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Research Article

Nephroprotective Effect of the Methanolic Fruit Extract of *Musa balbisiana* Colla against Carbon Tetrachloride-Induced Toxicity in Swiss Albino Mice

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ABSTRACT

Renal failure is the loss of renal functions owing to multiple factors, including oxidative stress, and it is a global concern, as it costs millions of lives every year. Carbon tetrachloride is a standard toxicant exploited to induce nephrotoxicity in experimental animals to study oxidative stress-related renal injury. This study was conducted to determine the nephroprotective activity of a methanolic extract derived from the unripe fruit pulp of *Musa balbisiana* Colla (MBME) in carbon tetrachloride-induced toxicity in Swiss albino mice. Multiple groups of mice were treated with a single dose of carbon tetrachloride (1-mg/kg body weight) intraperitoneally once in a week followed by oral administration of the methanolic fruit extract of *M. balbisiana* Colla (MBME) at two different doses (200 and 400 mg/kg body weight) consecutively for 28 days. Treatment with carbon tetrachloride reduced body weight along with enzymatic and non-enzymatic antioxidants in the renal tissue, elevated kidney weight, renal biomarkers, lipid peroxidation, IL-6, TNF-α, and TGF-β levels in the blood serum, impaired antioxidant system and up-regulated TGF-β expression in the kidney tissue, and brought histological changes in the architecture of renal tissues. Our data revealed that the oral administration of the methanolic fruit extract of *M. balbisiana* Colla (MBME) dose wisely restored kidney and body weight, renal biomarkers, enzymatic and non-enzymatic antioxidants in the renal tissue, reduced lipid peroxidation, interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α), and transforming growth factor-β (TGF-β) levels in the blood serum, re-established antioxidant system and down-regulated TGF-β expression in the kidney tissue, and re-established the architecture of renal tissues. Thus, this study reported the nephroprotective effects of methanolic fruit extract of *M. balbisiana* Colla (MBME) in carbon tetrachloride-induced toxicity in Swiss albino mice through possible antioxidant and anti-inflammatory activity.

Introduction

Kidneys are the organs of the human body and perform immense important functions such as maintenance of homeostasis in the body by eliminating waste substances like NH_3, regulation of fluid and electrolyte balance, the metabolic acid-base equilibrium in the blood, and production or modification of hormones that regulate blood pressure, calcium, and potassium levels, and red blood cell production.[1,2] Hence, exposure of the kidneys to any form of toxicity may lead to critical pathophysiological conditions resulting in detrimental ailments and may cause morbidity. So it is very essential to comprehend the causal mechanism and recognize the risk factors associated with such renal toxicities for the adoption of preventive measures and assurance of proficient medication.

Renal injury, or toxicity, can be prompted by non-steroidal anti-inflammatory drugs (NSAID), ACE inhibitors and angiotensin II receptor blockers, aminoglycoside antibiotics, radiographic contrast dye (RCD), heavy metals, etc.[3] Drug-induced nephrotoxicity induced by drugs has a very complex pathophysiologic mechanism frequently facilitated through alteration of intraglomerular

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hemodynamics, impaired tubular secretion, inflammation, uric acid deposition, rhabdomyolysis, and thrombotic microangiopathy. Patients with underlying renal insufficiency are susceptible to developing nephrotoxicity.[4] Kidney disease is a global public health problem, affecting over 750 million persons worldwide.[5]

CCl_4 is a potential intoxicant of animals. Henceforth, it is an important investigational standard for the replication of oxidative stress induced by many pathophysiological factors inside the animal body. CCl_4 has the ability to induce nephrotoxicity in animals based on its molecular features. CCl_4 triggers oxidative stress upon its exposure, leads to the production of free radicals which impair DNA, proteins, and brings about lipid peroxidation in renal tissues.[6-14] Animal body employs enzymatic and non-enzymatic antioxidant systems in order to resist oxidative stress. Artificial administration of antioxidants may prevent tissues from the destructive consequence of oxidative stress and thus it can be beneficial in the prevention of chronic kidney diseases.[6]

There is a lack of scientific validation of pro-active behaviors of specific natural products from such traditional medicinal plants. A medicinal plant *Musa balbisiana* Colla (Musaceae family) found in Southeast Asia is full of nutritional and therapeutic value. Most of the parts of this plant are edible, but among them its fruit is widely consumed throughout the world and it is rich in many bioactive compounds. Till now, its protective activity against renal damage or injury has not yet been investigated.

M. balbisiana Colla banana pulp has anti-peroxidative, antioxidant activity antibacterial activity against *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Escherichia coli.*[8,9] *M. balbisiana* unripe banana extract shows anti-ulcer efficacy.[10] The dried fruit pulp powder of *M. balbisiana* Colla prevents cardiac hypertrophy *via* modulation of inflammation along with oxidative stress in the hypertrophic heart.[11] The ethanolic fruit pulp extract of ripe *M. balbisiana* Colla is full of antioxidants act as an anti-gout agent.[12] The methanolic, ethanolic, and aqueous banana pulp extracts of *M. balbisiana* Colla display antioxidant, anti-inflammatory, antibacterial, antifungal, and anti-diabetic activity[13] (Nhon Hoang *et al*. in 2023). Thus, with this backdrop, the goal of our current study was set to explore the potential nephroprotective effect of the methanolic extract from the unripe fresh fruit pulp of *M. balbisiana* Colla (MBME) in Swiss albino mice subjected to CCl_4 intoxication.

Materials and Methods

Chemicals

CCl_4, glutathione (GSH), and thiobarbituric acid (TBA), were bought from Sisco Research Laboratories Pvt. Ltd. (SRL) - India. The kits for the determination of blood urea nitrogen (BUN), urea, uric acid, and creatinine, the kits for the determination of superoxide dismutase (SOD), catalase (CAT), GSH and malondialdehyde (MDA) and Elisa kits for the detection of serum cytokine IL-6 were purchased from Sigma Aldrich (Saint Louis, MO, USA) and Elisa kit for TGF-β was bought from ThermoFischer Scientific (Waltham, MA USA). All the chemicals used during the experiments were of analytical grade.

Plant Collection and Identification

For the purpose of this study, fresh unripe fruit pulp of *M. balbisiana* Colla has been selected and collected during the month of November 2021 during the month of the Kamrup Metropolitan district of Assam, India. The plant species was identified through established literature and a voucher specimen was prepared for the purpose of authentication and submitted to the Herbarium of the Department of Botany, Gauhati University, Assam, possessing the accession number GUBH20010 for future reference.

Preparation of Fruit Pulp Extract

The collected fruit pulp of *M. balbisiana* Colla was cleaned, cut into pieces, air-dried, and later powdered in the finest form. For extraction purposes, 10 g of fine powder was dissolved in 100 mL methanol in the ratio 1:10. Cold maceration extraction was used for this purpose with the usage of solvents of analytical grades followed by filtration with Whatman no.1 filter paper. The filtrate was subjected to evaporation by a rotary evaporator at 30°C until it was dried and the extract (MBME) was collected and stored at 4°C for future usage.[15]

Experimental Animals

The experiments performed in order to evaluate the nephroprotective activity of fruit pulp of *M. balbisiana* Colla involved the usage of healthy adult mice in a weight range of 20 to 30 g. All the experimental processes involved in this study were reviewed and received approval from the Institutional Ethical Committee for Animal Welfare with reference no. *GUIEC/2021/038* and the experiments were performed as per the current guidelines of laboratory animals of Institutional Animal Ethics Committee (IAEC) bearing reference no. IAEC/Per/2022/PP-IAEC/ 2022-4/01)

Assignment of Animals

Male Swiss albino mice were arbitrarily assigned into five groups, each containing six healthy mice (n = 6) for this study. In order to facilitate identification, each of them was marked and group I was considered was the normal control group (provided distilled water 1-mL/kg b.w. orally for 28 days and olive oil 1-mL/kg b.w. orally twice in a week),[16] group II was designated as CCl_4 group (a single dose of 1-mL/kg b.w. CCl_4 dissolved in olive oil 1:1 v/v, intraperitoneally once in a week), group III as

reference drug silymarin group (a single dose of 1-mL/kg b.w. CCl_4 dissolved in olive oil v/v, intraperitoneally once in a week and 100 mg/kg b.w. silymarin orally daily for 28 days), group IV as low dose MBME group (CCl_4+MBME, a single dose of 1-mL/kg b.w. CCl_4 dissolved in olive oil v/v, intraperitoneally weekly and 200 mg/kg b.w. MBME every day orally for 28 days) and group V as high dose MBME group (CCl_4+MBME, a single dose of 1-mL/kg b.w. CCl_4 dissolved in olive oil v/v, intraperitoneally once in a week and 400 mg/kg b.w. MBME every day orally for 28 days). The doses of CCl_4 were administered compliant with the suggested chronic oral exposure reference dose (RfD) for CCl_4 (CASRN 56-23-5).[6,17,18]

Housing and Nutrition

The animals were accommodated in cages along with sawdust litter. They were provided with food and water. The temperature was regulated at the optimum level and the lighting condition was maintained in such a way that a cycle of twelve hours of sunlight was followed by twelve hours of darkness in alternation. Each cage was identified by labeling its cage number, the weight of the animals, information about the provided drug, route of administration, and the level of dosage. The animals were provided proper food and water as per requirement.

Blood and Tissue Sample Collection

The sacrifice of animals was done by using ketamine [19] and blood was withdrawn through a cardiac puncture in order to carry on the analysis of serum biochemical parameters. In order to get clear serum, the blood samples were subjected to centrifugation at 2500 rpm for 10 minutes and stored at -20°C for further use. Also, kidneys were removed and, weighed and washed by using 0.001 mol/100 mL phosphate-buffered saline (PBS pH 7.4), then dissected and a part of it was fixed in 10% formaldehyde solution for histopathological studies and the other part was homogenized (10% w/v) in 0.025 mmol/mL tris-KCl buffer, centrifuged at 2500 rpm for 10 minutes, collected the supernatant and stored at -80°C for the estimation of antioxidant enzymes in renal tissue and estimation of lipid peroxidation.[16,18]

Analysis of Serum Biochemical Markers

For the purpose of assessment of kidney function in different treatment groups of animals, analysis of serum biochemical markers such as blood urea nitrogen (BUN), urea, uric acid, and creatinine performed by commercially available kits from Sigma Aldrich (St. Louis, MO, USA). All the assessments were carried out in triplicates.

Estimation of Antioxidant Enzymes in Renal Tissue and Estimation of Lipid Peroxidation

The activity of enzymatic antioxidants SOD, catalase and non-enzymatic antioxidant GSH along with MDA, a thiobarbituric acid is considered as an index of lipid peroxidation and was determined by commercial colorimetric assay kits according to the manufacturer's instructions (Sigma Aldrich (St. Louis, MO, USA) using the supernatant of kidney homogenate. The experiments were performed in triplicates.

Estimation of Serum Cytokines

The serum levels of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta (TGF-β) in CCl_4 intoxicated mice with nephrotoxicity were assessed with commercially available respective cytokine-specific ELISA kits according to the manufacturer's guidelines from Sigma Aldrich (Saint. Louis MO, USA). By using a microplate reader, the absorbance of the product was measured at 450 nm. The estimation of serum cytokines was conducted in triplicates.

Immunohistochemistry of Renal Tissues

Thin (3 µm) paraffin sections of renal tissue were used for performing immunohistochemistry as per the established method.[19] These sections were subjected to deparaffinization and rehydration in different alcohol grades followed by antigen retrieval, staining and incubation with anti-TGF-β (MA1-21595 Invitrogen, diluted in the ratio 1:1500) at a very low temperature (4°C) for the whole night. Then these were treated with goat anti-rabbit secondary antibodies, dehydrated and mounted, respectively. The slides were visualized by microscope (Leica DM3000, China) at 40X magnification and the analysis of the images was conducted using ImageJ software (National Institutes of Health).

Histopathological Study of Renal Tissues

The renal tissue, was fixed in 10% formaldehyde solution right after the sacrifice of the animals, and these were further processed for histopathological analysis according to standard protocol.[20] The tissues were dehydrated in a series of different grades of alcohol and embedded in paraffin, respectively. Then these were incised into pieces of five µm of thickness and finally stained with hematoxylin-eosin stain.

Statistical Analysis

The results obtained from the experiments were expressed as mean ± SD (standard deviation) and $p<0.05$ was considered as statistically significant. The results were analyzed by the use of GraphPad Prism 10.0.0 (153). One-way ANOVA and t-test were performed to evaluate *p-value* among and between the groups, respectively.

Results

Effect of MBME on Kidney Weight and Serum Biochemical Markers

CCl_4 dose caused a remarkable loss of kidney weight in mice as compared to the normal group ($p <0.05$), nevertheless,

Table 1: Effects of methanolic extract of fruit pulp of *M. balbisiana* Colla on the kidney weight and serum biochemical markers

Treatment groups	*Kidney weight (g)*	*Blood urea nitrogen (mg/dl)*	*Creatinine (mg/dl)*	*Urea (mg/dl)*	*Uric acid (mg/dl)*
Normal Control	0.32 ± 0.03	9.92 ± 1.68	0.40 ± 0.1	23.48 ± 2.77	1.09 ± 0.10
CC_L4	0.48 ± 0.03*	19.02 ± 2.25*	1.69 ± 0.11*	53.27 ± 3.87*	3.065 ± 0.46*
CCL_4+Silymarin 100 mg/kg	0.34 ± 0.02$^{\#}$	10.96 ± 1.64$^{\#}$	0.45 ± 0.08$^{\#}$	25.47 ± 4.38$^{\#}$	1.10 ± 0.09$^{\#}$
CCL_4+MBME 200 mg/kg	0.41 ± 0.01*$^{\#}$	14.90 ± 1.69*$^{\#}$	1.09 ± 0.08*$^{\#}$	37.10 ± 4.67*$^{\#}$	2.15 ± 0.29*$^{\#}$
CCL_4+MBME 400 mg/kg	0.35 ± 0.01$^{\#}$	11.96 ± 1.89$^{\#}$	0.50 ± 0.07$^{\#}$	26.98 ± 3.71$^{\#}$	1.20 ± 0.07$^{\#}$

All the values are manifested as mean ± SD and analyzed by ANOVA, followed by Welch's t test. p <0.05 is considered as statistically significant, the *p-value* is marked by * *vs.* control and $^{\#}$ *vs.* CCl_4. All the assessments were conducted in triplicates and the number of animals in each group was six (n = 6).

this loss was well-compensated by low and high dose of MBME (Table 1). The estimated levels of uric acid, BUN, urea, and creatinine in all treatment groups were documented in Table 1. CCl_4 dose administration markedly increased the levels of serum creatinine urea, uric acid, and BUN as compared with the normal control (p <0.05). The low dose (200 mg/kg) and high dose (400 mg/kg) of MBME reduced the levels of these biomarkers in a dose-dependant manner in contrast to the CCl_4 group (p <0.05).

Effect of MBME on Serum Renal Antioxidant Enzymes and Lipid Peroxidation

The assessment of the levels of renal antioxidant enzymes is reported in Table 2. A substantial decline in the renal antioxidant enzyme levels and non-enzymatic antioxidant GSH content and significant escalation in the level of MDA content (generated by lipid peroxidation) was observed in the animals administered with CCl_4 (p <0.05, compared to normal control). A remarkable raise of renal antioxidant enzymes and reduction in MDA was noticed in low and high dose of MBME group in contrast to the CCl_4 group (p <0.05).

Effect of MBME on Serum Cytokines

The estimated levels of serum cytokine IL-6, TNF-α and TGF-β in all treatment groups are depicted in Fig. 1. A noticeable elevation in the IL-6, TNF-α and TGF-β in the CCl_4 toxic group (p <0.05 vs. normal control) was marked, while the control group maintained the normal range. Administration of MBME at low and high dose depleted the levels of these serum cytokines in contrast to CCl_4 group (p <0.05).

Effect of MBME on Immunohistochemical Study of TGF-β in Renal Tissues

The immunohistochemical photomicrographs of the renal tissues of all the experimental groups were presented in Fig. 2[A-F]. The renal tissues of the nephrotoxic group exhibited the highest expression of TGF-β (p <0.05, when compared to the normal control). Administration of MBME effectively reduced expression of TGF-β in the renal tissues dose wise (p <0.05, when compared to the nephrotoxic group).

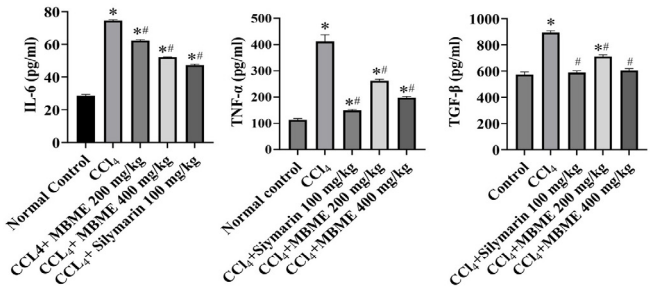

Fig. 1: Effects of MBME on serum cytokines IL-6, TNF-α and TGF-β. All the values are manifested as mean ± SD and analyzed by ANOVA, followed by Welch's t test. p<0.05 is considered as statistically significant, the *p-value* is marked by * vs. control and $^{\#}$ vs. CCl_4. All the assessments were conducted in triplicates and the number of animals in each group was six (n = 6). IL-6: Interleukin-6, TNF-α: Tumor necrosis factor-alpha and TGF-β: Transforming growth factor

Effect of MBME on Histopathological Study of Renal Tissues

The histopathological micrographs of renal tissue from the normal control revealed normal renal cellular architecture with zero modification, as displayed in Fig. 3[A]. In contrast, major alterations in the structure of glomeruli, and distortion of distal convoluted tubule (DCT), and proximal convoluted tubule (PCT) with narrower lumen were pretty obvious in the Fig. 3[B]. Such pathological changes occurred by the administration of toxic CCl_4 have been reverted in the low dose (CCl_4+MBME 200 mg/kg) and high dose (CCl_4+MBME 400 mg/kg) groups of MBME in a dose-dependent fashion as observed in 3[C] plus 3[D] in that order, while standard drug silymarin reduced the degree of histopathological alterations to maximum 3[E].

Discussion

This current study exploited carbon tetrachloride (CCl_4) for the induction of nephrotoxicity in the experimental animals, resulting in alterations of kidney and body weight along with renal tissue architecture, reduction of enzymatic antioxidants and non-enzymatic antioxidant in the renal tissue, elevation in the renal biomarkers in the serum, lipid peroxidation, pro-inflammatory and

Table 2: Effects of methanolic extract of fruit pulp of *M. balbisiana* Colla on antioxidant enzymes and lipid peroxidation

Treatment groups	*SOD (U/mg protein)*	*CAT (U/mg protein)*	*GSH (µmol/mg protein)*	*MDA (µmol/g tissue)*
Normal Control	60.5 ± 1.87	49.12 ± 1.60	6.69 ± 1.56	0.69 ± 0.12
CCL_4	32.00 ± 1.67*	19.27 ± 1.39*	2.35 ± 0.88*	1.46 ± 0.16*
CCL_4+Silymarin 100 mg/kg	58.76 ± 4.48#	47.82 ± 1.84#	5.53 ± 0.54#	0.76 ± 0.16#
CCL_4+MBME 200 mg/kg	47.58 ± 2.88*#	32.36 ± 2.71*#	3.92 ± 0.57*#	1.19 ± 0.06*#
CCL_4+MBME 400 mg/kg	57.78 ± 3.31#	46.45 ± 2.64#	5.11 ± 0.79#	0.84 ± 0.11#

All the values are manifested as mean ± SD and analyzed by ANOVA, followed by Welch's t test. $p < 0.05$ is considered as statistically significant, the *p-value* is marked by * *vs.* control and # *vs.* CCl_4. All the assessments were conducted in triplicates and the number of animals in each group was six (n = 6). SOD: superoxide dismutase, CAT: catalase, GSH: glutathione MDA: malondialdehyde.

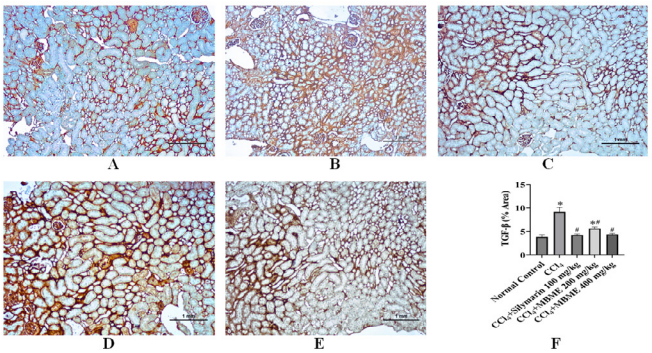

Fig. 2: Photomicrophotographs of immune-stained renal tissues against TGF-β [A-E] and comparison of the percent area of TGF-β in all the experimental groups [F]. [A] normal control, [B] CCl_4 group, [C] CCl_4+silymarin 100 mg/kg) group, [D] low dose (CCl_4+MBME 200 mg/kg) MBME group, [E] high dose (CCl_4+MBME 400 mg/kg) MBME group (scalar bar: 1mm). TGF-β: transforming growth factor beta

pro-fibrogenic cytokines, and the TGF-β expression in the renal tissue. Upon exposure, CCl_4 accumulates and causes oxidative stress in the renal tissue upon exposure via the enzyme cytochrome P450 which forms free radicals.[6] CCl_4 leads to renal damage by excessive free radical production.[21-23] Oxidative stress is disparaging of renal tissue, as it causes inflammation and renal tissue injury.[24] Many natural products and plant extracts protect the kidneys from damage and diseases by their antioxidant and anti-inflammatory properties.[23-25]

CCl_4 was reported to reduce kidney weight in experimental animals.[26] In our investigation, the mice provided with CCl_4 treatment exhibited a decline in the whole body weight together with kidney weights. The oral administration of MBME to mice (200 and 400 mg/kg) demonstrated considerable improvement from CCl_4-induced loss of whole body weight and kidney weight.

Elevated level of BUN, urea, creatinine, and uric acid are associated with renal disease progression.[21,27] Creatine phosphate breaks down to creatinine within muscle. A creatinine clearance test is executed to inspect the advancement of renal disease.[28] If the creatinine level exceeds the normal range, it connotes the loss of 50% functional capacity of the kidneys.[29] Urea forms *via* catabolism of proteins and amino acids and its elevation in the serum is indicative of acute renal injury (AKI). BUN in higher concentrations points out with kidney disease.[28] Plasma uric acid levels higher than normal concentration specify chronic kidney disease.[30] MBME treatment reduced the elevated concentration of these renal biomarkers in a dose-wise manner. Reports on the lowering of the renal biomarkers by other medicinal plants have been published by several studies.[21,22]

Oxidative stress contributes to acute renal injury by declining the endogenous enzymatic antioxidants, such as SOD, and catalase along with non-enzymatic antioxidant GSH, which take part in the detoxification of free radicals. CCl_4 administration causes excessive ROS production, leading to endogenous anti-oxidant depletion and an increase of MDA contents through higher lipid peroxidation. SOD and catalase are the antioxidant enzymes implicated in the superoxide radical dismutation, converting these to hydrogen peroxide (H_2O_2) and finally oxygen, and water in an order.[31,32] GSH, a non-enzymatic antioxidant, serves as a cofactor for multiple enzymes committed to detoxification and protects against oxidative stress.[33] Also, CCl_4 induces the production of MDA through lipid peroxidation via generating free radicals. Thus, a rise in MDA levels signals renal tissue damage.[6] CCl_4 dosage lowered the SOD, catalase, and GSH levels and raised the MDA level in the toxic renal tissues in our study. Treatment with MBME

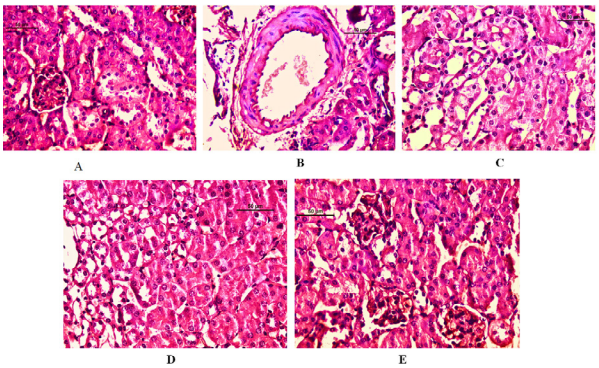

Fig. 3: Photomicrographs of histological slides of renal tissue depicting the effects of methanolic fruit extract of *M. balbisiana* Colla (40X). A: Normal Control, B: CCl_4 group, C: CCL_4+MBME 200 mg/kg, D: CCl_4+MBME 400 mg/kg, E: CCl_4+Silymarin 100 mg/kg. Scalar bar: 50 m

raised the levels of these enzymes and GSH and declined the MDA contents, which validates its nephroprotective efficacy. Similar findings have been reported by previous studies.[17,22,34]

A toxicant CCl_4 trigger the activation of TNF-α and TGF-β that results in apoptosis and fibrosis, respectively. CCl_4 also increases the generation of IL-6 by free radical production.[35] IL-6 signals local and systemic renal tissue inflammation during AKI and its higher serum level is also related to the progression of chronic kidney disease (CKD).[36] Our study revealed higher levels of TNF-α, TGF-β and IL-6 in the CCl_4-intoxicated group and administration of MBME effectively reduced these levels. Various studies on different compounds or medicinal plants reported similar outcomes.[37,38]

TGF-β is a key mediator of renal fibrosis. It increases extracellular matrix protein contents and starts epithelial-mesenchymal transition within the tubular cells, that leads to deterioration of cells and tubulointerstitial fibrosis.[30,39] Our investigation reveals that CCl_4 mediated up-regulation of TGF-β in the renal tissues. Administration of MBME caused down-regulation of TGF-β compared with the nephrotoxic group. Comparable information on the down-regulation of TGF-β has been reported earlier.[40,41] Toxic CCl_4 causes enormous architectural changes in the renal tissue by elevation of renal biomarkers, reduction of renal antioxidant enzymes, lipid peroxidation, and augmentation of pro-inflammatory (IL-6 and TNF-α) and pro-fibrotic cytokine TGF-β.[30,42] The biochemical findings in our investigation correlated with the histological findings. Destruction of the renal tissues was the highest in the CCl_4 group. Treatment with MBME reduced such destruction and established near-normal structure dose-wise.

On the basis of the findings of our investigation, MBME displayed nephroprotective efficacy against CCl_4-induced renal injury in the Swiss albino mice, as observed in the study of renal biomarkers and parameters, immunohistochemistry, and histology. MBME attenuated the serum levels of BUN, urea, uric acid, creatinine, and MDA, and enhanced the serum levels of SOD, catalase, GSH. It down-regulated serum level of TNF-α, IL-6, and TGF-β along with the TGF-β concentration in the renal tissue. Thus, MBME attenuates CCl_4-induced nephrotoxicity through its antioxidant and anti-inflammatory property. As a future prospect of this study, isolation of the active compound responsible for this activity and the discovery of a novel drug against nephrotoxicity is a sought-after.

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