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Research Article

ACUTE AND SUBACUTE TOXICITY STUDIES OF ETHANOLIC EXTRACT OF *MIRABILIS JALAPA* LINN IN WISTAR ALBINO RATS

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ABSTRACT

Objective: The objective of the study was to evaluate the toxicological potential of the ethanolic extract of leaves of *Mirabilis jalapa* linn through acute and subacute toxicity studies in albino Wistar rats.

Methods: For acute toxicity studies, the ethanolic extract of *M. jalapa* was given up to 2000 mg/kg and then the animals were observed for 14 days to find out any adverse effect or death. For sub-acute toxicity studies, the exact was given for 28 days and the following parameters were observed such as changes in body weight, food intake, water intake, hematological parameters, biochemical parameters, lipid profile, urine analysis, and histopathological studies were undertaken.

Results: Single oral administration of 2000 mg/kg of the ethanolic extract of *M. jalapa* produced no mortality or signs of toxicity. During subacute toxicity there were no changes in body weight, food intake and water intake were observed. There were no changes in lipid profile, hematological parameters, and biochemical parameters. In histopathological changes, there were no structural changes in treated groups when compared to control.

Conclusion: The leaves of ethanolic extract of *M. jalapa* is safe when administered for 28 days.

There were no deaths or signs of toxicity in treated rats during acute toxicity studies and subacute toxicity studies.

Keywords: *Mirabilis jalapa*, Hematological parameters, Histological changes, Toxicity studies.

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INTRODUCTION

Mirabilis jalapa linn is a herbal medicine commonly called Four O Clock or Marble of Peru [1].

Synonyms are *Mirabilis lindheimeri*, *Mirabilis dichotoma*, *Mirabilis odorata,* and Common Names are Clavillia, four-o'clocks, jalap, maravilla, bonina. In Tamil Nadu, it is known as *andhi mandhaarai* and in Andhra Pradesh it is called as *Chandrakantha indraganti,* and in Karnataka it is called as *sanje mallige,* in Malayalam it is called as *Naalumani poovu*, in Maharashtra it is called as *gulabakshi,* in Odisha it is called as *rangani,* and in Punjab it is called as *sham di sohnap*, which means "evening beauty" [2]. Branching stems grows to an extent of 36" with tall and erect in size and it is a perennial herb which is bushy and tender. Generally grows in various colors such as pink, rose, red, magenta, yellow, and white and different color appears in same plant. The flower blossom around 4 O' Clock and continue to blossom till morning. Size of the leaves is in Ovate and Dark Green color and generally appears as heart shape at the base [3]. It is used traditionally for purgative, emetic, genitourinary system disorders, muscle pain, abdominal colic, inflammation, in skin disease, as anesthetic, as anthelmintic, in bronchitis, in kidney problems, and in blood and vaginal disease [4].

METHODS

Plant collection and authentication of plant material

The leaves of the plant of *M. jalapa* were collected from in and around Kanyakumari district. The plant was identified and authenticated by Dr. Jeyaraman, Assistant Professor of Botany, Chennai.

Extraction process

The leaves were shade dried at room temperature. The dried leaves were subjected to size reduction to a coarse powder using dry grinder and pass through sieve. The powder was packed in Soxhlet apparatus and extracted successively with ethanol (60–80°C) [5]. The extract was dried at 45°C in hot air oven till solid to semi-solid mass is obtained and is stored in air tight container in a refrigerator below 10°C [6].

Experimental animals

Albino rats (Wistar rats) weighing (150–250 g) were placed in a polypropylene cages in a controlled room temperature 24±1°C and relative humidity of 60–70% in animal house. The animals were maintained in standard pellet diet and water *ad libitum*. They were acclimatized to laboratory condition for 7 days before commencement of the experiment [7].

Animal experimentation protocols were approved by Institutional Animal Ethical Committee in K.K. College of Pharmacy, Chennai with the reference no. (Ref: KKCP/2015/038).

Drug and preparation of stock solution

The ethanolic extract of *M. jalapa* was prepared in 1% carboxymethylcellulose solution in distilled water before oral administration to animals. It was used within 7 days and stored at 8°C while for further use, freshly prepared solution was used. The vehicle alone served as control.

Toxicity studies

Acute oral toxicity (OECD -423 Guidelines)

Three animals are used for each step. The dose level used as the starting dose was selected from one of four fixed levels, 5, 50, 300, and 2000 mg/kg body weight [8]. Animals were observed individually for the signs of toxicity all observations are systematically recorded with individual records being maintained for each animal. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.

Table 1: Body weight (g) of albino rats exposed to ethanolic extract of *Mirabilis jalapa* Linn for 28 days

Dose (mg/kg/day)	Days				
	1	7	14	21	28
Control	1 40.24±1.02	142.22±1.06	148.04±1.46	150.11±0.44	152.01±0.21
200 mg/kg M.E	144.11±0.01	142.24±1.65	140.22±1.21	138.20±0.11	136.12±0.01
400 mg/kg M.E	142.01±0.02	140.20±1.02	139.01±1.12	138.01±1.04	136.22±0.48

Values are mean of a six animals ± S.E.M (Dunnett's test) *p<0.05;**p<0.01.n=6

Table 2: Water (ml/day) intake of albino rats exposed to for 28 days

Dose (mg/kg/day)	Days (ml/rat)				
	1	7	14	21	28
Control	44.01±0.11	46.22±0.04	45.21±0.32	46.13±0.97	46.64±0.11
200 mg/kg M.E	48.42±0.63	52.08±0.14	55.67±0.12	58.20±1.24	60.12±1.02
400 mg/kg M.E	50.28±0.61	54.22±0.44	58.12±1.08	62.01±1.44	64.02±1.21

Values are mean of a six animals±S.E.M (Dunnett's test) *p<0.05;**p<0.01.n=6

Table 3: Food (g/day) intake of albino rats exposed to ethanolic extract of *Mirabilis jalapa* Linn for 28 days

Dose (mg/kg/day)	Days (g/rats)				
	1	7	14	21	28
Control	40.10±1.12	42.46±1.21	42.24±0.24	44.20±1.20	46.12±1.04
200 mg/kg M.E	42.20±0.44	45.28±0.18	47.22±1.02	50.16±1.02	52.11±0.22
400 mg/kg M.E	45.21±0.12	46.22±1.606	48.12±0.13	50.24±1.08	54.12±0.22

Values are mean of a six animals±S.E.M (Dunnett's test)*p<0.05;**p<0.01.n=6

Table 4: Hematological parameters after 28 days treatment with ethanolic extract of *Mirabilis jalapa* Linn in rats

Parameters	Control	200 mg/kg	400 mg/kg
Red blood cell (mm^3)	7.18±0.24	8.02±1.44	9.46±1.28
HB (%)	14.22±0.10	15.42±0.28	16.48±1.0212
Leukocyte ($\times 10^6$/ml)	10144±200.12	10228±241.27	10302±252.24
Platelets/ul	1432±21.22	1411±30.20	1462±32.01
Mean corpuscular volume (gl)	55.02±1.02	56.02±2.22	57.12±2.08

Values are mean of a six animals ± S.E.M (Dunnett's test)* p<0.05;**p<0.01.n=6

Table 5: Effect of treatment with ethanolic extract of *Mirabilis jalapa* Linn on biochemical parameters

Dose (mg/kg)	Control	200 mg/kg	400 mg/kg
Total bilirubin (mg/dl)	0.210±0.11	0.214±0.10	0.216±0.16
Bilirubin direct (mg/dl)	0.1±0.02	0.1±0.04	0.1±0.04
Bilirubin indirect (mg/dl)	0.1±00	0.1±00	0.1±00
ALP (U/L)	382.02±10.22	360.46±10.12	340.12±12.04
Serum glutamate oxaloacetate transaminase (U/L)	160.02±1.06	160.11±1.04	159.26±1.12
Serum glutamic-pyruvic transaminase (U/L)	46.14±0.20	46.20±0.24	46.46±0.02
Total protein (g/dl)	10.01±0.08	9.02±0.11	9.01±1.48
Albumin (g/dl)	3.10±0.02	3.10±0.04	3.12±0.04
Globulin (g/dl)	6.08±0.12	5.84±0.24	5.42±0.24

LFT: Liver function test. Values are mean of a six animals±S.E.M (Dunnett's test)* p<0.05;**p<0.01.n=6

Table 6: Renal function test

Dose (mg/kg)	Control	200 (mg/kg)	400 (mg/kg)
Urea (mg/dl)	56.21±1.23	55.80±1.02	53.09±1.21
Creatinine (mg/dl)	0.74±0.01	0.74±0.02	0.76±0.04
Uric acid (mg/dl)	1.6±0.02	1.6±0.04	1.6±0.06
Na m.mol	136.4±1.02	138.2±1.02	140.01±1.20
K m.mol	18.01±1.14	19.02±1.08	19.42±1.22
Cl m.mol	96.04±1.02	99.20±0.12	100.04±0.22

Values are mean of a six animals±S.E.M (Dunnett's test) *p<0.05;**p<0.01.n=6

Table 7: Lipid profile

Parameters	Control	200 mg/kg	400 mg/kg
Blood glucose (mg/dl)	126.20±0.02	126.42±1.24	126.12±1.24
Triglycerides(mg/kg)	80.12±1.22	79.12±1.01	78.11±0.14
High-density lipoprotein (mg/dl)	12.08±1.02	12.44±1.20	12.88±1.28
Total cholesterol (mg/kg)	40.20±1.01	38.24±1.24	38.16±1.28
Low-density lipoprotein (mg/dl)	38.10±1.04	40.12±1.42	40.00±1.04
Very-low-density lipoproteins (mg/dl)	15.62±1.02	15.22±1.08	15.01±1.24
Total cholesterol/High-density lipoprotein ratio (g/dl)	2.02±0.22	2.44±1.20	3.12±0.04

Values are mean of a six animals±S.E.M (Dunnett's test) *p<0.05;**p<0.01.n=6

Sub-acute oral toxicity study (OECD – 407 guidelines)

From the results of acute toxicity, studies in Wistar rats indicated that *M. jalapa* was nontoxic and no behavioral changes were observed up to the dose level of 2000 mg/kg body weight. The test doses were selected as 200 and 400 mg/kg [9]. Ten rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 days. The test substance was freshly

prepared every day for 28 days [10]. The control animals were administered vehicle only.

Body weight

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated [11].

Food and water consumption

The quantity of food consumed by groups consisting of six animals for different doses was recorded at weekly interval. Food consumed per animal was calculated for control and the treated dose groups.

Clinical signs

All animals were observed daily for clinical signs. The time of onset, intensity, and duration of these symptoms, if any, were recorded.

Mortality

All animals were observed twice daily for mortality during entire course of study.

Terminal studies

Laboratory investigations

Following laboratory investigations were carried out on day 29 in fasted animal's over-night. On 29th day, the animals were anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: One with EDTA for immediate analysis of hematological parameters, and the other without any anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 min to obtain the serum. Serum was stored at 20°C until analyzed for biochemical parameters.

Hematological investigations

Blood samples of control and experimental rats were analyzed for hemoglobin content, total red blood corpuscles, white blood corpuscles count, mean corpuscular volume, and platelets. Then, the mean values are calculated [12].

Biochemical investigations

Serum and urine was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, albumin, globulin, bilirubin, urea, uric acid, creatinine, potassium, chlorine, and sodium levels were carried using standard methods. Activities of glutamate oxaloacetate transaminase/aspartate aminotransferase, glutamate pyruvate transaminase/alanine amino transferase, and alkaline phosphatase were estimated as per the colorimetric procedure [13].

Urine analysis

Urine samples were collected in week 4 and in week 6 and for the estimation of normal parameters.

Necropsy

All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, adrenals, spleen, brain, heart, uterus, and testes/ovaries were recorded.

Histopathology

Histopathological investigation of the vital organs was done. The organs include stomach, heart, kidneys, liver, and spleen of the animals were preserved they were subjected to histopathological examination [14].

Statistical analysis

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and biochemical parameters were subjected to One-way ANOVA followed by Dunnett's test.

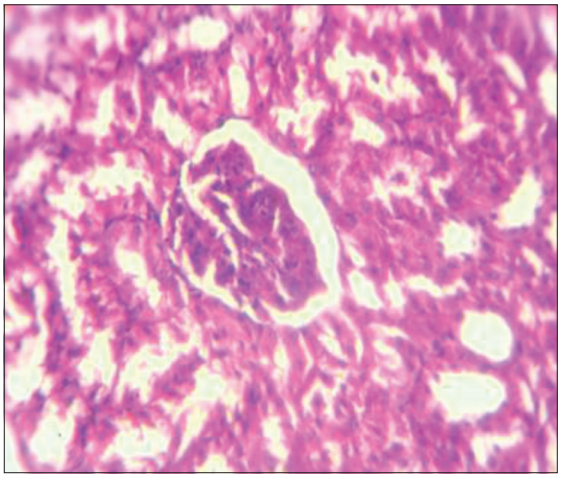
RESULTS

Acute oral toxicity studies

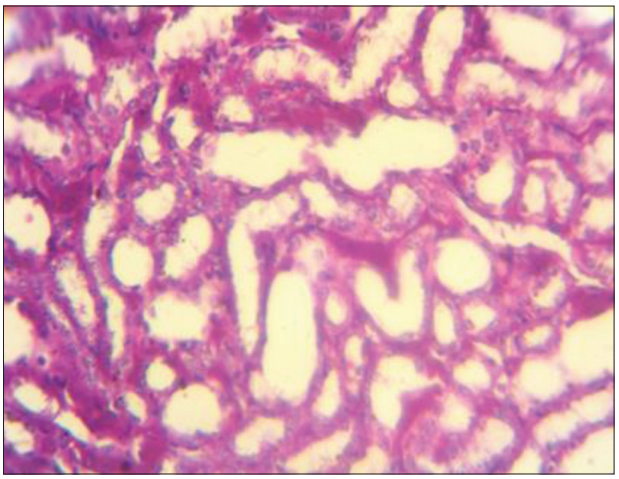
There were no signs of toxicity up to 2000 mg/kg of the extract; it shows the absence of pilo erection, righting reflux, seizures, any stains of urine, loss of righting reflux, alopecia, edema, lacrimation, etc.

Subacute oral toxicity studies

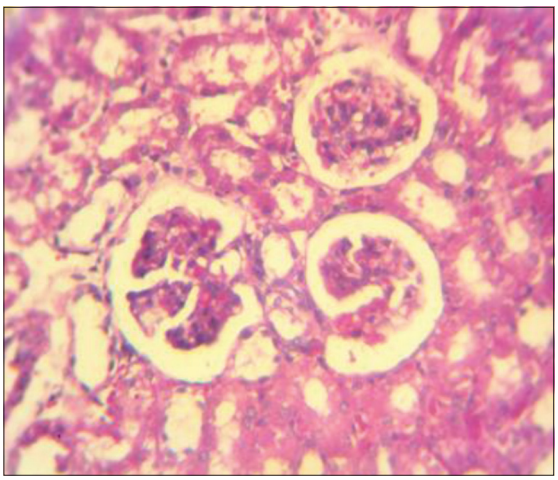
Administration of ethanolic extract of mirabilis jalapa 200mg/kg and 400mg/kg during 28 days had no significant changes on body weight (Table 1), water intake (Table 2), food intake (Table 3) when compared



Control



***Mirabilis jalapa* Linn (200 mg/kg)**



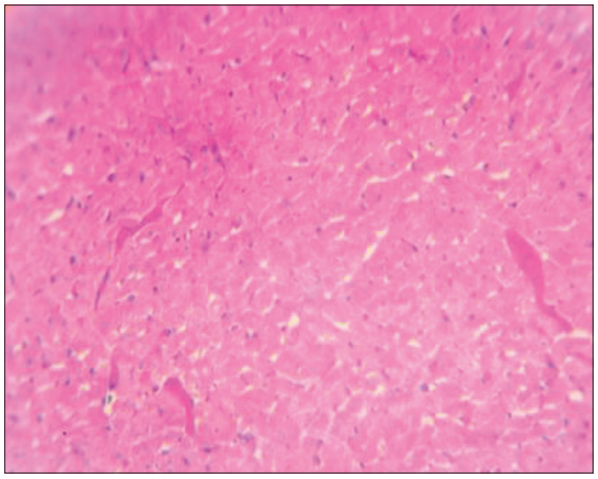
***Mirabilis jalapa* Linn (400 mg/kg)**

to control group. The effect of the extract on hematological parameters are shown in (Table 4) result in no significant changes when compared to the control group. The results of biochemical parameters (Table 5), showed no significant difference in all parameters when compared to control. Administration of the extract for 28 days had no significant changes in renal function test (Table 6), lipid profile (Table 7) urine analysis (Table 8) when compared to control group. The effect of different doses of administration of ethanolic extract of mirabilis jalapa on organ weight (Table 9) showed no significant changes when compared to control.

Histopathological Studies

Kidney

Heart



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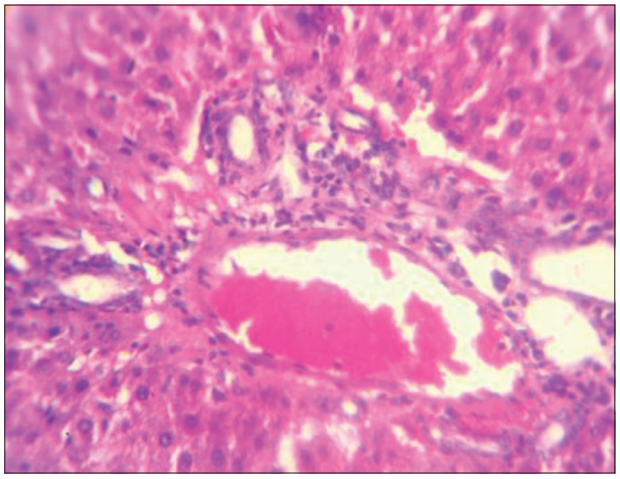

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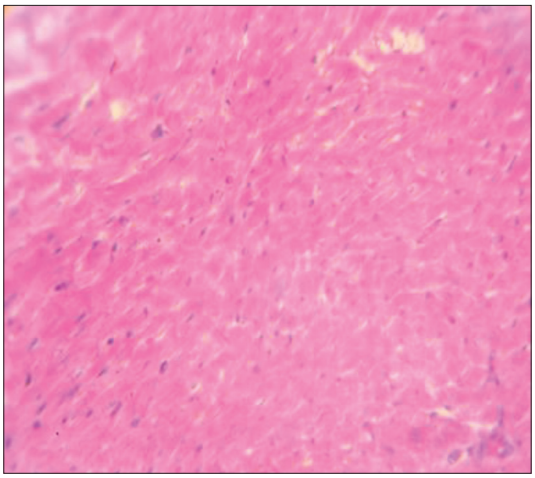
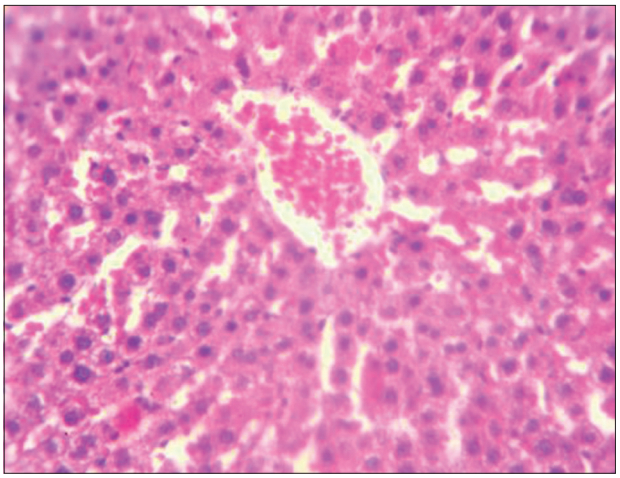
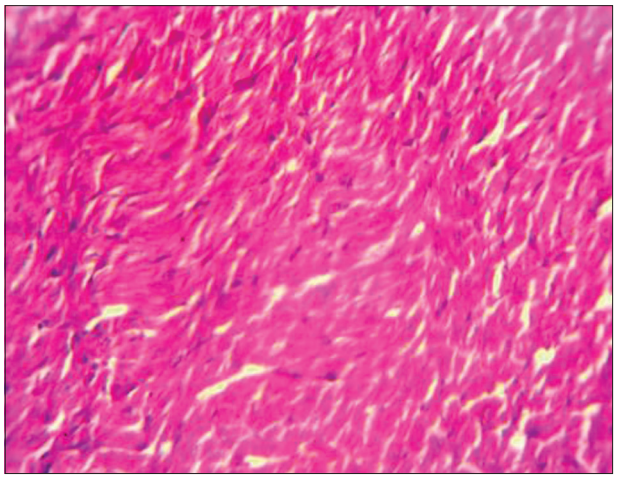


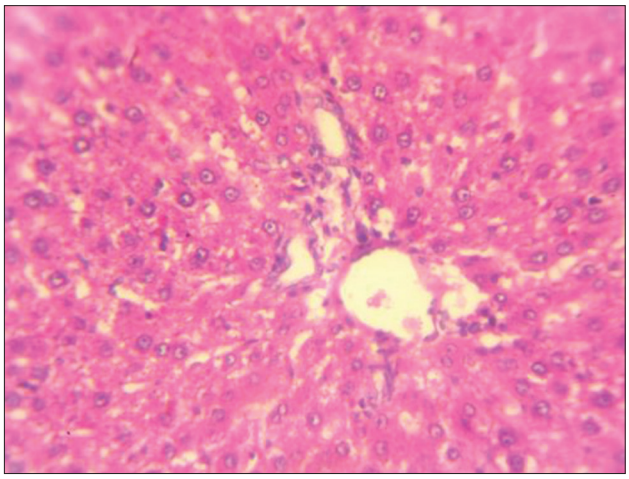
***Mirabilis jalapa* Linn (200 mg/kg)**



***Mirabilis jalapa* Linn (200 mg/kg)**



***Mirabilis jalapa* Linn (400 mg/kg)**

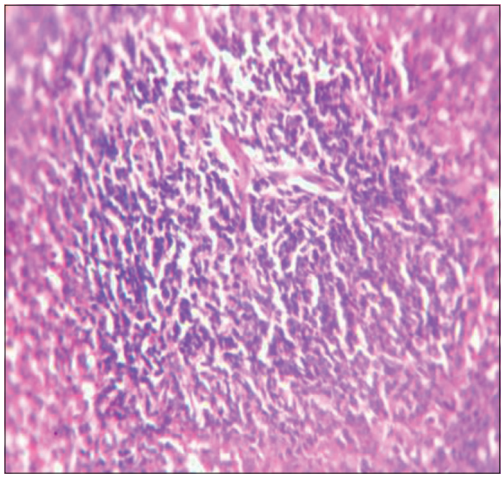


***Mirabilis jalapa* Linn (400 mg/kg)**

Liver
Spleen
Stomach

DISCUSSION

In acute toxicity studies, it showed no toxic signs up to 2000 mg/kg body weight and does not produce any mortality or toxic effect. In subacute toxicity studies, the extract 200 mg/kg and 400 mg/kg have been given for a period of 28 days. During this period, there was a gradual normal increase in the mean body weight of the extract and control group. There were no changes in food intake and water intake of treated groups. In assessment of hematological parameters, the readings are all within the reference range for rats. In biochemical parameter, the extract 200 mg/kg and 400 mg/kg of did not show any significant changes in extract



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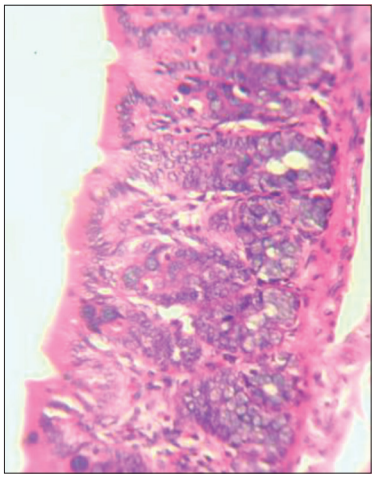
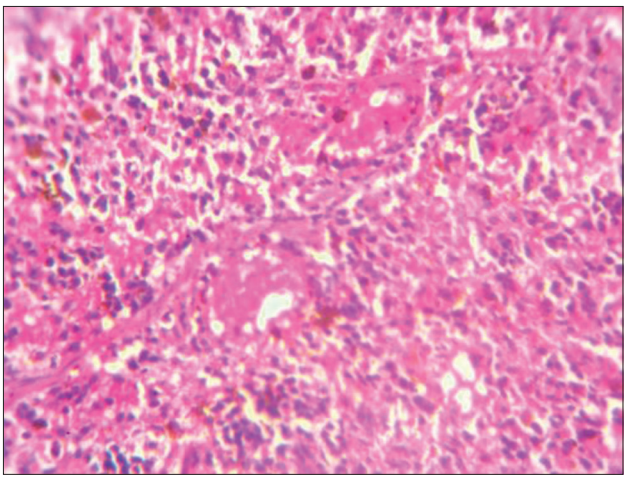
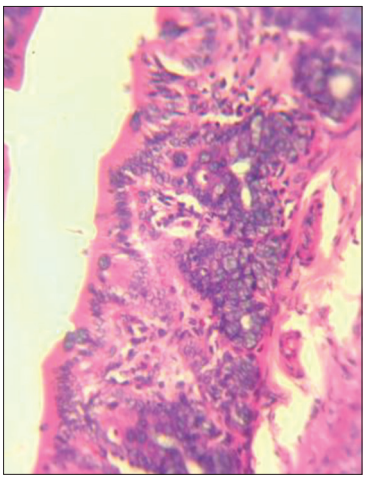


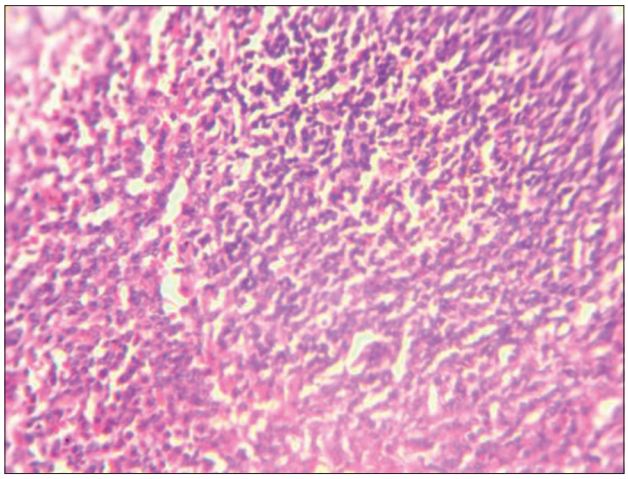
Control



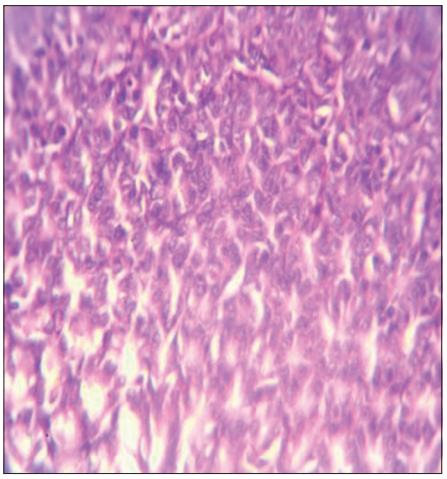
***Mirabilis jalapa* Linn (200 mg/kg)**



***Mirabilis jalapa* Linn (200 mg/kg)**



***Mirabilis jalapa* Linn (400 mg/kg)**



***Mirabilis jalapa* Linn (400 mg/kg)**

Table 8: Urine analysis

Parameters	Control	200 mg/kg	400 mg/kg
Transparency	Clear	Slightly turbid	Slightly turbid
Specific gravity	1.010	1.010	1.010
PH	>7.2	>7.4	>7.4
Protein	Nil	2+	2+
Glucose	Nil	Nil	Nil
Bilirubin	−ve	−ve	−ve
Ketones	−ve	−ve	−ve
Blood	Absent	Absent	Absent
Urobilinogen	Normal	Abnormal	Abnormal
Pus cells	0-cells/HPF	0-cells/HPF	1-cells/HPF
Red blood corpuscles	Nil	Nil	1-cells/HPF
Epithelial cells	Nil	1-cells/HPF	Nil
Crystals	Nil	Nil	Nil
Casts	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen
Color	Yellow	Yellow	Yellow

Values are mean of a six animals±S.E.M (Dunnett's test)* p<0.05;**p<0.01.n=6

Table 9: Effect of oral administration of ethanolic extract of *Mirabilis jalapa* Linn on organ weight

Dose (mg/kg)	Control	200mg/kg	400mg/kg
Liver (g)	4.22±0.20	4.84±0.12	5.10±0.89
Heart (g)	0.60±0.01	0.60±0.02	0.62±0.02
Lung (g)	1.44±0.02	1.42±0.04	1.42±0.06
Spleen (g)	0.54±0.02	0.54±0.04	0.56±0.08
Ovary (g)	1.54±0.08	1.56±0.02	1.58±0.02
Testes (g)	1.26±0.06	1.28±0.26	1.32±0.18
Brain (g)	1.42±0.12	1.44±0.01	1.52±0.14
Kidney (g)	0.64±0.01	0.66±0.02	0.68±0.02
Stomach (g)	1.34±0.1	1.38±0.10	1.38±0.11

Values are mean of a six animals±S.E.M (Dunnett's test) *p<0.05;**p<0.01. n=6

treated groups. In histopathogical studies, liver was excised from all treated groups to analyze the injuries in bile ducts, hepatic vein, and portal area. It showed that there was no modification detected when compared to control group. In spleen, heart, intestine, there were no structural changes when compared to control. Kidney tissues also examined for any morphological changes. There were no morphological changes when compared to control group.

CONCULSION

In administration of the ethanolic extract of *M. jalapa* 200 mg/kg and 400 mg/kg for a period of 28 days, there was no changes in food intake, water intake, biochemical parameters, lipid profile, renal function, and urine analysis when compared to control. There were no signs of toxicity observed in heart, kidney, stomach, spleen, and liver. The ethanolic extract of *M. jalapa* is a safer and nontoxic and further studies have to be carried out for its medicinal and therapeutic efficacy.

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AUTHOR'S CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Nil.

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