divided by total number of rats per group, G1: No treatment, G2: Paraffin, G3: *M. citrifolia* 2000 mg/kg, G4: *M. citrifolia* 5000 mg/kg, *M. citrifolia: Morinda citrifolia*

RESULTS

Acute dermal toxicity

General sign and behavior of the rats

The toxic effects of ethanolic fruit extract of *M. citrifolia* on the presentation and the general observable example of the rats are demonstrated in Tables 2 and 3, individually. No poisonous signs or mortality were seen in any creatures, which made due up to 14 days in the wake of applying of the concentrates once on the 1st day at single measurements level of 2000 and 5000 mg/kg body weight. The observable examples of creatures were watched initially 6 hrs and pursued by 14 hrs in the wake of applying the concentrates. The creatures in both vehicle-treated and fruit-essence treated classes were typical and did not show any critical changes in conduct, skin impacts, breathing, disability in nourishment admission and water utilization, postural variations from the norm and losing of hair. In the treated classes, during the initial 6 hrs fast pulse was seen in the wake of applying the concentrate yet it got to be typical and this may be because of the anxiety of treatment.

Organs and body weight

The selected organs of control and fruit concentrate-served rats are shown in Fig. 2. There were no gross lesions seen in the liver, spleen, and kidneys of all rats. Body weights and weights of liver and kidneys of the rats are shown in Fig. 3 and Table 4, respectively. No considerable variations were observed in the weight of the body. All rats had shown a standard enhancement in the weight of the body, which was not seriously ($p>0.05$) variant between both control and treated groups. Similar to the body weights, there were no vital ($p>0.05$) variations in the changes of relative organ weights between groups.

Table 3: Behavioral patterns and general appearance of rats in all groups

Abnormal sign	Control group		Treatment groups	
	G1 (6 hrs)	G1 (14 hrs)	G2-G4 (6 hrs)	G2-G4 (14 hrs)
Skin and fur	No change	No change	No change	No change
Eyes	No change	No change	No change	No change
Mucous membrane	No change	No change	No change	No change
Behavioral patterns	No change	No change	Tachycardia	No change
Salivation	No change	No change	No change	No change
Lethargy	No change	No change	No change	No change
Sleep	No change	No change	No change	No change
Diarrhea	No change	No change	No change	No change
Coma	No	No	No	No
Tremors	No	No	No	No

G1: No treatment, G2: Paraffin, G3: *M. citrifolia* 2000 mg/kg, G4: *M. citrifolia* 5000 mg/kg. NO: Not observed, *M. citrifolia: Morinda citrifolia*

Table 4: Organ relative weights of rats in all groups

Group	Liver (g)	Kidneys (g)
G1	0.024±0.005	0.005±0.0005
G2	0.020±0.007	0.006±0.0004
G3	0.020±0.007	0.006±0.0007
G4	0.024±0.008	0.006±0.0007

Values are expressed as mean±SD (n=5 for each group), relative organ weight was calculated by organ weight/body weight×100%, G1: No treatment, G2: Paraffin, G3: *M. citrifolia* 2000 mg/kg, G4: *M. citrifolia* 5000 mg/kg, SD: Standard deviation, *M. citrifolia: Morinda citrifolia*

Evaluation
All the erythron and leukon parameters were normal in all rats as shown in Table 5.

Blood biochemistry
Table 6 shows the serum biochemical parameters of liver and muscle enzymes, kidney parameters and protein concentration of female rats of control and *M. citrifolia* fruit extract groups. There were no significant (p>0.05) changes observed in all biochemical parameters.

Histopathology examination
Microscopic structures of liver, kidneys and skin depicted in Fig. 4 show insignificant distinctions between the control and treatment classes. The microscopic exploration uncovered that, all the organs from the fruit-essence treated rats did not demonstrate any modifications in cell arrangements or any adverse impacts when seen under the light microscope utilizing numerous amplification powers.

Sub-acute dermal toxicity
Normal manifestation and behaviour of the rats
The lethal impacts of ethanolic fruit-concentrate of *M. citrifolia* on the emergence and the general observable example of rats are indicated in Tables 7 and 8, individually. No harmful signs or mortality were seen in any creatures that made due up to 28 days in the wake of applying of every ethanol separate day by day at three separate measurements; 500, 1000, 2000 mg/kg body weight. The observable examples of creatures were watched initial 6 hrs and took after by 14 hrs in the wake of applying the concentrates. The creatures in both vehicle-treated and concentrate treated gatherings were ordinary and did not show any critical changes of conduct, skin impacts, breathing, debilitation in nourishment admission and water utilization, postural irregularities and losing of hair.

Table 5: Elytron and leadon parameters and plasma protein concentration of rats in all groups

Parameter	Unit	G1	G2	G3	G4
RBC	$\times 10^{12}$/L	8.25±0.28	8.56±0.61	8.31±0.21	8.53±0.85
Hb	g/L	171±7.91	166±7.79	163±7.38	177±14.25
PCV	L/L	0.45±0.00	0.45±0.01	0.45±0.01	0.45±0.01
MCV	flu	58.2±0.83	57.8±1.30	57.8±1.30	57.8±1.40
PP	g/L	75.4±2.07	77.8±1.92	75.2±1.30	77.0±1.58
WBC	10^9/L	7.82±0.87	7.68±0.93	7.56±1.40	7.08±1.15
Neutrophils	10^9/L	1.45±1.14	1.47±0.83	1.45±1.09	1.37±0.89
Lymphocytes	10^9/L	5.66±1.14	5.66±1.64	5.56±1.14	5.22±1.48
Monocytes	10^9/L	0.31±1.00	0.32±1.30	0.30±0.70	0.26±1.30
Eosinophil's	10^9/L	0.21±0.83	0.12±0.89	0.16±0.83	0.14±1.22
Basophils	10^9/L	0.07±0.70	0.09±0.83	0.07±0.70	0.05±0.83
Platelets	10^9/L	869±40.70	913±28.10	971±19.00	936±31.00

Values are expressed as mean±SD (n=5 for each group). G1: No treatment; G2: Paraffin; G3: *M. citrifolia* 2000 mg/kg; G4: *M. citrifolia* 5000 mg/kg. None of the values were significantly different at p>0.05, *M. citrifolia: Morinda citrifolia*, SD: Standard deviation

Organs and body weight
The selected organs of control and fruit extract-treated rats are shown in Fig. 5. There were no gross abnormalities observed in liver, spleen and kidneys of all rats. Body weights and weights of liver and kidneys of the rats are shown in Fig. 6 and Table 9, respectively. No vital transformations in the weight of the body were observed. All rats had shown standard accession in the weight of the body which was not seriously (p>0.05) different between both control and treated groups. Similar to the body weights, there were no serious (p>0.05) variations in the changes of relative organ weights between groups.

Hematology evaluation
All the erythron and leukon parameters were normal in all rats as shown in Table 10.

Blood biochemistry
Table 11 shows the serum biochemical parameters of liver and muscle enzymes, kidney parameters and protein concentration of female rats of control and *M. citrifolia* fruit extract groups. There were no significant (p>0.05) changes observed in all biochemical parameters.

HISTOPATHOLOGY EXAMINATION

Microscopic structures of liver, kidneys, and skin depicted in Fig. 7 show inconsiderable variations between the control and treatment classes. The microscopic observation revealed that, all the organs from the fruit concentrate conducted rats had no exposure of any alterations in cell structures or any unfavorable outcomes during observing employing a number of magnification powers through light microscope.

DISCUSSION

Phytotheraputic items from therapeutic plants have ended up all around well-known in essential human services, especially in developing countries, whereas some of these have been erroneously viewed as protected on the grounds that they get from a common source. In any case, these bioactive items from restorative plants should be protected without any trading off wellbeing impact and in this way generally utilized likewise as arrangement toward oneself [12]. Then again, there is a glaring absence of experimental studies on the danger and unfavorable impact of the vast majority of these cures. Consequently, supplementary toxicity inquiries are required, not just to distinguish the scope of dosages that could be hence proposed, in addition to uncover the conceivable clinical signs evoked by the substances under scrutiny. By and large, *in vivo* toxicity studies demonstrate the toxicological investigation of numerous restorative plants and their potencies for subjective and quantitative assessment by means of histopathology, intense and sub-intense toxicity studies. Intense and sub-intense toxicity testing in rats can be utilized to assess regular solutions for distinctive pharmacological exercises, considering the fundamental preface that pharmacology is essentially toxicology at a lower measurement [13].

Table 6: Serum biochemical parameters of liver, muscle enzymes, kidneys, and protein concentration of rats in all groups

Parameter	Unit	G1	G2	G3	G4
ALT	(U/L)	41.2±1.30	43.4±1.14	43.4±2.07	42.2±0.83
ALP	(U/L)	74.0±2.73	73.2±2.58	74.0±1.58	74.4±2.70
AST	(U/L)	134.6±2.96	139.0±2.23	137.0±1.41	135.6±3.28
CK	(U/L)	172.8±2.68	174.6±1.81	172.2±1.30	173.2±2.58
Urea	(mmol/L)	6.44±0.25	6.30±0.30	6.46±0.27	6.50±0.18
Creatinine	(μmol/L)	36.5±1.35	37.5±1.46	36.8±1.39	36.9±2.29
TP	(g/L)	72.8±1.92	73.8±1.92	73.8±1.30	73.4±1.67
Albumin	(g/L)	37.7±0.97	38.1±0.79	38.3±0.63	38.0±0.63
Globulin	(g/L)	32.7±0.72	33.1±0.75	32.4±1.14	32.8±1.72
A/G ratio	(g/L)	1.14±0.03	1.14±0.03	1.17±0.06	1.15±0.07

Values are expressed as mean±SD (n=5 for each group). G1: No treatment; G2: Paraffin; G3: *M. citrifolia* 2000 mg/kg; G8: *M. citrifolia* 5000 mg/kg. None of the values were significantly different at p>0.05, *M. citrifolia: Morinda citrifolia*, SD: Standard deviation

Table 7: Mortality rate of rats after applied with topical ethanolic fruit extract of *M. citrifolia* at 500 mg/kg, 1000 mg/kg and 2000 mg/kg body weight, once, for the sub-acute dermal toxicity study

Group	Mortality rate*(%)
G1	0
G2	0
G3	0
G4	0
G5	0

*Mortality rate is number of dead rats divided by total number of rats per group, G1: No treatment, G2: Paraffin, G3: *M. citrifolia* 500 mg/kg, G4: *M. citrifolia* 1000 mg/kg, G5: *M. citrifolia* 2000 mg/kg, *M. citrifolia: Morinda citrifolia*

Table 8: Behavioral patterns and general appearance of rats in all groups

Abnormal sign	Control group		Treatment groups	
	G1 (6 hrs)	G1 (14 hrs)	G2-G5 (6 hrs)	G2-G5 (14 hrs)
Skin and fur	Unchanged	Unchanged	Unchanged	Unchanged
Eyes	Unchanged	Unchanged	Unchanged	Unchanged
Mucous membrane	Unchanged	Unchanged	Unchanged	Unchanged
Behavioral patterns	Unchanged	Unchanged	Tachycardia	Unchanged
Salivation	Unchanged	Unchanged	Unchanged	Unchanged
Lethargy	Unchanged	Unchanged	Unchanged	Unchanged
Sleep	Unchanged	Unchanged	Unchanged	Unchanged
Diarrhea	Unchanged	Unchanged	Unchanged	Unchanged
Coma	No	No	No	No
Tremors	No	No	No	No

G1: No treatment, G2: Paraffin, G3: *M. citrifolia* 500 mg/kg, G4: *M. citrifolia* 1000 mg/kg, G5: *M. citrifolia* 2000 mg/kg. NO: Not observed, *M. citrifolia: Morinda citrifolia*

Table 9: Organ relative weights of rats in all groups

Group	Liver (g)	Kidneys (g)
G1	0.026±0.002	0.0062±0.0002
G2	0.031±0.002	0.0062±0.0002
G3	0.028±0.003	0.0064±0.0002
G4	0.028±0.004	0.0063±0.0003
G5	0.026±0.002	0.0063±0.0002

Values are expressed as mean±SD (n=5 for each group), Relative organ weight was calculated by organ weight/body weight×100%, G1: No treatment, G2: Paraffin, G3: *M. citrifolia* 500 mg/kg, G4: *M. citrifolia* 1000 mg/kg, G5: *M. citrifolia* 2000 mg/kg, *M. citrifolia: Morinda citrifolia*, SD: Standard deviation

The general thought is that natural medications are, by nature, amazingly protected and free from symptoms is false. Plants have several segments and some are exceptionally harmful, for example, the most cytotoxic against tumor plant-inferred medications, digitalis, the pyrrolizidine alkaloids, ephedrine, phorbol esters, and so forth. Notwithstanding, the negative impacts of most home grown medications are reasonably less incessant when the medications are utilized properly contrasted and manufactured medications, yet decently controlled clinical trials have now affirmed the presence of such negative impacts cannot be avoided [14]. A harmful substance may inspire intriguing pharmacological impacts at lower non-poisonous measurements. Harmfulness results from creature studies will be critical in absolutely judging the security of medicinal plants in the event that they are found to have sufficient potential for advancement into pharmacological items [15]. As utilization of medicinal plants continues to enhance, test screening of the poisonous quality of these plants is critical to guarantee the security and adequacy of those characteristic sources. On the other hand, intense and sub-intense lethality studies do not locate consequences for indispensable capacities like the cardiovascular, focal apprehensive and respiratory systems, which are not typically evaluated amid the study, and these ought to be assessed before they are utilized in humans [16].

In addition, acute and sub-acute toxicity are predominantly to acquire a proper dosage for fleeting and long haul toxicity tests and to figure out which organs are influenced toward the end of the treatment. Consequently, this study was especially intended to further examine poisonous quality of ethanolic foods grown from the ground concentrate of *M. citrifolia* by utilizing intense and sub-intense dermal toxicity examination. All the techniques were performed keeping in view as per the suitable OECD rule. It was carried out to estimate the safety of ethanolic fruit extract of *M. citrifolia* that was applied locally to male and female rats. As indicated previously, no clinical signs of toxicity and deaths were observed throughout the experimental period. The expenditure of ethanolic fruit extracts of *M. citrifolia* was showing well and did not create any organ or systemic toxicity when applied to the male and female rats at the dose levels of 2000 mg/kg and 5000 mg/kg in the acute dermal study and dose levels of 500, 1000 and 2000 mg/kg in sub-acute dermal study. In this study, the rats in the control and treated classes got vehicles and rough concentrate, individually. The rats were checked day by day until day 14 in intense study and day 28 in sab intense study for any lethal signs and mortality. The clinical side-effect is one of the major vital perceptions to show the lethality impacts on organs in the treated gatherings [17].

Amid the 14 and 28 days of period intense and sub-intense harmfulness assessment, the rats hinted at no unmistakable pain,

Table 10: Erythron and leukon parameters and plasma protein concentration of rats in all groups

Parameter	Unit	G1	G2	G3	G4	G5
RBC	$\times 10^{12}$/L	8.60±0.44	8.93±0.56	8.49±0.56	8.11±0.45	8.63±0.67
Hb	g/L	170±8.57	172±3.83	170±12.5	163±10.9	169±8.11
PCV	L/L	0.45±0.00	0.46±0.01	0.45±0.01	0.43±0.02	0.46±0.01
MCV	fl	57.6±1.75	57.8±0.75	57.1±1.47	56.6±1.50	56.8±1.16
MCHC	g/L	342±7.31	345±7.74	346±9.78	346±5.63	347±8.88
PP	g/L	77.6±1.86	75.3±1.03	77.3±1.50	76.0±2.00	75.5±1.37
WBC	10^9/L	7.45±1.31	8.01±0.74	7.00±0.90	7.15±1.40	7.48±0.83
Neutrophils	10^9/L	1.49±0.98	1.61±0.75	1.36±0.54	1.43±1.16	1.45±1.22
Lymphocytes	10^9/L	5.43±1.54	5.81±1.36	5.18±1.41	5.24±1.03	5.48±1.21
Monocytes	10^9/L	0.29±1.09	0.29±1.21	0.26±1.16	0.22±0.40	0.29±0.63
Esoinophils	10^9/L	0.17±0.81	0.17±0.75	0.12±0.75	0.15±1.16	0.16±0.75
Basophils	10^9/L	0.04±0.81	0.10±0.81	0.05±0.75	0.07±0.89	0.07±0.63
Platelets	10^9/L	864±44.9	837±27.3	856±30.9	836±62.3	817±47.2

Values are expressed as mean±SD (n=5 for each group). G1: No treatment; G2: Paraffin; G3: *M. citrifolia* 500 mg/kg, G4: *M. citrifolia* 1000 mg/kg, G5: *M. citrifolia* 2000 mg/kg. None of the values were significantly different at p>0.05, *M. citrifolia: Morinda citrifolia*, SD: Standard deviation, WBC: White blood count, RBC: Red blood count, PCV: Packed cell volume, Hb: Hemoglobin, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration

Table 11: Serum biochemical parameters of liver, muscle enzymes, kidneys, and protein concentration of rats in all groups

Parameter	Unit	G1	G2	G3	G4	G5
ALT	(U/L)	44.1±8.35	42.0±6.41	42.6±5.68	42.5±4.72	44.3±4.36
ALP	(U/L)	135±12.3	138±10.1	124±12.6	122±18.2	127±16.1
AST	(U/L)	149±12.4	150±16.5	163±17.7	146±11.0	149±13.3
CK	(U/L)	201±22.1	221±26.3	195±35.6	211±39.0	204±23.2
Urea	(mmol/L)	7.71±0.91	6.91±0.57	7.15±1.13	7.75±1.03	7.20±1.20
Creatinine	(μmol/L)	55.5±2.25	55.5±3.08	52.0±2.19	55.1±2.13	53.3±2.87
TP	(g/L)	72.2±4.09	75.5±4.37	71.5±2.72	70.1±5.45	70.2±4.23
Albumin	(g/L)	40.8±1.15	43.8±2.62	40.2±2.73	38.0±5.06	39.4±2.63
Globulin	(g/L)	31.4±3.23	31.6±5.26	31.3±2.22	32.1±2.69	30.7±2.75
A/G ratio	(g/L)	1.25±0.13	1.36±0.26	1.25±0.16	1.16±0.19	1.25±0.13

Values are expressed as mean±SD (n=5 for each group). G1: No treatment; G2: Paraffin; G3: *M. citrifolia* 500 mg/kg, G4: *M. citrifolia* 1000 mg/kg, G5: *M. citrifolia* 2000 mg/kg. None of the values were significantly different at p>0.05, *M. citrifolia: Morinda citrifolia*, SD: Standard deviation, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, TP: Total protein, CK: Creatine kinase

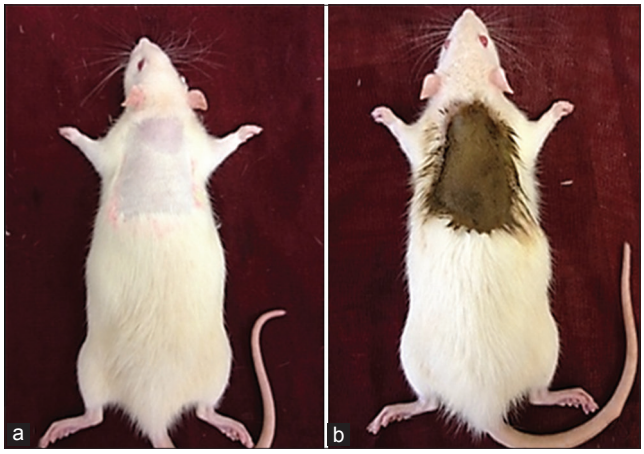

Fig. 1: (a) Clipped area of the skin; (b) Clipped area applied with fruit extract. For each of treatment group, approximately 0.5 mL (dosage 0.25 mg/mL) of different concentrations of fruit extract was applied topically on the skin of the rats

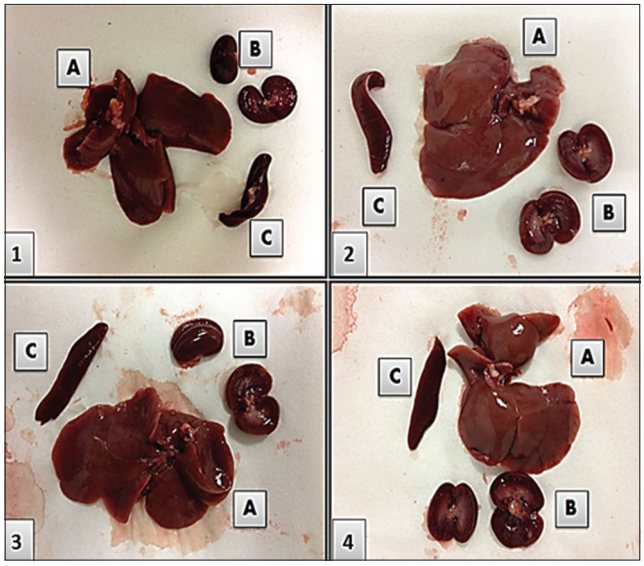

Fig. 2: Normal gross appearance of selected organs of the rats (1): No treatment group; (2): Paraffin group; (3): Morinda citrifolia (2000 mg/kg) group; (4): Morinda citrifolia (5000 mg/kg) group. (a): Liver; (b): Kidneys; (c): Spleen

nor were there any discernible manifestations of poisonous quality or deaths. The greater part of the rats put on weight, and showed no critical changes in conduct. Furthermore, the physical appearance countenances, for example, skin, fur, and eyes were discovered to be unchanged. Whilst the body weight of the rats demonstrated an expansion, this shows that the application of the rough concentrate had immaterial level of poisonous quality on the development of the test animals. In addition, determination of nourishment admission and water utilization is crucial in the investigation of wellbeing of an item with helpful purposes, as legitimate admission of supplements are huge to the physiological status of the creatures, and to the achievement of the best possible reaction to the medications tried [18].

In this study, the sustenance admission and water utilization was additionally not influenced by neighborhood application of the ethanolic tree grown foods concentrate and it did not prompt ravenousness concealment or different harmful impacts. Accordingly, this shows there was no unsettling influence in carb, protein, or fat digestion system [19]. By and large, the changes of body weight pick up and inward organ weights of rats would reflect the lethality after presentation to the dangerous substances [20]. Body weight changes are markers of unfavorable impacts of medications and chemicals and will be noteworthy if the body weight reduction happened is more than 10% from the starting weight [21,22]. Organ weight likewise is a vital record of physiological and obsessive status in creatures. The relative organ weight is major to finding, whether the organ was presented to the damage or not. The liver, kidney, and spleen are the essential organs influenced by metabolic response brought on by toxicant [23]. The entire perception of systemic organs of both control and treated classes demonstrate that there were no progressions seen in gross perception of systemic organs of both control and treated classes. In this study, the relative and total weight of organs in both control and treated classes expanded fundamentally showing that the concentrate supported the organs. Henceforth, body weight remained the same in both control and treated classes without measurably critical contrasts. Local utilization of the ethanolic fruit concentrate of *M. citrifolia* neglected to demonstrate any unfavorable impacts on organs weight of exceptionally vital organs. Subsequently, it can be recommended that, *M. citrifolia* products of the fruit concentrate is basically nontoxic.

The hematopoietic framework is extremely delicate to poisonous mixes, and serves as a vital list of the physiological and neurotic status in both creatures and people [24]. Following 14 and 28 days of treatment with ethanolic products of the fruit concentrate of *M. citrifolia* there were no progressions in the hematological parameters between the treatment and control bunches. This shows that there were no huge changes of serum levels of all blood organic chemistry parameters thus, confirming the non-dangerous nature of ethanolic fruit concentrate of *M. citrifolia*. This discovering additionally underpins the utilization of this herb in conventional pharmaceutical by customary healers. Be that as it may, the typical scope of this

parameter can be changed by the admission of poisonous plants [25] which was not tried in this study. Separated from that, histological investigation was carried out to further affirm the change in cell structure of the organs. The histological examination is the "highest level" for assessing treatment-related neurotic changes in tissues and organs [26]. In this study, histopathological assessment of intense and sub-intense dermal toxicity demonstrated that the ethanolic products of the fruit concentrate of *M. citrifolia* did not unfavorably influence the morphology of the rats' organs. This discovering concurs with the aftereffects of hematological and biochemical investigation. By and large, the histopathology investigation worked together with the consequences of body weight and organ weight. The ethanolic fruit concentrate of *M. citrifolia* did not result in toxicity towards the organs as there was no structural harm to the tried organs of liver, kidney, and skin of the rats amid the length of time of the study. The liver is the primary target organ of intense and sub-intense toxicity, where it was presented to the outside substances that were retained in the entrails and metabolized to different mixes, which might be hepatotoxic to the rats [27].

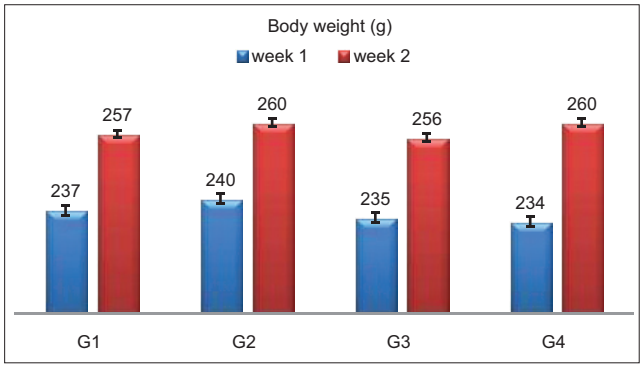

Fig. 3: Mean body weight (g) of rats in all groups. Data collected were recorded and presented as mean ± standard error of mean. G1: No treatment; G2: Paraffin; G3: Morinda citrifolia 2000 mg/kg; G4: Morinda citrifolia 5000 mg/kg. None of the numbers were seriously variant at p>0.05.

In this study, the liver histology uncovered ordinary hepatocytes, entryway and focal veins, and bile conduits and also hepatic supply route, and did not result in any modification to the structure of the liver cells between the control and treated classes. Conversely, the histological examination study directed by Harizal and his team [28] utilizing *Mitragyna speciosa* concentrate uncovered less extreme morphological changes in livers of mice treated with concentrate at measurements level (100 and 500 mg/kg). Then, an alternate study by Salawu [29] utilizing *Crossopteryx febrifuga* watched provocative changes histologically in the liver by invasion of lymphocytes at entryway and focal veins of rats treated at measurements level (500 and 1000 mg/kg) and demonstrated that the concentrate applied harmful impacts on the liver. The liver is equipped for recovering harmed tissue; thus, the liver capacity may not be weakened at an early stage taking after an affront from a toxicant [29]. Likewise, the intense toxicity study led on *C. fistula* case extricate and histological examination of the organs of rats treated with concentrate at measurements of (1000 mg/kg) uncovered that there was no potential toxicity or harm to the cell structure of liver, kidney, and testes. In addition, there was no corruption, incendiary response, fibrosis or neighborhood greasy degeneration saw in liver and the course of action of cell structure. This is just about like the organs of rats in the control bunch [30]. In this study, the morphology of liver cells in both control and treated classes were ordinary and no structural harms were watched. What's more, kidney micrograph in this study demonstrates that no antagonistic impacts were seen in all classes and the glomeruli and containers seemed typical and the Bowman's space is likewise unmistakably checked.

Interestingly, the study directed by Alade [31] uncovered the histology of kidneys saw with central proximal tubular epithelial corruption. In the interim, there was variety in the lungs between the control and rats treated with *B. monandra* leaf remove at measurement (4 g/kg). Separated from that, the study directed by Akanmu on *C. fistula* cases concentrate uncovered that there were

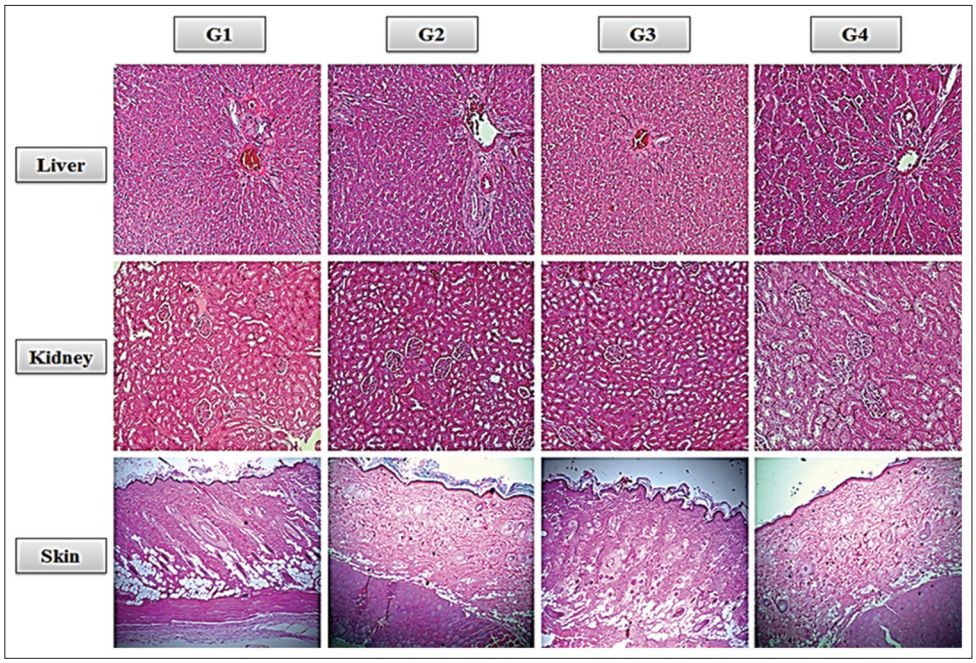

Fig. 4: Histological sections of liver, kidney and skin of rats in all groups. G1: No treatment; G2: Paraffin; G3: Morinda citrifolia 2000 mg/kg; G4: Morinda citrifolia 5000 mg/kg. Selected photomicrographs ×10 and ×20. Hematoxylin-eosin staining (scale bar: 200 µm).

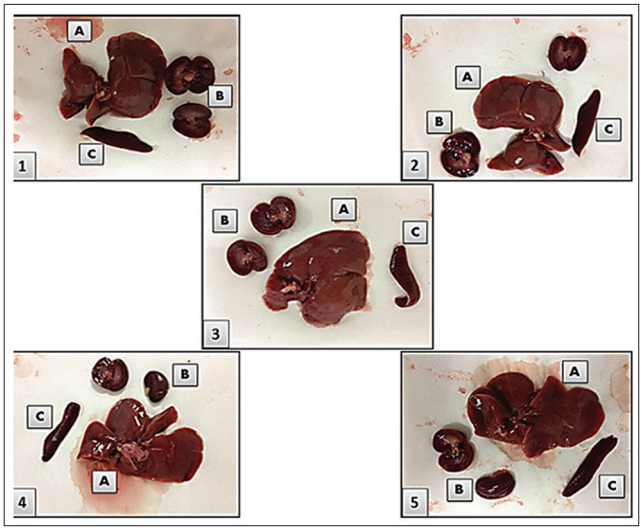

Fig. 5: Normal gross appearance of selected organs of the rats (1): No treatment group; (2): Paraffin group; (3): Morinda citrifolia (500 mg/kg) group; (4): Morinda citrifolia (1000 mg/kg) group; (5): Morinda citrifolia (2000 mg/kg). (a): Liver; (b): Kidneys; (c): Spleen.

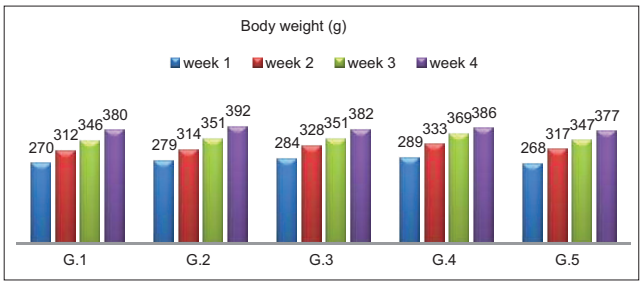

Fig. 6: Mean body weight (g) of rats in all groups. Data collected were recorded and presented as mean ± SEM. G1: No treatment; G2: Paraffin; G3: *Morinda citrifolia* 500 mg/kg, G4: *Morinda citrifolia* 1000 mg/kg, G5: *Morinda citrifolia* 2000 mg/kg. None of the values were significantly different at p>0.05.

slight changes in the histology of the kidneys from the rats treated with concentrate at dosage (1000 mg/kg), where a portion of the glomeruli and the proximal tubules were seen to extend without any harm contrasted with the controls [30]. Moreover in this study, the tiny investigation of the skin of the rats treated with concentrates did not show any progressions in the layers of the skin at the epidermis, dermis, and hypo dermis layers contrasted with control rats. Conversely, an intense and sub-endless dermal toxicity study carried out by Korani investigated the potential toxicity of colloidal nanosilver in lab animals [32].

In that study, there were some histopathological irregularities in skin, liver, and spleen of all test classes at dosage (100, 1000 and 10000 µg/ml). Diminished thickness of epidermis and dermis, expanded levels of Langerhans cells, aggravation, diminished papillary layer with customary collagens strands, decreased thickness of epidermis and dermis, diminished papillary layer with standard collagen filaments, acidophilic cytoplasm in muscle strands, degenerative filaments, and expanded levels of macrophages in endomysium were some of histopathological irregularities of dermal toxicity study conveyed by Korani and his group. In the present intense and sub-intense dermal toxicity ponders, the histopathological evaluation was performed on all the examples in the control and treatment bunches. No minuscule injuries attributable to the treatment were identified in the rats that got the ethanolic foods grown from the ground remove generally. The most imperative result is that in any of the measurement assembles no treatment-related changes watched for the histological examinations uncovered the nonexistence of toxicity to the liver, kidney, and skin of rats.

CONCLUSION

The outcomes of this study propose that ethanolic products of the soil concentrate of *M. citrifolia* do not result in any obvious *in vivo* danger. No death or indications of poison presence were seen in rats treated with the concentrate at measurements of 2000 and 5000 mg/kg (intense danger study), furthermore at dosages of 500 mg/kg, 1000 mg/kg and 2000 mg/kg (sub-intense lethality study), in this manner securing its being safe during usage. The histology exploration disclosed no progressions in the architectures of the chosen organs of the rats in both control and treated classes. Subsequently, it is presumed that fruit-concentrate of *M. citrifolia* can be utilized as topical restorative operators at those measurements, particularly in

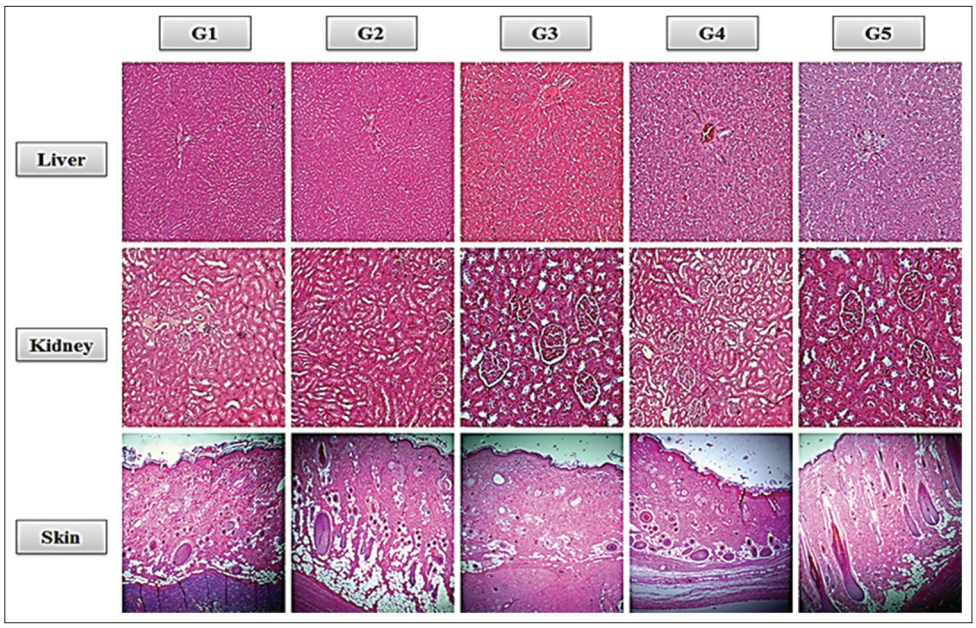

Fig. 7: Histological sections of liver, kidney and skin of rats in all groups G1: No treatment; G2: Paraffin; G3: Morinda citrifolia 500 mg/kg, G4: Morinda citrifolia 1000 mg/kg, G5: Morinda citrifolia 2000 mg/kg. Selected photomicrographs ×10 and ×20. hematoxylin-eosin staining (scale bar: 200 µm).

provincial groups where customary medications are exorbitant in view of their high cost.

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REFERENCES

1. Asante-Duah K. Public Health Risk Assessment for Human Exposure to Chemicals. Vol. 6. Springer Science & Business Media; 2002.
2. Morton JF. The ocean-going noni, or Indian mulberry (*Morinda citrifolia*, Rubiaceae) and some of its "colorful" relatives. Econ Bot 1992;46(3):241-56.
3. Elkins R. Hawaiian Noni (*Morinda Citrifolia*): Prize Herb of Hawaii and the South Pacific. Houghton Mifflin Harcourt; 1998.
4. Dixon AR, McMillen H, Etkin NL. Ferment this: The transformation of noni, a traditional polynesian medicine (*Morinda citrifolia*, Rubiaceae). Econ Bot 1999;53(1):51-68.
5. Ross IA. Chemical constituents, traditional and modern medicinal uses. Medicinal Plants of the World. Vol. 3. New York: Springer; 2007.
6. Cardon, D. Le monde des teintures naturelles. Belin, Paris. Chemistry 2003;49:4478-81.
7. Wang MY, Su C. Cancer preventive effect of *Morinda citrifolia* (Noni). Ann N Y Acad Sci 2001;952(1):161-8.
8. Solomon N. The tropical fruit with 101 medicinal uses. Allergy 1999;3:86.
9. McClatchey W. From Polynesian healers to health food stores: changing perspectives of *Morinda citrifolia* (Rubiaceae). Integr Cancer Ther 2002;1(2):110-20.
10. Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, *et al. Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. Acta Pharmacol Sin 2002;23(12):1127-41.
11. Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM. Toxicological studies of hydromethanolic leaves extract of *Grewia crenata*. Int J Pharm Sci Drug Res 2012;4:245-9.
12. Vaghasiya YK, Shukla VJ, Chanda SV. Acute oral toxicity study of *Pluchea arguta* boiss extract in mice. J Pharmacol Toxicol 2011;6(2):113-23.
13. Sasidharan S, Darah I, Jain K. *In vivo* and *in vitro*. Toxicity study of *Gracilaria changii*. Pharm Biol 2008;46(6):413-7.
14. Kirithikar KR, Basu BD, An IC. Indian Medicinal Plants. Vol. 1. Dehradun, India: International Book Distributors; 1995. p. 371-2.
15. Moshi MJ, van den Beukel CJ, Hamza OJ, Mbwambo ZH, Nondo RO, Masimba PJ, *et al*. Brine shrimp toxicity evaluation of some Tanzanian plants used traditionally for the treatment of fungal infections. Afr J Tradit Complement Alternat Med 2008;4(2):219-25.
16. Syahmi AR, Vijayarathna S, Sasidharan S, Latha LY, Kwan YP, Lau YL, *et al*. Acute oral toxicity and brine shrimp lethality of *Elaeis guineensis* Jacq. (oil palm leaf) methanol extract. Molecules 2010;15(11):8111-21.
17. Eaton DL, Klaassen CD. Principles of toxicology. Casarett and Doull's Toxicology: The Basic Science of Poisons. Vol. 1. New York: McGraw-Hill; 1996. p. 13-48.
18. Iversen PO, Nicolaysen G. Water for life. J Norwegian Med Assoc 2003;123:3402-5.
19. Klaassen CD, editor. Casarett and Doull's Toxicology: The Basic Science of Poisons. Vol. 1236. New York, NY: McGraw-Hill; 2013.
20. Carol SA. Acute, sub-chronic and chronic toxicology. In: Michael JD, Mannfred AH, editors. CRC Handbook of Toxicology. Boca Raton, FL, USA: CRC Press Inc.; 1995. p. 51-104.
21. Raza M, Al-Shabanah OA, El-Hadiyah TM, Al-Majed AA. Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of swiss albino mice. Sci Pharm 2002;70(2):135-45.
22. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of D-methylphenidate and D,L-methylphenidate in Sprague-Dawley rats. Toxicology 2002;179:183-96.
23. Dybing E, Doe J, Groten J, Kleiner J, O'Brien J, Renwick AG, *et al*. Hazard characterisation of chemicals in food and diet: dose response, mechanisms and extrapolation issues. Food Chem Toxicol 2002;40(2):237-82.
24. Adeneye AA, Ajagbonna OP, Adeleke TI, Bello SO. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. J Ethnopharmacol 2006;105(3):374-9.
25. Ajagbonna OP, Onifade KI, Suleiman U. Haematological and biochemical changes in rats given extract of *C. pocera*. Sokoto J Vet Sci 1999;1:3-12.
26. OECD. OECD Guideline for Testing of Chemicals. Repeated Dose 28-Day Oral Toxicity Study in Rodents. Paris, France: Organisation for Economic Co-operation and Development; 1995.
27. Rhiouani H, El-Hilaly J, Israili ZH, Lyoussi B. Acute and sub-chronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. J Ethnopharmacol 2008;118(3):378-86.
28. Harizal SN, Mansor SM, Hasnan J, Tharakan JK, Abdullah J. Acute toxicity study of the standardized methanolic extract of *Mitragyna speciosa* Korth in rodent. J Ethnopharmacol 2010;131(2):404-9.
29. Salawu OA, Chindo BA, Tijani AY, Obidike IC, Salawu TA, Akingbasote AJ. Acute and sub-acute toxicological evaluation of the methanolic stem bark extract of *Crossopteryx febrifuga* in rats. Afr J Pharm Pharmacol 2009;3:621-6.
30. Akanmu MA, Iwalewa EO, Elujoba AA, Adelusola KA. Toxicity potentials of *Cassia fistula* fruits as laxative with reference to Senna. Afr J Biomed Res 2004;7(1):23-6.
31. Alade GO, Akanmu MA, Obuotor EM, Osasan SA, Omobuwajo OR. Acute and oral sub-acute toxicity of methanolic extract of *Bauhinia monandra* leaf in rats. Afr J Pharm Pharmacol 2009;3:354-8.
32. Korani M, Rezayat SM, Ghamami SG. Silver nanoparticle induced muscle abnormalities: A sub-chronic dermal assessment in guinea pig. J Pharm Health Sci 2012;1:21-9.