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ORIGINAL ARTICLE

# Effect of Phloretin Treatment Ameliorated the Cisplatin-Induced Nephrotoxicity and Oxidative Stress in Experimental Rats

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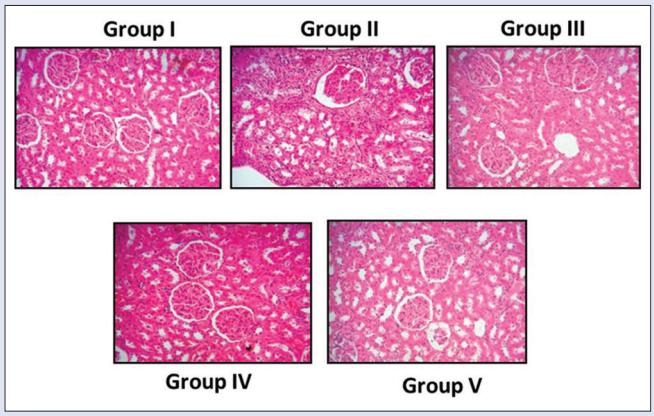
## ABSTRACT

**Background:** The cisplatin is one of the widely employed platinum-based drugs as the chemotherapeutic substance for the treatment of various cancers such as bladder cancer, prostate cancer, cervix cancer, and lung cancer. Hence, the present work was designed to test the curative efficacy of phloretin against the cisplatin-induced nephrotoxicity in rat model. **Materials and Methods:** Male Wistar albino rats were divided into five groups and the Group I rats were served as control, Group II rats were injected with cisplatin (8 mg/kg), Group III rats were administered with the low dose of phloretin (25 mg/kg), Group IV rats were administered with the high dose of phloretin (50 mg/kg), and the Group V rats were administered with the standard drug silymarin (50 mg/kg) for 10 days. After the experimental period, the animals have been sacrificed and collected all the samples for further analysis. **Results:** In the current study, the cisplatin was exhibited the severe renal damage in Wistar albino rats by possessing the various clinical complications such as increased serum urea, proteinuria and creatinine levels, decreased antioxidant enzymes level, increased DNA fragmentation, elevated oxidative stress, and severe injury to renal tissues. In addition, the cisplatin increased the poly (ADP-ribose) polymerase-1 and caspase-3 activity and pro-inflammatory cytokines level such as tumor necrosis factor alpha and interleukin-1 β. Whereas, the treatment with the low dose and high dose of phloretin (25–50 mg/kg) was exhibited a significant ($P < 0.05$) attenuation against the cisplatin-induced nephrotoxicity in experimental rats. **Conclusion:** The phloretin treatment significantly protected the kidneys against cisplatin-induced renal damages in rats.

**Key words:** Cisplatin, DNA fragmentation, histopathology, nephrotoxicity, oxidative stress, phloretin

## SUMMARY

- Phloretin was possessed the variety of therapeutic properties such as antioxidant, anti-inflammatory, and anticancer activities
- The phloretin treatment was significantly protected the kidneys against the cisplatin stimulated renal damages in rats.




**Abbreviations used:** SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; TBARS: Thiobarbituric acid reactive substances; TNF-α: Tumor necrosis factor alpha; IL-1 β: Interleukin-1 beta.

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## INTRODUCTION

The kidneys are the most imperative organs of the human body. They are very essential for the maintenance of a variety of body fluids volume, regulations of fluid osmolality, balancing of acid-base concentrations, maintaining concentrations of various electrolytes, and removal or excretion of toxins from the body through the urine.[1] Nevertheless, the severe injury of kidneys which is induced by various drugs was an undoubtedly a serious clinical complication. The chemotherapy is a most hopeful and alternative treatment strategy for various cancers. Chemotherapy means the administration of various chemical substances in alone or in combination to kill the malignancy cells. The potential toxicity and other unfavorable effects were linked to these chemical substances. These are the main limitations which restricting the chemotherapy for the cancer treatment.[2,3]

The cisplatin is one of the widely employed platinum-based drugs as the chemotherapeutic substance for the treatment of various cancers such as bladder cancer, prostate cancer, cervix cancer, and lung cancer. Nonetheless, the cisplatin was associated with the various severe side effects such as nephrotoxicity, ototoxicity, neurotoxicity, and in some cases ocular toxicity. Among these severe effects, the nephrotoxicity is a very often and drug dose restricting side effect with the cisplatin employed treatment. The irretrievable renal damage was occurred in one third of the cisplatin administered patients. The nephrotoxicity of the cisplatin was aroused through many ways such as inflammation, increased oxidative stress, damage of mitochondria, and apoptosis.[4,5]



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Nevertheless, the high morbidity and mortality rate was associated with the acute renal damage which is induced by the cisplatin administration. The mechanisms underlying in the cisplatin-induced acute renal failure was multi steps process which involving the oxidative stress, apoptosis, and inflammation. The earlier research studies have shown that the inflammation was taking part in the pathophysiology of cisplatin-induced nephrotoxiciy.[5-7] More than 25% of the cancer patients cannot receive as cisplatin as a chemotherapeutic drug due to its nephrotoxicity effects. There are various clinical conditions such as the nephropathy, continual polunephritis, glomerulonephritis, and disruptive uropathy was associated by the cisplatin-stimulated nephrotoxicity.[8]

The high-level accumulation of cisplatin in the kidney and the frequent excretion through the urine by the active excretory process may also contribute the nephrotoxicity by cisplatin. The cisplatin was stimulated the tissue necrosis and apoptosis in the epithelial cells of tubules in inner cortex which is the main site of cisplatin accumulation. The cisplatin was mainly induce the injury to the S3 region of proximal tubules of the kidney.[9,10] The herbal plants are considered as safe, satisfactory, cheap, and trustable sources for the biologically active constituents for therapeutic purposes. The curative potentials of herbal plants and their products on the renal and urinary tract diseases have been studied widely for the efficiency.[11,12] A wide variety of phytochemicals was studied extensively for the therapeutic potential. Among these compounds, the phloretin (dihydrochalcone flavonoidic compound) is biologically active compound which is present in the peel of fruits and vegetables such as berries, apples. The phloretin was possessed the variety of therapeutic properties such as antioxidant, anti-inflammatory, and anticancer activities.[13-15] Nevertheless, there are no scientific researches to claim the curative effects of phloretin against the nephrotoxicity. Hence, the present work was designed to test the curative efficacy of phloretin against the cisplatin-induced nephrotoxicity in the rat model.

## MATERIALS AND METHODS

### Chemicals and drugs

All the chemicals, reagents, and drugs which include Phloretin, Cisplatin, ELISA kits, and other diagnostic kits were commercially procured from the Sigma Aldrich (USA) and used for the study.

### Animals

The male Wistar albino rats weighing from 220 to 230 g were procured and maintained in the polypropylene confines under the laboratory conditions (26°C ± 1°C, air moisture 60%–70%, light and dark sequence were 12 h). All the animals were feed with the commercially purchased rat pelleted food with water *ad libitum*. The animals were getting used to laboratory acclimatization for 7 days in prior to experiments.

### Experiment design

Male Wistar albino rats were divided into five groups, and every group contains 6 animals ($n = 6$). The Group I rats were served as control and it was feed with commercial pelleted diet and received a normal saline solution for 10 days. The group-II rats were administered with the normal saline solution for 10 days and on the 7$^{th}$ day, a single injection of cisplatin (8 mg/kg) was administered by intraperitoneally (i.p.) for the stimulation of nephrotoxicity. The Group III rats were administered with the low dose of phloretin (25 mg/kg) for 10 days and on the 7$^{th}$ day a single injection of cisplatin (8 mg/kg) through by i.p., was given. The Group IV rats were administered with the high dose of phloretin (50 mg/kg) for 10 days and on the 7$^{th}$ day a single injection of cisplatin (8 mg/kg) through i.p., was given. The Group V rats were administered with the standard drug silymarin (50 mg/kg) for 10 days and on the 7$^{th}$ day a single injection of cisplatin (8 mg/kg) through by i.p., was given. After the completion of the experimental regimen, the urine was collected from the experimental rats by natural urination. Then, the experimental rats were anesthetized by administering the ketamine hydrochloride through intraperitoneal. Then the animals were sacrificed and the blood as well as tissue samples were collected/kept in the -80° C for the further experiments.

### Analysis of renal function markers

The urine was collected using metabolic cages from the experimental rats by natural urination. The blood samples were collected from the femoral vein after the scarification of experimental animals and immediately centrifuged at 3000 rpm for 6 min. Then the serum was collected and used for the experiments. Then the level of serum urea, creatinine, protuninuria, and albumin was measured by the previously described methods.[16]

### Preparation of tissue homogenate

The 100 mg of collected kidney sample was homogenized with the 10-fold of 100 mM of potassium phosphate buffer plus 1 mM of ethylenediaminetetraacetic acid (EDTA) (pH-7.4). The homogenate was centrifuged at the 13000 rpm for 30 min at 4°C. After centrifugation, the resulting supernatant was separated and used for the analysis of antioxidant enzymes level and other examinations.

### Determination of activities of antioxidant enzymes

#### *Measurement of superoxide dismutase*

The effectiveness of the superoxide dismutase (SOD) enzyme activity in cell lysate was evaluated by the method described by Kakkar *et al.*[17] The 0.2 ml of cell lysate was diluted to 0.4 ml with the double $dH_2O$ followed by the addition of 1 ml of ethanol and 0.6 ml of ice cold chloroform. This reaction solution was shaken well for 2 min at 4°C. The reaction mixture included sodium pyrophosphate buffer (480 µl), phenazine methosulfate (40 µl), nitroblue tetrazolium (120 µl) was diluted for enzyme preparation. A reaction was initiated by the addition of nicotinamide adenine dinucleotide (NADH) (80 µl). The reaction mixture was incubated at 30°C for 2 min. After incubation, the reaction was terminated by the addition of 0.4 ml of acetic acid. Then the 1.6 ml of butanol was added to the reaction mixture and shaken continuously for 1 min and set aside. Then, the reaction mixture was centrifuged to separate the butanol layer from the reaction mixture. The absorbance was taken 520 nm.

#### *Assay of catalase*

To assess the catalase (CAT) enzyme activity in cell lysate was determined by previously described method by Beers and Sizer.[18] The 0.1 ml cell lysate was mixed with 1.9 ml phosphate buffer (pH-7.4). Then 1 ml of hydrogen peroxide was added. Then the absorbance was taken at the 240 nm in 1 min interval for 3 min. The control solution was placed on the reference that containing 0.1 ml of cell lysate and 2.9 ml of the phosphate buffer. Finally, the activity of the CAT enzyme was calculated by the extinction of molar coefficient at 4.37 nm.

#### *Assay of glutathione*

The glutathione (GSH) activity cell lysate was assayed by the method of Sedlak and Lindsay.[19] The portion of the tissue homogenate was weighed and mixed with EDTA solution. Then, the double $dH_2O$ and trichloroacetic acid (50%) were mixed with the homogenate, then it was centrifuged. Then the upper layer was collected added to the tris buffer and DTNB (Ellman's reagent) and shaken vigorously. Finally, the absorbance was taken at 412 nm.

## Determination of lipid peroxidation

The lipid peroxidation level in the kidney tissues of experimental rats was assessed using the spectrophotometric method by measuring the thiobarbituric acid reactive substances. This assay was carried out using commercially procured malondialdehyde (MDA) assay kits (Sigma Aldrich, USA) according to the manufacturer's instructions.

## Determination of proinflammatory cytokines and nitric oxide level

The cisplatin-induced production of pro-inflammatory cytokines in the renal tissues of experimental rats such as tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1 β) and nitric oxide (NO) level was determined using a commercially procured ELISA kits (Sigma Aldrich, USA). The standard and detection antibodies were reconstituted with the sterile deionized water. The serial dilutions of the sample and standard were prepared according to instructions. The wells of the microplate were washed twice with washing buffer and the 100 µl of the prepared sample and standard was loaded. The diluted detection antibody was added, and then the color development enzyme and solution were added. Then the absorbance was measured by using the microplate reader.[20]

## Assay of DNA fragmentation

The collected renal tissues of experimental rats were washed with the phosphate buffer solution, and the pulverized tissues were homogenized with the phosphate buffer. The resulting homogenate was centrifuged for 4000 rpm for 15 min for 6 times. The pellet (cell lysate) was collected for the assay. The cells were reconstituted with the lysis buffer (50 µl) which containing 50 mM/L of Tris-HCL (pH-8.0) and the sodium dodecyl sulphate (0.5%) and incubated at room temperature for 30 min. Then the 2 µl of 10 mg/ml of DNase free RNase was added and it was incubated at room temperature for 2 h. Then the 5 µl of Proteinase K was added to the samples and further incubated for 90 min at 50°C. The DNA precipitation was dissolved with the 20 µl of Tris-EDTA buffer and used to quantification by spectrophotometric method. The equal concentration of DNA was loaded with the 1% agarose gel which is stained with ethidium bromide and proceeded to run the DNA. Then the agarose gel was observed under the ultra violet light to detect the fragmentation of DNA.

## Assay of poly (ADP-ribose) polymerase-1 activity

The activity of poly (ADP-ribose) polymerase-1 (PARP1) was determined with commercially procured ELISA based colorimetric diagnostic kit (Sigma Aldrich, USA). The substrate for the PARP1 was loaded with the microplate, and the prepared renal tissue homogenate was loaded followed by the adding of PAR rabbit polyclonal antibody. Then it was incubated for 2 h at room temperature, and after the incubation period, the HRP coupled anti-rabbit IgG antibody solution derived from goat was added into the wells. Finally, the luminescent color developing agent was mixed and the resulting light emission was measured at 380 nm.

## Measurement of caspase-3 activity

The activity of rat specific caspase-3 of the renal tissue homogenate was determined using the commercially procured ELISA kit (Sigma Aldrich, USA) according to the instructions of the manufacturer.

## Histological analysis of renal tissue

The renal tissues of the experimental animals were collected after the scarification, and a portion of renal tissue was washed thrice with the isotonic saline solution. The washed renal tissue was fixed with the 10% of neutral formalin solution for 48 h. Then it was embedded in the paraffin wax. After that, the sections of 5–6 µm thickness were made from the paraffin blocks using microtome and stained with the mixture of Periodic acid and Schiff reagents. Finally, the slides of renal tissue were observed under microscope to detect the cisplatin-induced cellular destructions and damages of the kidney of rats.

## Statistical analysis

Obtained experimental results were depicted as mean ± standard error of mean. The statistical variations between the experimental animals were calculated by the one way analysis of variance by the tukey's Kramer test with the $P < 0.05$ comparison to the control group and $P < 0.01$ to the cisplatin-treated group using SPSS (version 19) (SPSS Inc., Chicago, IL, USA).

# RESULTS

## Effects of phloretin on cisplatin-induced nephrotoxicity markers in rats

The phloretin treatment was significantly attenuates the cisplatin-induced deleterious alterations in serum nephrotoxicity markers. The cisplatin administered animals exhibited the significant ($P < 0.01$) elevation in the serum urea, creatinine, and proteinuria level which in contrast to the control animals. The cisplatin administration also showed the significant ($P < 0.01$) decrease in the serum albumin than the normal control group. Whereas, the treatment with the phloretin was potentially ameliorates the cisplatin-induced changes in nephrotoxicity markers. The low-dose and high-dose treatment of phloretin (25–50 mg/kg) was significantly ($P < 0.05$) decreased the urea, proteinuria, and creatinine level in the serum of experimental rats. Phloretin also increased the serum albumin level, which can be compared to the cisplatin administered animals [Figure 1]. The high-dose treatment of phloretin (50 mg/kg) was exhibited the significantly ($P < 0.05$) increased amelioration effects than the low-dose treatment (25 mg/kg). The standard drug silymarin (50 mg/kg) treatment also possessed the significant ($P < 0.05$) reversible effects against the cisplatin-induced nephrotoxicity [Figure 1].

## Effects of phloretin on cisplatin-induced oxidative stress markers level in rats

The phloretin treatment was significantly ($P < 0.05$) ameliorated the cisplatin-induced oxidative stress in the experimental rats. The cisplatin administered rats were possessed the significant ($P < 0.01$) reduction in the antioxidant enzymes levels such as SOD, GSH, and CAT in the serum of experimental rats which in contrast to the control animals. The cisplatin also showed the significant ($P < 0.01$) increase in the lipid peroxidation level than the normal control group. While the phloretin treatment was significantly attenuates the cisplatin-induced oxidative stress in rats. The low-dose and high-dose treatment of phloretin (25–50 mg/kg) was significantly ($P < 0.05$) decreased the cisplatin-induced oxidative stress level in experimental rats. Phloretin was also decreased the cisplatin-induced lipid peroxidation when compared to the cisplatin-treated group [Figure 2]. The high-dose treatment of phloretin (50 mg/kg) was possessed the significantly ($P < 0.05$) increased attenuation activity than the low-dose treatment (25 mg/kg). The standard drug silymarin (50 mg/kg) also exhibited the significant ($P < 0.05$) activity against the cisplatin-induced oxidative stress [Figure 2].

## Effects of phloretin on cisplatin-induced nitric oxide and pro-inflammatory cytokines level in rats

The treatment with phloretin was possessed the significant ($P < 0.05$) reversible effects against the cisplatin-induced elevation in NO and pro-inflammatory cytokines level in the experimental rats. The administration of cisplatin to the rats was exhibited the significant ($P < 0.01$) elevation in the IL-1 β, TNF-α, and NO in the experimental rats, which in contrast to the normal control rats [Figure 3]. Whereas, the treatment with the phloretin was significantly ($P < 0.05$) decreased the IL-1 β, TNF-α, and NO levels in the experimental rats. The low-dose and high-dose treatment of phloretin (25–50 mg/kg) was possessed the significant ($P < 0.05$) decrease in the cisplatin-induced elevation of IL-1 β, TNF-α, and NO in experimental rats [Figure 3]. The high-dose treatment of phloretin (50 mg/kg) was exhibited the significantly ($P < 0.05$) increased amelioration effects when compared to low-dose treatment (25 mg/kg). The standard drug silymarin (50 mg/kg) also showed the significantly ($P < 0.05$) decrease in the cisplatin-induced elevation of IL-1 β, TNF-α and NO in experimental rats [Figure 3].

## Effects of phloretin on cisplatin-induced DNA fragmentation level in rats

The phloretin treatment was significantly ($P < 0.05$) prevented the cisplatin-induced DNA fragmentation level in experimental rats. The cisplatin administered animals were possessed the significantly ($P < 0.01$) elevated level of DNA fragmentation in the experimental rats which in contrast to the normal control rats [Figure 4]. While the treatment with the phloretin was exhibited the significantly ($P < 0.05$) reduced level of DNA fragmentation in the experimental rats. The low-dose and high-dose treatment of phloretin (25–50 mg/kg) was showed the significant ($P < 0.05$) decrease in the cisplatin-induced DNA

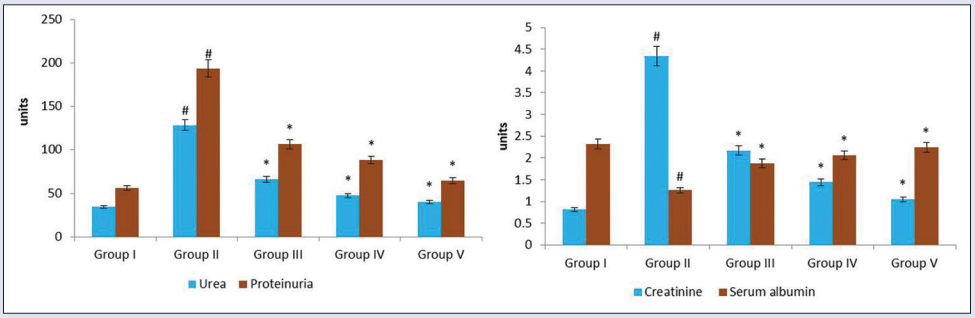


**Figure 1:** Effects of phloretin on cisplatin-induced nephrotoxicity markers in experimental rats. Results are depicted as mean ± standard error of mean of six animals ($n = 6$). $^{\#}P < 0.05$ as compare to the control group. $^{*}P < 0.01$ as compare to the Cisplatin treated group

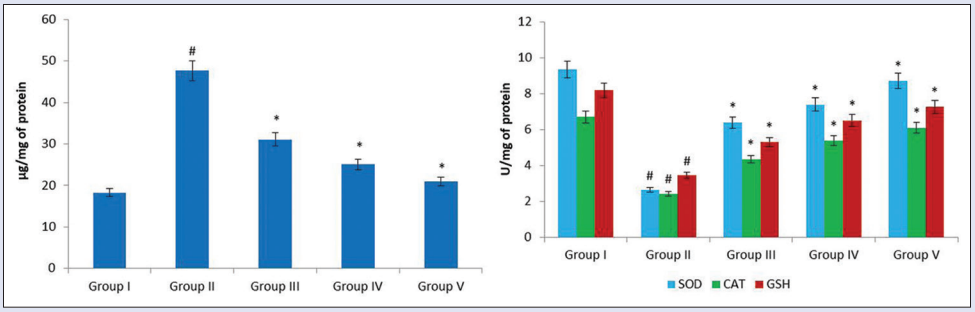


**Figure 2:** Effects of phloretin on cisplatin-induced oxidative stress markers level in experimental rats. Results are depicted as mean ± standard error of mean of six animals ($n = 6$). $^{\#}P < 0.05$ as compare to the control group. $^{*}P < 0.01$ as compare to the Cisplatin treated group

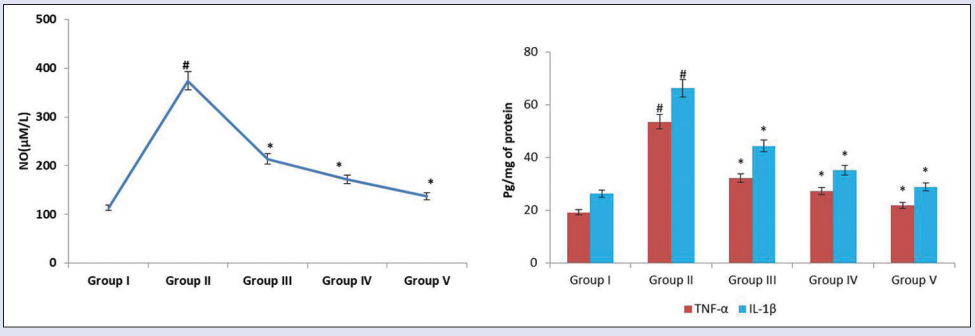


**Figure 3:** Effects of phloretin on cisplatin-induced NO and pro-inflammatory cytokines level in experimental rats. Results are depicted as mean ± standard error of mean of six animals ($n = 6$). $^{\#}P < 0.05$ as compare to the control group. $^{*}P < 0.01$ as compare to the Cisplatin treated group

fragmentation in experimental rats [Figure 4]. The high-dose treatment of phloretin (50 mg/kg) was possessed the significantly ($P < 0.05$) increased attenuation effects when compared to low-dose treatment (25 mg/kg). The standard drug silymarin (50 mg/kg) also effectively prevented the cisplatin-induced DNA fragmentation in experimental rats [Figure 4].

## Effects of phloretin on poly (ADP-ribose) polymerase-1 and caspase-3 activity in rats

The phloretin was significantly ($P < 0.05$) reduced the cisplatin-induced increased activity of PARP-1 and caspase-3 in the experimental rats. The cisplatin administered animals were exhibited the significantly ($P < 0.01$) increased activity of PARP-1 and caspase-3 in the experimental rats which in contrast to the normal control rats [Figure 5]. Whereas, the phloretin treatment was exhibited the significant ($P < 0.05$) reduction in the activity of PARP-1 and caspase-3 in experimental rats. The treatment with the low-dose and high dose of phloretin (25–50 mg/kg) was possessed the significantly ($P < 0.05$) decreased the activity of PARP-1 and caspase-3 in experimental rats [Figure 5]. The treatment with the high dose of phloretin (50 mg/kg) was demonstrated the significant ($P < 0.05$) attenuation activity when compared to low-dose treatment of phloretin (25 mg/kg). The standard drug silymarin (50 mg/kg) also exhibited the effective reducing activity against the cisplatin-induced PARP-1 and caspase-3 activity in experimental rats [Figure 5].

## Effects of phloretin on renal histology of rats

The phloretin treatment was distinctly possessed the protective effects against the cisplatin-induced renal cellular damage and inflammation in the experimental rats. The administration of cisplatin to the rats was exhibited the distinct signs of renal toxicity such as degeneration of granular vacuoles, infiltration of inflammatory cells, congestion, glomerulus atrophy, inflammation between the tubules and tissue necrosis of the renal cells in the kidney of experimental rats which in contrast to the normal control rats [Figure 6]. While the phloretin treatment was possessed the significant protective effects against the cisplatin-induced renal damage in experimental rats. The treatment with the low-dose and high dose of phloretin (25–50 mg/kg) was possessed the effective protection against the renal damage which is induced by cisplatin in experimental rats [Figure 6]. The treatment with the high dose of phloretin (50 mg/kg) was demonstrated the increased attenuation effects than the low-dose treatment of phloretin (25 mg/kg). The standard drug silymarin (50 mg/kg) also showed the protective effects against the cisplatin-induced renal cellular damage and tissue necrosis in experimental rats [Figure 6].

# DISCUSSION

The administration of cisplatin was stimulates the injury of renal tubules by the various mechanisms, such as free radicals generation, hypoxia, apoptosis, and inflammation. In the current study, the cisplatin was exhibited the severe renal damage in Wistar albino rats by possessing the various clinical complications such as increased serum urea, proteinuria and creatinine levels, decreased antioxidant enzymes level, increased DNA fragmentation, elevated oxidative stress and severe injury to renal tissues. In addition, the cisplatin was also increased the PARP-1 and caspase-3 activity and proinflammatory cytokines level such as TNF-alpha and IL-1 β. Whereas, the treatment with the low-dose and high dose of phloretin (25–50 mg/kg) was possessed the significantly ($P < 0.05$) attenuates the cisplatin-induced nephrotoxicity in experimental rats.

The cisplatin was a one of the often employed anticancer drugs in chemotherapy, but the Cistoplastin is widely known for its deleterious nephrotoxicity effects. The most cancer patients who administered with

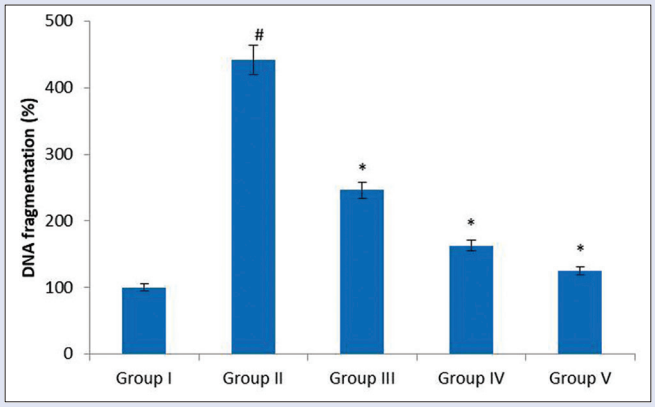


**Figure 4:** Effects of phloretin on cisplatin-induced DNA fragmentation level in experimental rats. Results are depicted as mean ± standard error of mean of six animals ($n = 6$). $^{\#}P < 0.05$ as compare to the control group. $^{*}P < 0.01$ as compare to the Cisplatin treated group

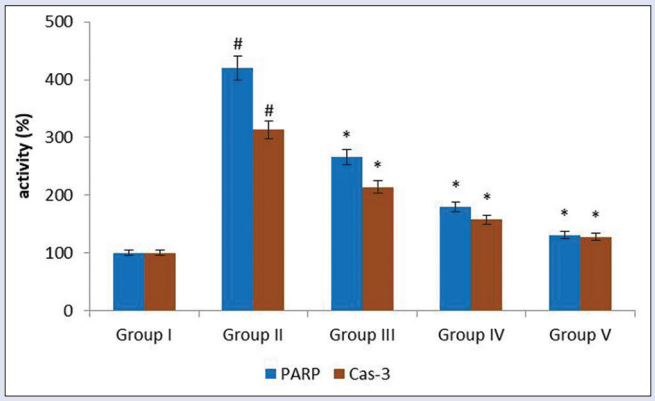


**Figure 5:** Effects of phloretin on poly (ADP-ribose) polymerase-1 and caspase-3 activity in experimental rats. Results are depicted as mean ± standard error of mean of six animals ($n = 6$). $^{\#}P < 0.05$ as compare to the control group. $^{*}P < 0.01$ as compare to the Cisplatin treated group

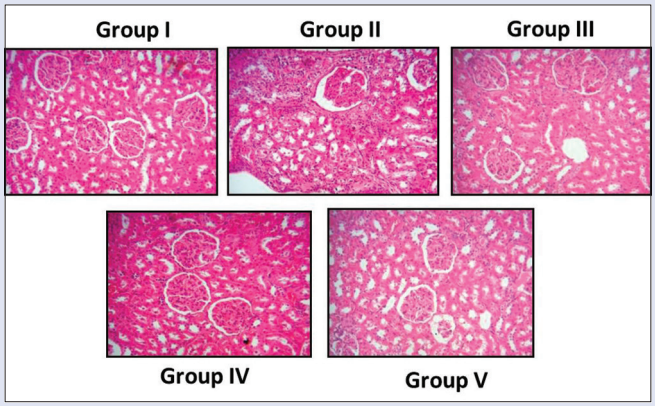


**Figure 6:** Effects of phloretin on renal histology of experimental rats. Control rats show the normal architecture whereas cisplatin-induced kidney damage. The treatment with the high-dose of phloretin (50 mg/kg) was demonstrated the increased attenuation effects than the low-dose treatment of phloretin (25 mg/kg). The standard drug silymarin (50 mg/kg) also showed the protective effects against the cisplatin-induced renal cellular damage and tissue necrosis in experimental rats

Cistoplastin were frequently suffered from the severe renal damage or failure. The cisplatin was possessed the inflammation, apoptosis, and increased oxidative stress which often leads to the nephrotoxicity.[1,21] The level of serum creatinine was a noteworthy indicator of the functions of kidneys. The creatinine is a waste product of muscle energy metabolism, is produced at a constant rate that is proportional to the muscle mass of an individual.[22] The serum creatinine level is a widely employed to determine the function of kidneys. The diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the normal level in the serum. In chronic renal failure and uremia, an eventual reduction occurs in the excretion of creatinine by both the glomeruli and the tubules.[23]

The urea is a nitrogen containing end product of protein and amino acids catabolism. It was produced by the liver and dispersed throughout the intracellular and extracellular fluids. The urea was filtered from the blood by glomeruli of the kidneys and partly reabsorbed with the water.[24] The determination of serum urea level was widely employed method to examine the renal functions.[25] The serum levels of urea, uric acid, and creatinine can be used as an effective indicator of renal dysfunction. The phloretin treatment was significantly attenuates the cisplatin-induced deleterious alterations in serum nephrotoxicity markers. The cisplatin administered rats were exerted the significant ($P < 0.01$) increase in the serum urea, creatinine and proteinuria levels in rats. The cisplatin administration also showed the significant ($P < 0.01$) decrease in the serum albumin than the normal control group. Whereas, the treatment with the phloretin was potentially ameliorates the cisplatin-induced changes in nephrotoxicity markers. The low-dose and high-dose treatment of phloretin (25–50 mg/kg) was significantly ($P < 0.05$) decreased the urea, proteinuria and creatinine level in the serum of experimental rats. Phloretin also increased the serum albumin level than the cisplatin administered animals [Figure 1].

The increased oxidative stress was a major factor of pathophysiology of cisplatin-stimulated kidney damage of failure.[26] The increased oxidative stress was indicating the reduction of antioxidant enzymes such as GSH, CAT and SOD and elevated level of MDA.[1] The increased oxidative stress level can lead to demolishes of cellular proteins, membrane lipids, and nucleic acids and finally results the tissue necrosis.[27] The cisplatin administered rats were possessed the statistically significant ($P < 0.01$) reduction in the antioxidant enzymes levels such as SOD, GSH, and CAT in the serum of experimental rats which in contrast to the control animals.

The cisplatin also showed the statistically significant ($P < 0.01$) increase in the lipid peroxidation level than the normal control group. The significant reduction in the antioxidant enzymes level was indicating the increased oxidative stress. Whereas the phloretin treatment was significantly attenuates the cisplatin-induced oxidative stress in rats by significantly ($P < 0.05$) elevating the antioxidant enzymes level. The low-dose and high-dose treatment of phloretin (25–50 mg/kg) was significantly ($P < 0.05$) decreased the cisplatin-induced oxidative stress level in experimental rats. Phloretin was also decreased the cisplatin-induced lipid peroxidation when compared to the cisplatin-treated group [Figure 2]. The high-dose treatment of phloretin (50 mg/kg) was possessed the significantly ($P < 0.05$) increased attenuation activity than the low-dose treatment (25 mg/kg). The standard drug silymarin (50 mg/kg) also exhibited the significant ($P < 0.05$) activity against the cisplatin-induced oxidative stress [Figure 2].

The exposure to the cisplatin can stimulate the release of pro-inflammatory cytokines such as TNF-alpha and IL-1 β, and it could lead to the inflammation in the kidney tissues.[21] The TNF-α is an important pleiotropic proinflammatory cytokine which is synthesized by endothelial cells, glomerular cells, and dendritic cells of the renal tubules.[28] The TNF-α, IL-1 β, and IL-6 stimulated cytotoxicity or inflammatory reaction was exhibited by the various mechanisms such as elevated production of reactive oxygen species (ROS). The increased ROS can demolish the segments of cell such as proteins, lipids, and nucleic acids. The increased production of TNF-α besides with the other pro-inflammatory cytokines such as IL-1 β and IL-6 was playing a imperative role in the progression and development of nephrotoxicity.[29]

In the current study, the administration of cisplatin to the rats was exhibited the significant ($P < 0.01$) elevation in the IL-1 β, TNF-α, and NO in the experimental rats, which in contrast to the normal control rats [Figure 3]. Whereas, the treatment with the low-dose and high-dose treatment of phloretin (25–50 mg/kg) was significantly ($P < 0.05$) decreased the IL-1 β, TNF-α and NO levels in the experimental rats [Figure 3]. The high-dose treatment of phloretin (50 mg/kg) was exhibited the significantly ($P < 0.05$) increased amelioration effects when compared to low-dose treatment (25 mg/kg). The phloretin treatment was also significantly ($P < 0.05$) prevented the cisplatin-induced DNA fragmentation level and reduced the PARP-1 and caspase-3 activity in experimental rats. The cisplatin administered animals were exhibited the significantly ($P < 0.01$) elevated level of DNA fragmentation and increased activity of PARP-1 and caspase-3 in the experimental rats which in contrast to the normal control rats. Whereas phloretin treatment was exhibited the significantly ($P < 0.05$) reduced level of DNA fragmentation and decreased the PARP-1 and caspase-3 activity in rats.

The prescription of cisplatin was restricted to the cancer patients due to its deleterious nephrotoxicity effects, and more than 30% of cancer patients were experienced the renal failure because of cisplatin administration.[30] The cisplatin has the low molecular weight, and hence, it can be easily penetrates to the membrane of glomerular base and accumulates in the inner medulla of proximal tubules and the outer cortex. The pharmacological complications of cisplatin in renal tissues were possessed in the form of tissue necrosis in tubules through the increased oxidative stress, apoptosis, vasoconstriction of the renal vasculars and inflammation. The many studies highlighted that the tissue necrosis in the tubules was the important histological phenomenon of the cisplatin-stimulated nephrotoxicity.[30-33] In the present study, the phloretin treatment was distinctly possessed the protective effects against the cisplatin-induced renal cellular damage and inflammation in the experimental rats.

The administration of cisplatin to the rats was exhibited the distinct signs of renal toxicity such as degeneration of granular vacuoles, infiltration of inflammatory cells, congestion, glomerulus atrophy, inflammation between the tubules and tissue necrosis of the renal cells in the kidney of experimental rats which in contrast to the normal control rats [Figure 6]. While the phloretin treatment with low- and high-dose (25–50 mg/kg) was possessed the significant protective effects against the cisplatin-induced renal damage in experimental rats [Figure 6]. The observed results of the current research work were clearly exhibits that the phloretin significantly attenuates the cisplatin-induced nephrotoxicity in the experimental rats.

## CONCLUSION

The phloretin treatment was significantly protected the kidneys against the cisplatin-stimulated renal damages in rats. Hence, the further research on the phloretin may lead to the development of the novel drug for the effective treatment of nephrotoxicity.

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## Conflicts of interest

There are no conflicts of interest.

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