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Research Article

NEPHROPROTECTIVE ACTIVITY OF LEAVES OF *MIRABILIS JALAPA.L* BY ACETAMINOPHEN INDUCED NEPHROTOXICITY

Sharmila Shaik*, M. Dhanalakshmi, T. Jayasree
Department of pharmacology, Krishna Teja Pharmacy College, Chadalawada Nagar, Renigunta Road, Tirupathi-517 507, Andhra Pradesh, India.
Email:sharmilashaik.mpharm@gmail.com

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Abstract:

Mirabilis Jalapa. Linn (Nyctaginaceae) is an herbaceous medicinal plant used to treat wound healing, stomachic, liver problems, dysentery, diarrhea, muscular pain and abdominal colic in India and other Asian countries. Acetaminophen (APAP) is a commonly used antipyretic and analgesic agent which, at high doses, causes liver and kidney necrosis in man and animals. The aim of this study was to investigate the nephroprotective and antioxidant activities of ethanol extract of *MJ* at two dose levels of 250 and 500 mg/kg B/W on acetaminophen (APAP) induced toxicity in male albino rats. APAP significantly increased levels of serum urea, hemoglobin (Hb), total leukocyte count, packed cell volume, creatinine, DLC, and mean corpuscular volume, raised body weight, and reduced levels of neutrophils, mean corpuscular Hb content, mean corpuscular hematocrit, granulocytes, uric acid, and platelet Concentration. *Mirabilis Jalapa. Linn* (MJ) inhibited the hematological effects of APAP. MJ significantly increased activities of renal superoxide dismutase, catalase, glutathione, and glutathione peroxidase and decreased malondialdehyde content of APAP treated rats. Apart from these, histopathological changes also showed the protective nature of the MJ extract against APAP induced necrotic damage of renal tissues. In conclusion it was observed that the ethanol extract of MJ conferred nephroprotective and antioxidant activities by histopathological and biochemical observations against APAP induced renal damage in rats.

Key words: *Mirabilis Jalapa.Linn*, nephrotoxicity, oxidative stress, acetaminophen.

Introduction:

Acetaminophen (APAP) also known Paracetamol, N-acetyl p-aminophenol is widely used analgesic and antipyretic agents belonging to the Para amino phenol class of non steroidal anti inflammatory drugs (NSAIDS) that is safely

employed for a wide range of treatments [1], overdose of APAP in human is fairly common and is often associated with hepatic [2-4] and renal damage[5-7] . Although nephrotoxicity is less common than hepatotoxicity in APAP overdose, renal tubular damage and acute renal failure can occur even in the absence of liver injury [8-10] and can even lead to death in humans and experimental animals [11-12]. At therapeutic doses, APAP is metabolized via glucuronidation and sulfuration reactions occurring primarily in the liver, and results in water-soluble metabolites that are excreted via the kidney. As a result of the metabolic conversion of APAP by the microsomal P-450 enzyme system, a highly reactive intermediate, N-acetyl-p-benzoqunione imine (NAPQI) is produced. NAPQI directly reacts with glutathione (GSH) and at overdoses of APAP, the depletion of cellular GSH occurs. This allows NAPQI to bind to cellular proteins and initiate lipid peroxidation, leading to renal injury. Previous evidence suggests that oxidative stress with increased generation of reactive oxygen species, depletion of reduced glutathione (GSH) and lipid peroxidation play a crucial role in the development of APAP-induced hepatic and renal damage [13].

Studies are going on throughout the world for the search of protective molecules that would provide maximum protection to the liver, kidney as well as other organs and practically very little or no side effects would be exerted during their function in the body[14-15]. A number of herbs are traditionally used in different countries in response to drug or toxin induced hepatic and renal disorders [16].

Mirabilis jalap Linn, a traditional medicinal herb belonging to the family Nyctaginaceae in found throughout India. It is commonly known as four O' clock plant [17]. *Mirabilis jalapa.L* is a perennial herb or under shrub. An erect herb to about one meter high, native of Peru, but now dispersed throughout the tropics. The plant is decorative and a favorite garden plant with red, white, yellow, pink, purple and orange flowers. Which survive under conditions of neglect in England, France and some parts of the Africa [18].Leaf is used as anti- inflammatory, boils, to heal wound as external application, bruises and also for allaying itching in urticaria .Roots as purgative. Roots thickened and tuberous up to 1 m high, stems swallow in clusters, funnel – shaped, simple or double, fragrant ellipsoid and one seeded [19]. The main constituents of MJ were found belonging to alphaamyrins, arabinose, beta-amyrins, camposterol, daucosterol, flavonoids, phenolic compounds and alkaloids [20].

However, the nephroprotective effects of this plant extract have not been shown in scientific research work. Keeping this in view the present study is aimed to evaluate the nephroprotective and antioxidant activities of ethanolic extract of *Mirabilis jalapa.Linn* against APAP induced toxicity in rats.

2. Materials and Methods:

Plant material:

The leaves of *Mirabilis Jalapa.Linn* was collected from Tirupathi, Chittoor district, Andhra Pradesh, India and the plant material was taxonomically identified and authenticated by the Dr.Madhava chetty(Research Officer) botany, Andhra Pradesh. Voucher specimen (AECBT-4/2011-2012) of this plant has been retained in the Krishna Teja Pharmacy College, Tirupathi and Andhra Pradesh, India.

Extraction:

The leaves was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (90% v/v) in soxhlet apparatus at 60^{o}C. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in vacuum desiccators [21].

Phytochemical screening:

The presence of phytochemical constituents in the ethanolic extract of leaves of *Mirabilis jalapa.L* was tested by using the standard methods. These standard methods revealed the presences of glycosides, flavonoids, steroids, tannins, saponins, triterpenoids and alkaloids [20].

Animals:

Studies were carried out using Wistar albino male rats (150-200g), obtained from Raghavendra Enterprises, Bangalore India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2^{o}C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Poultry Research Station, Nandhanam, India and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Acute toxicity studies:

The Acute Toxicity study has been found that the no mortality or any signs of behavioral changes or toxicity observed after oral administration of the ethanolic extract of leaves up to the dose of 2000mg/kg bodyweight in mice according to the OECD, 423 guidelines.

Acetaminophen induced nephrotoxicity in rats:

Animals were randomized and divided into four groups (I-IV) of six animals in each group. Group I served as untreated control and is fed orally with normal saline 5ml/kg body weight daily for 14 days. Group II rats were similarly treated as group I. Group III and IV animals were treated with 250 mg/kg and 500mg/kg body weight of the ethanol extract of *Mirabilis jalapa.L* for 14 days, respectively. On the 14^{th} day, acetaminophen suspension was given by oral route, in a dose of 750 mg/kg body weight to all rats except the rats in group I.

Hematological study:

After 48 h, animals were sacrificed by chloroform anesthesia. Blood samples were collected by cardiac puncher under diethyl ether anesthesia, using 21 gauge (21 G) needles mounted on a 5ml syringe (Hindustan syringes and medical devices ltd, Faridabad, India.) into ethylene diamine tetra-acetic acid (EDTA) – coated sample bottles for analyzed Hematological parameters like full blood count (FBC), hemoglobin, (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet concentration (PLC) and Total leucocyte count (TLC). These parameters were analyzed using automatic hematological system (Sysmex Hematology – Coagulation system, Model MO-1000 I, Trans Asia, Japan).

Sampling and biochemical analysis:

Following termination of the experiment on the day 7, the rats were fasted overnight for 14 hours. Blood samples were collected by cardiac puncture with 21G needle mounted on 5 ml syringe (under diethyl ether anesthesia) and centrifuged for 10min at 5000 rpm. The obtained clear sera were stored at −20 ◦C for subsequent measurement of blood urea, creatinine and uric acid levels using colorimetric assay kits, Bayer (Seamon) according to the manufacturer's instructions.

Preparation of renal homogenate:

The kidneys were removed and dissected free from the surrounding fat and connective tissue. Each kidney was longitudinally sectioned, and renal cortex was separated and kept at −8oC. Subsequently, renal cortex was homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The renal cortical homogenates were centrifuged at 5000 rpm for 10 min at 4oC. The resulting supernatant was used for the determination of malondialdehyde (MDA) content, reduced glutathione (GSH) levels and antioxidant enzyme levels such as superoxide dismutase

(SOD), catalase (CAT), glutathione reductase (GRD) and glutathione peroxidase (GPX) activity using colorimetric assay.

Biochemical estimation of markers of oxidative stress:

MDA content was measured according to the earlier method reported. SOD activity was determined according to the previous report CAT activity was determined from the rate of decomposition of H2O2 by the reported method [22-23]. GPx activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3 [24]. Glutathione reductase activity was assayed according to the previous reports. Protein content in the tissue was determined by the method reported earlier using bovine serum albumin (BSA) as the standard [25].

Histopathological examination:

Pieces of kidney from each group were fixed immediately in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (50–100%) alcohol, embedded in paraffin, cut into 4–5 μm thick sections and stained with hematoxylin–eosin. The sections were evaluated for the pathological symptoms of nephrotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc.

3. Results:

Effect of Mirabilis jalapa. L extract on serum urea, uric acid and creatinine concentrations:

Serum urea and creatinine concentrations were significantly increased ($p < 0.01$) in the APAP treated group of animals compared to the normal animals indicating the induction of severe nephrotoxicity (Figures 2 and 3). Treatment with the ethanol extract of *Mirabilis jalapa.L* showed significant ($p < 0.05$ and $p < 0.01$) (Group III and IV) decrease in concentrations of serum urea and creatinine compared to the APAP treated group. However the levels of uric acid (UA) significantly decreased ($p<0.01$) in the APAP treated groups (Group II, Figure 4), when compared to the control group. Treatment with ethanol extract of *Mirabilis jalapa.L* significantly ($p < 0.05$ and $p < 0.01$) (Group III and IV respectively) increased the uric acid levels, compared to the APAP treated group.

Effect of ethanol extract of *Mirabilis jalapa.L* on hematological parameters:

APAP caused a significant ($P<0.01$) increase in the levels of Hb, PCV, DLC and MCV (Figures 2 and 3) (Group II) when compared to the normal control group (Group I), resulting in acetaminophen associated nephropathy. Administration of ethanol extract of *Mirabilis jalapa.L* significantly (Group III and Group IV; $p<0.05$, $p<0.01$ respectively) decreased the Hb, PCV, DLC and MCV levels as compared to the APAP induced group (Group II)

(Figure 2 and 3). Further, in APAP treated group (Group II), the levels of PLC, MCHC, MCH and lymphocyte are decreased significantly ($p<0.01$) when compared with normal (Group I) (Figures 1, 2 and 4). Administration of *Mirabilis jalapa.L* ethanol extract ensures that these levels are retrieved normally, significantly ($P<0.05$, $P<0.01$) when compared with Group 2.

Effect of the Mirabilis jalapa.L extract on kidney antioxidant status:

The activity of CAT in the APAP treated group was significantly ($p<0.01$) decreased when compared to the normal animals (Group I). Treatment with the ethanol extract of *Mirabilis jalapa.L* significantly ($p < 0.05$ and $p < 0.01$) (Group III and IV) prevented decrease in the level of catalase activity (Figure 1) compared to the APAP induced rat (Group II). Likewise, the decreased GPx activity as a result of the treatment with APAP was also restored by the *Mirabilis jalapa.L* extract ($p < 0.05$ and $p < 0.01$) (Figure 2) for Group III and IV as compared to the normal group. Renal SOD activity was decreased significantly ($p<0.01$) in the APAP treated (group II) animals compared to normal group. Treatment with the ethanol extract of *Mirabilis jalapa.L* (250 and 500 mg/kg body wt) (Group III and IV) significantly ($p<0.05$ and $p<0.01$ respectively) elevated the SOD levels as compared to the APAP induced (Group II) animals (Figure 5). The GSH and MDA levels of APAP and extract treated animals are presented in (Figures 2 and 3). The GSH level reduced significantly ($p < 0.01$) along with increased in MDA concentration in the APAP treated group as compared to the Group I. However on treatment with *Mirabilis jalapa.L* ethanol extract, the GSH level was found to be enhanced significantly ($p<0.05$ and $p < 0.01$) and the MDA contents were reduced in Group III and IV as compared to the induced group (Group II) (Figure 2).

Histopathological studies:

The biochemical results were also confirmed by the histological pattern of normal kidney showing normal tubular brush borders and intact glomeruli and Bowman's capsule (Figure 6 (A)). Treatments with acetaminophen sever tubular necrosis and degeneration has shown in the renal tissue (Figure 6 (B)). The rats treated with ethanolic extract of *Mirabilis jalapa.L* (250mg/kg body weight) showed normal tubular pattern with a mild degree of swelling, necrosis and degranulation (Figure 6(C)) treatment with the extract (500 mg/kg body weight) ameliorated the toxic manifestations in the kidney (Figure 6 (D)).

Discussion: Acetaminophen over dose is often linked to many metabolic disorders including serum electrolyte, urea and creatinine dearrangements. Increased concentration of serum urea and creatinine are considered for investigating

drug induced nephrotoxicity in animals and man[26].The reason behind acetaminophen toxicology is the CYP-mediated conversion of acetaminophen to a highly reactive quinone imine, A^ acetyl-pbenzoquinone imine. The fundamental role of NAPQI in the toxicity of acetaminophen has been supported by many subsequent studies [27].

The vital function that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication by xenobiotics, makes the hematopoietic system unique as a target organ [28]. The various blood cells (erythrocytes, leucocytes, and platelets) are produced at a turnover rate of about 1 to 3 million per second in a healthy human adult and this value could be altered in certain physiological or pathological states including hemolytic anemia or suppressive inflammation[29]. Certain drugs including alkylating cytotoxic agents could also affect blood formation rate and the normal range of hematological parameters [30]. Treatment with APAP oral dose significantly increased the Hb, PCV, DLC & MCV levels. After administration of *Mirabilis jalapa.L* extract these levels are significantly decreased compare to the APAP induced group. Whereas the levels of granulocyte, MCH, MCHC and PLC were decreased significantly in the APAP treated group, compared to the normal control group. However after administration of *Mirabilis jalapa.L* extract these levels are significantly increased compared to the APAP treated group. However this study shows that the *Mirabilis jalapa.L* extract could contain candidate molecules reversing the hematotoxic effect of acetaminophen, with ensuing improvement of hematopoiesis.

However the blood hematological parameters such as Hb, PCV, DLC and MCV values significantly increased the APAP induced groups, the other hematological parameters like MCHC, Neutrophils, PLC and MCH were decreased significantly. The pretreatment with *Mirabilis jalapa.L* extract significantly decreased the Hb, PCV, DLC and MCV values along with a significant increase in MCHC, Neutrophils, PLC, MCH contents when compared to APAP induced group.

Blood urea nitrogen is found in the liver protein that is derived from diet or tissue sources and is normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance. Elevation of urea and creatinine levels in the serum was taken as the index of nephrotoxicity. Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatinine breakdown. Thus serum urea concentration is often considered a more reliable renal function predictor than serum creatinine [31].

In the present study, administration of hepatotoxic and nephrotoxic doses of APAP to rats resulted in development of oxidative stress damage in hepatic and renal tissues. In this study, APAP induced nephrotoxicity showed a

significant (P<0.01) increase in the serum urea and creatinine concentrations in the Group II (APAP induced) rat when compared to the normal group (Group I). Moreover, oral administration of ethanolic extract of *Mirabilis jalapa.L* significantly (P<0.01) decreased in group III & IV when compared to the Group II. However the level of uric acid is significantly decreased (P<0.01) in the Group II rats when compared to Group I. Oral administration of plant extract significantly (P<0.01) increases the uric acid level in Group I when compared to the APAP induced rats (Group II).

Thus, oxidative stress and lipid peroxidation are early events related to radicals generated during the hepatic metabolism of APAP. Also the generation of reactive oxygen species has been proposed as a mechanism by which many chemicals can induce nephrotoxicity. Previous studies have clearly demonstrated that acute APAP overdose increases the lipid peroxidation and suppresses the antioxidant defense mechanisms in renal tissue. However in the APAP treated animals the MDA levels are increased significantly, when compared to normal control rats. On Administration of ethanolic extract of *Mirabilis jalapa.L,* the levels of MDA decreased significantly when compared to APAP induced rats. During kidney injury, superoxide radicals are generated at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism. The present study also demonstrated that acute APAP overdose resulted in a decrease in the SOD, CAT and GST activities, when compared with normal control rats. It is due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. When rat was treated with the *Mirabilis jalapa.L* extract the reduction of SOD, CAT and GST activity was increased significantly when compared with induced group (P<0.01)(Group II) [32].

Current evidence suggests that intracellular GSH plays an essential role in detoxification of APAP and prevention of APAP-induced toxicity in the liver and kidney. However, APAP was found to increase the microsomal superoxide and hydrogen peroxide production in mice. The generation of the reactive oxygen species appears as an early event which precedes intracellular GSH depletion and cell damage in APAP hepatotoxicity. APAP administration also caused a significant decrease in GSH content. Administration of *Mirabilis jalapa.L* extract helped to uplift the GSH depletion induced by APAP. APAP-induced nephrotoxicity was evidenced by biochemical measurements and histopathological changes that coincide with the observations of other investigators. The biochemical results were also confirmed by the histological findings which showed preservation of the glomeruli and the surrounding

Bowman's capsule and mildly swollen tubules. Other nephroprotective medicinal plants have been reported of inhibiting xenobiotic-induced nephrotoxicity in experimental animal models due to their potent anti-oxidant or free radicals scavenging effects. In addition, alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity. The protection offered by the extract could have been due to the presence of flavonoids and alkaloids [33].

The activity elicited by the extract might be due to its ability to activate antioxidant enzymes. The findings suggest the potential use of the ethanolic extract of *Mirabilis jalapa.L* as a novel therapeutically useful nephroprotective agent. Therefore, further studies to elucidate their mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases.

Table-I: Effect of treatment with ethanolic extract of *Mirabilis Jalapa* extract on the Biochemical levels in rats with acetaminophen (APAP)-induced nephrotoxicity.

Parameters	**Group-I**	**Group-II**	**Group-III**	**Group-IV**
Urea (UR) (mg/dl)	45.44±2.59	64.79±2.99**	60.41±2.73*	54.53±2.68**
Uric acid (UA)(mg/dl)	1.69±0.22	0.87±0.35**	1.21±0.20*	1.31±0.18**
Creatinine CR)(mg/dl)	1.01±0.07	2.84±0.84**	1.85±0.63*	1.12±0.32**

All values are mean ±S.D. (n=6). **p<0.01, *p<0.05 compared with control group by one-way ANOVA followed by Dunnett's t-test.

Table-II: Effect of treatment with ethanolic extract of *Mirabilis Jalapa* on the renal intracellular antioxidant levels in rats with acetaminophen (APAP)-induced nephrotoxicity

Antioxidant markers	**Group-I**	**Group-II**	**Group-III**	**Group-IV**
SOD(units of activity/mg protein)	0.85±0.02	0.62±0.32**	0.72±0.02*	0.81±0.021**
CAT (micromoles of H_2O_2decomposed/mg protein/min)	20.52±0.43	9.5±0.28**	14±0.24*	19.32±0.76**
Malondialdehyde(µM/mg protein)	43.24±1.8	98.35±5.1**	65.38±2.2*	54.07±3.5**
Reduced Glutathione(nM/mg protein)	28.45±0.7	10.80±1.3**	24.40±0.9*	25.90±0.4**
GPx(U/mg protein)	7.95±0.21	5.95±56**	7.10±0.5*	7.72±52

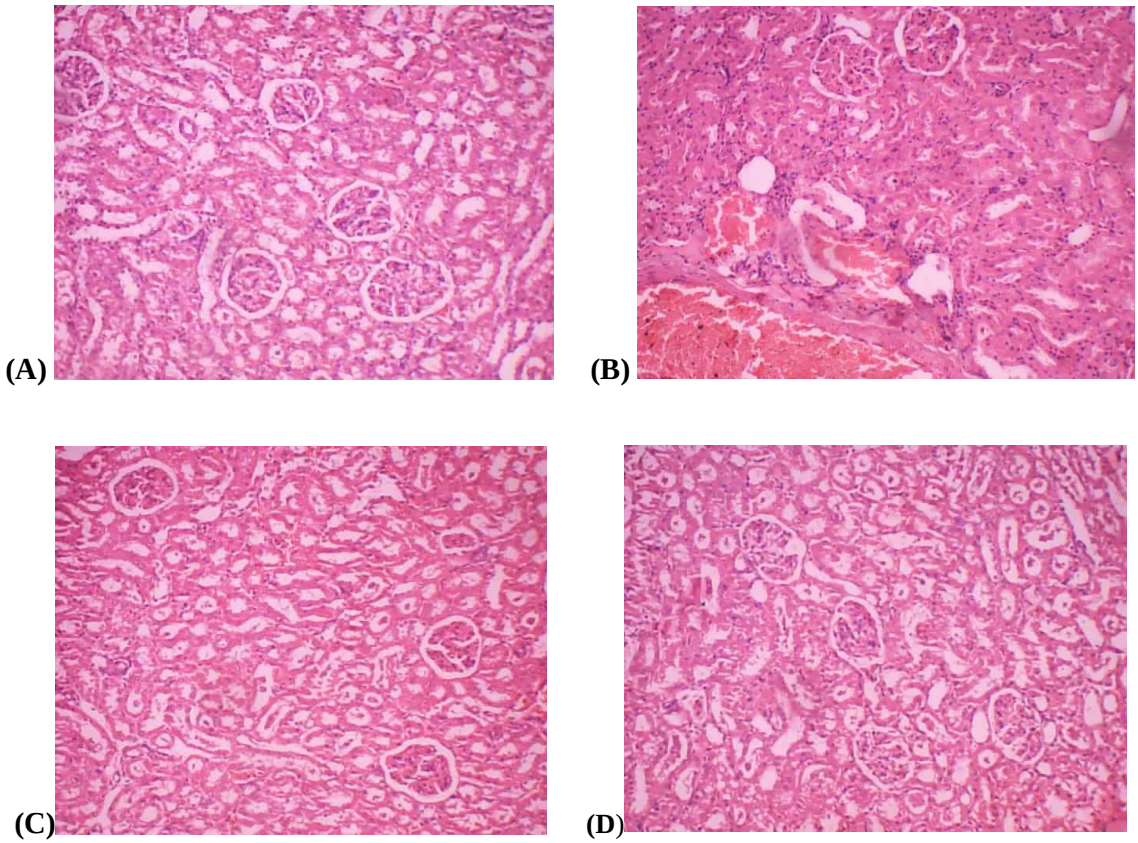
All values are mean ±S.D. (n=6). **p<0.01, *p<0.05 compared with control group by one-way ANOVA followed by Dunnett's t-test.

Table-III: Effect of treatment with ethanolic extract of Mirabilis Jalapa on the blood hematological parameters, in rats with acetaminophen (APAP)-induced nephrotoxicity.

Parameters	**Group-I**	**Group-II**	**Group-III**	**Group-IV**
PCV (%)	58.06±0.29	50.01±0.83**	54.65±0.72*	57.45±0.48**
Hb(g/dl)	8.1±0.24	7.01±0.33**	7.56±0.04*	7.89±0.13**
TLC	8.17±0.47	6.29±0.35**	7.35±0.52*	7.89±0.51**
DLC				
Lymph (%)	56.71±6.22	70.21±3.18**	67.39±1.11*	64.95±4.09**
Neut (%)	23.38±0.85	14.45±1.70**	17.27±1.49*	20.27±1.72**
Gran (%)	15.01±0.80	5.73±0.53**	11.2±0.54*	13.1±1.02**
MCV(fL)	55.91±0.51	60.74±0.36**	59.69±0.58*	56.84±0.17**
MCH(pg)	19.17±0.96	14.23±0.74**	16.43±0.55*	17.56±0.88**
MCHC(g/dl)	34.91±0.72	28.08±0.65**	30.91±0.94*	32.68±1.43**
PLC(x10^3/µL)	593.17±19.9	516.33±81.5**	535.62±87.4*	570.67±53.6**

All values are mean ±S.D. (n=6). **p<0.01, *p<0.05 compared with control group by one-way ANOVA followed by Dunnett's t-test.

Fig.1 Nephroprotective effect of Mirabilis Jalapa extract. Histopathological observations (kidney sections stained with Hematoxylin- Eosin, magnification-100x) (A) Normal, (B) Acetaminophen, (C) Extracts 250mg/kg +APAP, (D) Extracts 500mg/kg +APAP.



Conclusion:

The results of the present investigation illustrated that ethanolic extract of the plant produced significant protection over Acetaminophen-induced alterations in serum marker enzymes and cellular damage. Combined effect of active principles present in the ethanolic extract of *Mirabilis jalapa.Linn* might offer protection against renal damage rendered by paracetamol in rats. Thus, ethanolic extract of leaves of *Mirabilis jalapa.Linn* exhibited significant nephro protective activity in rats. This supported the folklore use of the title plant in renal disorders.

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Corresponding Author:
Sharmila Shaik*,
Email: *sharmilashaik.mpharm@gmail.com*