International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2018.384.390

Research Article
Prophylactic Effect of Diacerein against Cisplatin-Induced Nephrotoxicity in Rats

[1]Asmaa Mohamed Abdel-Aziz, [1]Mohamed Abdellah Ibrahim, [1]Azza Ali El-Shiekh, [2]Nisreen AbdelTawab Osman, [1,3]Ayman Geddawy and [1]Aly Abdelrahman

[1]Department of Pharmacology, Faculty of Medicine, Minia University, 61111 Al-Minia, Egypt
[2]Department of Pathology, Faculty of Medicine, Minia University, 61111 Al-Minia, Egypt
[3]Department of Basic Medical Sciences, College of Medicine, Prince Sattam Bin Abdulaziz University, Al Kharj, Saudi Arabia

Abstract

Background and Objective: Cisplatin is an effective chemotherapeutic agent for solid tumors, however its use is limited by nephrotoxicity. The current study investigated the effect of diacerein in cisplatin-induced nephrotoxicity in rats. **Materials and Methods:** Rats were randomly divided into 5 groups; control (untreated), diacerein control (100 mg kg^{-1}), cisplatin (5 mg kg^{-1} i.p.), cisplatin+diacerein (50 mg kg^{-1}) and cisplatin+diacerein (100 mg kg^{-1}). Data were analyzed by one way ANOVA using GraphPad Prism. **Results:** Administration of cisplatin caused significant deterioration in renal function, designated by the increase in serum levels of both urea and creatinine, reduction in creatinine clearance, increase in microalbumin level and increase in fractionated sodium level. Cisplatin-induced renal injury was confirmed by histopathological finding in the form of damage of renal tubules. Cisplatin-induced renal damage was associated with significant increase in oxidative stress [increased renal contents of malondialdehyde (MDA), decreased reduced glutathione (GSH) and decreased activity of superoxide dismutase (SOD)] as well as by significant increase in the inflammatory mediator, tumor necrosis factor-α (TNF-α). **Conclusion:** Pretreatment with diacerein significantly ameliorated cisplatin-induced renal injury as evidenced both biochemically and histopathologically. The protective effect of diacerein was associated with amelioration of oxidative stress and reduction in TNF-α in renal tissue. Such data clarify that diacerein is a potential protective drug against cisplatin induced nephrotoxicity and its effect relies, at least partially, on its antioxidant and anti-inflammatory mechanisms.

Key words: Anticancer, cisplatin, nephrotoxicity, diacerein, oxidative stress, antioxidant, anti-inflammatory

Received: August 23, 2017 Accepted: November 23, 2017 Published: March 15, 2018

Citation: Asmaa Mohamed Abdel-Aziz, Mohamed Abdellah Ibrahim, Azza Ali El-Shiekh, Nisreen AbdelTawab Osman, Ayman Geddawy and Aly Abdelrahman, 2018. Prophylactic effect of diacerein against cisplatin-induced nephrotoxicity in rats. Int. J. Pharmacol., 14: 384-390.

Corresponding Author: Ayman Geddawy, Department of Pharmacology, Faculty of Medicine, Minia University, 61519 Minia, Egypt Tel: 0020863849288; Fax: 0020862342813

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cisplatin, a chemotherapeutic member of platinum coordination complex class of anti-cancer drugs, is an effective therapy for a variety of solid and metastatic malignancies. Despite effectiveness of cisplatin against tumors, its use is limited by a group of adverse effects such as severe vomiting, neurotoxicity, ototoxicty and importantly dose-related nephrotoxicity. Cisplatin cytotoxic effect on malignant cells, as well as its toxic effect on various organs is thought to be via several mechanisms including DNA inter- and intra-strand cross link formation, cell membrane lipid binding activities[1] and reduction in the antioxidant status[2].

Cisplatin-induced nephrotoxicity is associated with increased glomerular capillary permeability, heavy protein uria and degeneration of tubular epithelial cells that may lead to terminal renal failure[3].

Stratigies against nephrotoxicity due to cisplatin have been suggested including supplementation and hydration therapy. Previous studies proposed several agents; namely antioxidants for reducing cisplatin-induced toxicities[2,4]. However, the results of these studies did not achieve a full satisfaction.

Diacerein is an anti-inflammatory drug that ameliorates the course of osteoarthritis. It inhibits transcription of nuclear factor kappa-Beta (NF-κβ) that induced by the pro-inflammatory cytokine interleukin-1Beta (IL-1β). It also prevents the expression of the pro-inflammatory mediator inducible nitric oxide synthase (iNOS) and consequently the production of nitric oxide (NO). The later effect might explain its anti-osteoarthritic and anti-inflammatory properties[5]. Diacerein and its metabolite rhein have been recently reviewed for their anti-inflammatory and antioxidant activities[6]. The aim of the current study is to investigate the effect of diacerein in cisplatin-induced nephrotoxicity in rats.

MATERIALS AND METHODS

The study was carried out at the Department of Pharmacology, Faculty of Medicine, Minia university (from April, 2015-March, 2016) in accordance with the guide for the care and use of laboratory animals of the National Institute of Health.

Animals and drugs: Adult male albino rats (200-250 g), were obtained from the National Research Center (Giza, Egypt). Rats were housed in cages (3-4/cage) under standard laboratory conditions and fed rat chow diet and tap water. Diacerein powder was obtained from Eva pharma, Egypt. Cisplatin solution for injection (10 mg/10 mL) was purchased from Emic United Pharmaceutical (Cairo, Egypt).

Experimental design: The rats were allocated into 5 groups, 8 rats in each group as follow:

- **Group 1:** Normal control group received vehicle (carboxymethyl cellulose)
- **Group 2:** Diacerein control group received diacerein (100 mg kg^{-1}/day, p.o.)[7]
- **Group 3:** Cisplatin positive (nephrotoxic) control group received a single cisplatin (5 mg kg^{-1} i.p)[8]
- **Groups 4 and 5:** Cisplatin plus diacerein treated groups, cotreated with cisplatin plus diacerein either in 50 or 100 mg kg^{-1}/day, respectively

Diacerein was given from the 1st day for 14 consecutive days while cisplatin was injected at day 12 of the experiment. Rats were sacrificed at the 15th day.

Samples collection and preparation: At the end of the experimental period, rats were anaesthetized with urethane, weighed and sacrificed. Blood samples were collected from neck vessels by decapitation and left for 30 min to clot and then centrifuged (JANETZKI T30, Germany) for 10 min at 5000 rmp for separation of serum.

Kidneys were rapidly removed, dried, weighed and immediately frozen in liquid nitrogen. For each rat, half kidney was kept in 10% formalin for histopathological examination and second one and half were homogenized in ice-cold phosphate buffer and the homogenate was kept at -80°C for further measurements.

Measurements

Assessment of urea and creatinine: Urea level in serum urea and creatinine in serum and urine were assayed by using an enzymatic colorimetric urea kits (Biodiagnostic, Egypt and Human Co., Germany; respectively), according to the kit instruction.

Determination of creatinine clearance: Determined as following: Urinary creatinine(Cr_u) mg dL^{-1} × urine volume (v) mL/24 h divided on serum creatinine (Cr_s) mg dL^{-1} × 1440. According to the method described earlier[9].

Determination of the fractional excretion of sodium ($FENa^+$): The $FENa^+$ measure the percent of filtered Na^+ that

is excreted in the urine. This calculation is a marker that differentiates between pre-renal disease and acute tubular necrosis. The $FENa^+$ increased in case of acute tubular necrosis. It is calculated according to the following Eq.[9]:

$$FENa^+ = \frac{UNa^+ \times Serum\ Cr}{Serum\ Na^+ \times UCr} \times 100$$

Where:
UNa^+ = Sodium content in urine
UCr = Creatinine content in urine

Serum as well as urine contents of sodium were determined using flame photometer 400 (United Kingdom) for sodium.

Determination of urinary microalbumin level: Urinary albumin level was assayed by using a microalbuminria kit (BioSystems, Spain) according to the instruction of manufacturer.

Assessment of oxidative stress parameters in renal tissues: The reduced glutathione (GSH) was measured using colorimetric GSH kit (Biodiagnostic, Egypt).

The method is based on the reduction of the 5,5`-Dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorption can be measured at 405 nm.

Catalase was measured using colorimetric catalase kit (Biodiagnostis Co. Egypt). Catalase converts H_2O_2 to H_2O and O_2. In the presence of peroxidase, remaining H_2O_2 reacts with 3,5-Dichloro-2-hydroxybenzene sulfonic acid and 4-Aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample. Malondialdehyde level in renal tissues (MDA) is a reactive aldehyde that is a measure of lipid peroxidation. Renal contents of MDA were determined using the thiobarbituric acid method described earlier[10].

Measurement of tumor necrosis factor-α (TNF-α) in renal tissue: Renal tissue content of TNF-α was measured using Enzyme-Linked Immunosorbent Assay (ELISA) kit (Sigma-Aldrich, St. Louis, MO, USA) according the kit instruction. Samples were loaded into well plates precoated with rat-specific monoclonal antibodies, followed by addition of a secondary detection antibody. A substrate solution (Avidin-Biotin-Peroxidase Complex) and acidic stop solution were used to produce a yellow color that read at 450 nm.

Histopathological examination of kidney tissues: Renal tissues were collected after animal sacrificing, fixed in 10% formalin, processed routinely and embedded in paraffin. Paraffin-embedded tissues were sectioned at 5 mm thickness and stained with haematoxylin and eosin for histological assessment. Slides were examined by a pathologist who was blinded to the nature of treatments. Microscopic evaluation was done.

The severity of histopathological lesions was assessed semi-quantitatively by light microscopy of rat kidneys isolated from control group, rats treated with diacerein (50 mg kg^{-1}) or diacerein (100 mg kg^{-1}) alone, animals treated with cisplatin (5 mg kg^{-1}), or with cisplatin together with diacerein (50 mg kg^{-1}) or diacerein (100 mg kg^{-1}).

Score level 0 was considered normal. Scores +, ++ and +++ are mild, moderate and severe levels, revealing less than 25, 50 and 75% histopathological alterations of total fields examined, respectively. Slides obtained from 3 animals of each group, 5 fields/animal (40×).

Statistical analysis: The data were analyzed by one way analysis of variant (ANOVA) followed by Tukey's test. The values were represented as Mean±SEM. All statistical analysis was done using GraphPad Prism software, Inc. version 5. Statistical significance was determined at p value of <0.05.

RESULTS

Serum urea and creatinine: Experimental results revealed that rats receiving cisplatin (the nephrotoxic control) showed a significant increase ($p<0.05$) in serum levels of urea and creatinine compared to normal control group (Table 1). Pre-treatment of cisplatin-treated rats with low dose (50 mg kg^{-1}/day) or high dose (100 mg kg^{-1}) of diacerein resulted in reduction to normal levels of urea and creatinine compared to their respective cisplatin treated (the nephrotoxic control) group.

Creatinine clearance: Experimental results revealed that rats received cisplatin (the nephrotoxic control) showed a significant decrease ($p<0.05$) in creatinine clearance compared to normal control group (Table 1). Pre-treatment of cisplatin-injected rats with diacerein (50 mg kg^{-1}/day) or diacerein (100 mg kg^{-1}) resulted in a significant increase ($p<0.05$) in creatinine clearance compared to their respective cisplatin treated (the nephrotoxic control) group.

Table 1: Effect of diacerein on serum urea and creatinine, creatinine clearance, urinary microalbumin and fractionated sodium in rats exposed to cisplatin-induced nephrotoxicity

Groups	Serum urea (mg dL^{-1})	Serum creatinine (mg dL^{-1})	Creatinine clearance (mL min^{-1})	Urinary microalbumin (mg/24 h)	$FENa^+$ (%)
Control	38.50±2.7	1.15±0.06	0.37±0.04	2.5±0.4	1.8±0.2
Diacerein	39.24±5.3	1.20±0.08	0.40±0.05	2.3±0.4	1.7±0.3
Cisplatin	82.73±7.7[a]	3.15±0.13[a]	0.04±0.005[a]	5.2±0.2[a]	23±2.2[a]
Cisplatin+diacerein 50 mg	42.91±2.8[b]	1.19±0.09[b]	0.34±0.05[b]	2.6±0.3[b]	2.2±0.5[b]
Cisplatin+diacerein 100 mg	48.03±3.9[b]	1.28±0.07[b]	0.33±0.03[b]	2.6±0.4[b]	1.9±0.2[b]

Results are expressed as Mean±SEM (n = 8). [a]Significantly different (at p<0.05) from control group, [b]Significantly different (at p<0.05) from cisplatin group

Table 2: Effect of diacerein on renal reduced glutathione (GSH), catalase, malondialdehyde (MDA)and tumor necrosis factor-α (TNF-α) in rats exposed to cisplatin-induced nephrotoxicity

Groups	Renal GSH (μmol g^{-1} tissue)	Renal catalase (unit g^{-1} tissue)	Renal MDA (nmol g^{-1} tissue)	Renal TNF-α (ng g^{-1} tissue)
Control	4.9±0.18	63.1±6.3	36.40±1.8	23.30±1.01
Diacerein	4.4±0.67	66.4±6.6	47.62±3.4	23.14±1.1
Cisplatin	0.77±0.15[a]	12.14±1.8[a]	110.90±2.4[a]	102.60±2.5[a]
Cisplatin+diacerein 50 mg	4.50±0.35[b]	60.30±8.8[b]	39.25±3.8[b]	71.29±2.4[b]
Cisplatin+diacerein 100 mg	4.46±0.69[b]	67.08±5.8[b]	41.86±2.7[b]	59.70±2.5[b c]

Results are expressed as Mean±SEM (n = 8). [a]Significantly different (at p<0.05) from control group. [b]Significantly different (at p<0.05) from cisplatin group. [c]Significantly different (at p<0.05) from cisplatin+diacerein 50 mg group

Fractional excretion of sodium ($FENa^+$): Cisplatin (the nephrotoxic control) group showed a significant increase (p<0.05) in $FENa^+$ level compared to normal control group. (Table 1). Pre-treatment of cisplatin group with both doses of diacerein resulted in a significant decrease (p<0.05) in $FENa^+$ level compared to their respective cisplatin (the nephrotoxic control) group.

Urinary microalbumin: In cisplatin group, there was a significant increase (p<0.05) in 24 h urinary microalbumin level compared to control group (Table 1). Pre-treatment of cisplatin treated group with both doses of diacerein resulted in a significant decrease (p<0.05) in 24 h urinary microalbumin level compared to non-treated cisplatin group.

Oxidative stress parameters in renal tissue: Rats injected with cisplatin showed a significant decrease (p<0.05) in renal tissue level of GSH level compared to control group (Table 2). Pre-treatment of cisplatin group with either diacerein (50 mg kg^{-1}/day) or diacerein (100 mg kg^{-1}) resulted in a significant increase (p<0.05) in renal GSH level compared to cisplatin group. There was no significant change between groups pretreated with either diacerein 50 mg kg^{-1} plus cisplatin or diacerein 100 mg kg^{-1} plus cisplatin.

Cisplatin group showed a significant decrease (p<0.05) in renal catalase activity compared to control group (Table 2). Pre-treatment of cisplatin treated group with either dose of diacerein resulted in a significant increase (p<0.05) in renal catalase activity compared to their respective cisplatin group. No significant changes in catalase activity between low dose of diacerein plus cisplatin compared with or high dose of diacerein plus cisplatin.

Rats receiving cisplatin showed a significant increase (p<0.05) in renal MDA content compared to control group (Table 2). Pre-treatment of cisplatin group with either diacerein (50 mg kg^{-1}/day) or diacerein (100 mg kg^{-1}) resulted in a significant decrease (p<0.05) in renal MDA level compared to their respective cisplatin groups. There was no significant difference in renal tissue content of MDA between cisplatin rats pretreated either low or high dose of diacerein.

TNF-α in renal tissue: Data in Table 2 show that renal tissue content of TNF-α was significantly increased (p<0.05) in cisplatin group when compared with control rats. Cisplatin rats that pretreated with either low dose (50 mg kg^{-1}) or high dose (100 mg kg^{-1}) of diacerein reported significant reduction (p<0.05) of TNF-α content compared with nontreated cisplatin group. Rats that pretreated with high dose diacerein showed a significant reduction (p<0.05) in TNF-α level when compared with low dose pretreated rats.

Renal histopathology: Histopathological examination (Table 3 and Fig. 1) revealed that control, diacerein (100 mg kg^{-1}) groups had normal structure of renal glomeruli and cortical tubules. Cisplatin treated group presented with marked dilatation and degeneration of renal tubules that showed protein casts and cytoplasmic vaculation. Pre-treatment of cisplatin-treated group with diacerein

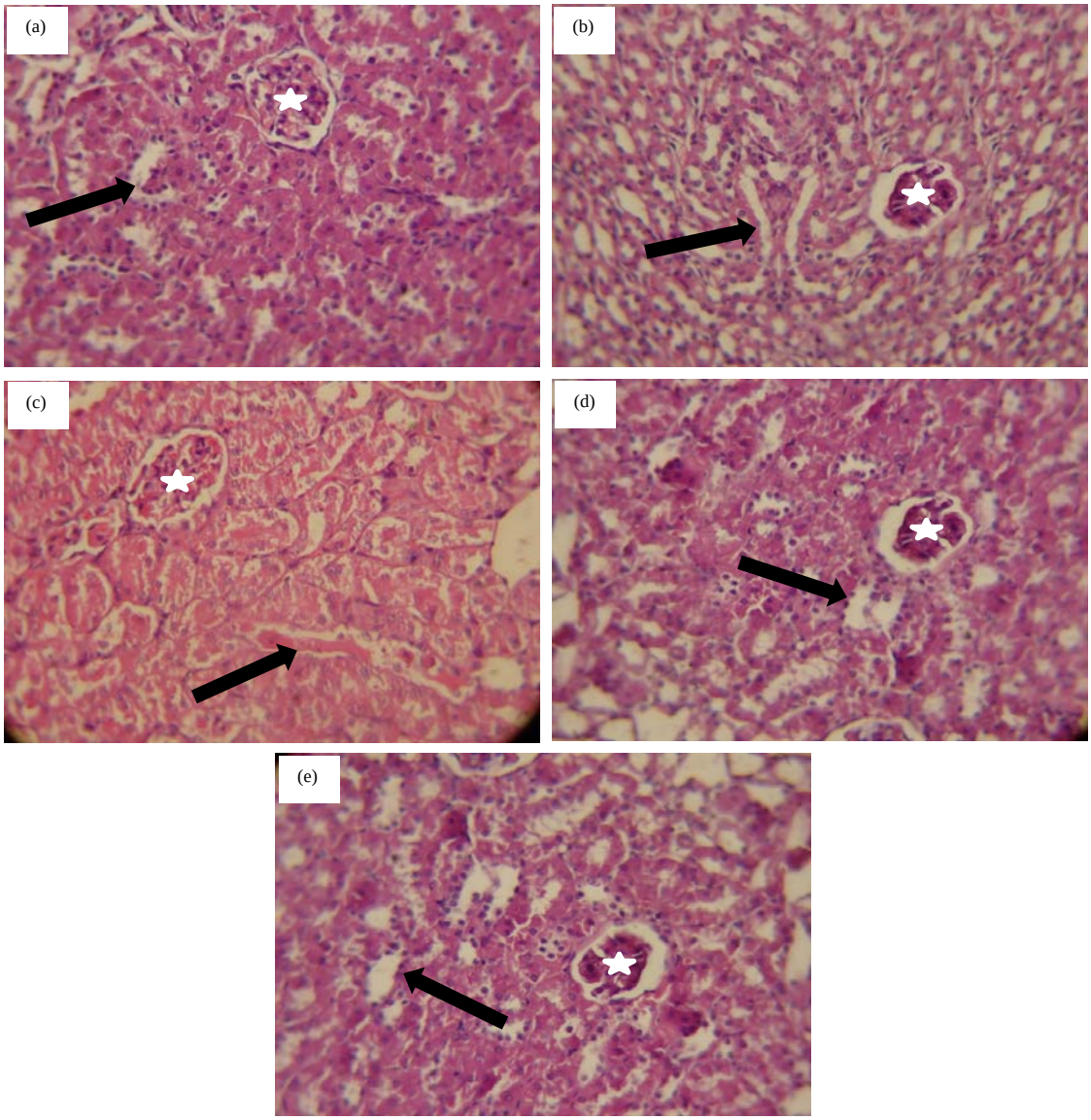

Fig. 1(a-e): Photomicrograph of kidney sections showing effect of diacerein on renal histopathology in rats exposed to cisplatin-induced nephrotoxicity (H and E, 40X). (a and b) Control and diacerein (100 mg kg^{-1}) groups, respectively, showing normal structure of renal glomeruli (Star) and cortical tubules (arrows). (c) Cisplatin-treated group showing normal renal glomeruli (Star) with severe degenerative changes observed in the renal tubules (arrows) with exfoliated cells, tubules are filled with protein casts and showing cystic dilatation with cytoplasmic vaculation. (d and e) Cisplatin-treated group with either diacerein (50 mg kg^{-1}/day) or diacerein (100 mg kg^{-1}) showing normal renal glomeruli (Star) with regeneration of some renal tubular epithelial cells lining, disappearance of protein casts and restoration of some tubular dilatation (arrows)

Table 3: Effect of diacerein on renal histopathological leasions in rats exposed to cisplatin-induced nephrotoxicity.

Groups	Tubular degeneration	Tubular dilatation	Cytoplasmic vaculation	Protein casts
Control	0	0	0	0
Diacerein	0	0	0	0
Cisplatin	++	+++	+	++
Cisplatin+diacerein 50 mg	+	+	0	0
Cisplatin+diacerein 100 mg	+	+	0	0

Slides obtained from 3 animals of each group, 5 fields/animal. Score level 0 was considered normal. Scores +, ++ and +++ are mild, moderate and severe levels, revealing less than 25, 50 and 75% histopathological alterations of total fields examined, respectively

(50 mg kg^{-1}/day) or diacerein (100 mg kg^{-1}) resulted in reversal of histopathological damage induced by cisplatin, with regeneration of renal epithelial cells lining of cortical tubules to somewhat to normal.

DISCUSSION

Results of the present study reveal that pretreatment with diacerein protected from cisplatin-induced nephrotoxicity as evidenced both biochemically and histopathologically. The protective effect of diacerein was associated with amelioration of oxidative stress and reduction in TNF-α in renal tissue. Data of the present study show that diacerein is a potential protective drug against cisplatin induced nephrotoxicity and its effect relies, at least partially, on its antioxidant and anti-inflammatory mechanisms.

Cisplatin continues to be an effective and widely used broad spectrum chemotherapeutic agent. However, its clinical use is limited because of its serious toxicities[11]. The mechanisms underlying cisplatin toxicity are complex; several studies have confirmed the contribution of oxidative stress[12] and inflammation[13] in the pathogenesis of cisplatin -induced organs damage.

The present results showed that a single dose of cisplatin (5 mg kg^{-1}) in rats induced marked nephrotoxicity manifested by elevation in serum levels of urea and creatinine, decrease in creatinine clearance, increase albumin uria and increase $FENa^+$ levels[14,15]. The increase in $FENa^+$ indicated that cisplatin caused tubular cells degeneration leading to a decrease in tubular capacity of Na^+ reabsorption and an increase in its fractional excretion as previously reported in rats with nephrotoxicity induced by cisplatin[16].

This nephrotoxicity was confirmed by histopathological changes in the form of tubular dilatation, cytoplasmic vaculation, tubular cells degeneration and the tubules were filled with protein casts. Similar findings were reported previously[3,17].

The results showed that cisplatin nephrotoxicity was associated with increased renal oxidative damage as well as with increased the inflammatory mediator TNF-α. Similar findings were previously reported in doxorubicin-induced nephrotoxicity[18].

The results of the current study revealed that administration of diacerein in the used doses before cisplatin attenuated cisplatin-induced nephrotoxicity. This was evidenced by a significant decrease in serum level of urea and creatinine, increase in creatinine clearance, decrease in $FENa^+$ and albumin uria. This was confirmed by improvement in the histological findings of cisplatin-induced nephrotoxicity.

In order to explain the mechanism of renal protection of diacerein, the oxidative stress parameters (GSH, catalase and MDA) were measured in renal tissue. The data of the present study reported that the protective effect of the used doses of diacerein was accompanied with a significant attenuation in oxidative stress parameters as they caused a significant decrease in the renal level of MDA and a significant increase in renal level of GSH and catalase. Administration of diacerein before cisplatin restored the kidney antioxidant parameters to almost control levels. These antioxidant properties of the drug could explain its nephroprotective effect in as similarly reported for other antioxidants[1,18].

The present study interestingly showed that diacerein significantly decreased rats body weight but it is worthy to note that further study is needed to explore the mechanism(s) by which diacerein decreased body weight, is it due to decreased food intake or increased energy expenditure. Diacerein is one of anthraquinone monomers isolated from rhubarb, which has been broadly used in the treatment of diabetic nephropathy and other chronic renal diseases. Rhein can inhibit metabolism in diabetic nephropathy mice, reduce urinary protein, ameliorate glomerular hypertrophy, narrow the area of the renal corpuscle and mesangium and suppress renal fibrosis[7].

At the molecular level, diacerein inhibits NF-κβ, leading to downregulation of downstream gene transcription, such as for genes encoding proinflammatory cytokines. Previous evidences demonstrated that IL-1 family of cytokines has important role in the regulation of responses associated with inflammatory stress. Diacerein downregulates the activity of IL-1β by decreasing the number of IL-1β receptors on the cell surface and by influencing the activities of NF-kappaβ and caspase-3 in the early phase of glomerulosclerosis[5,19].

Diacerein and its metabolite rhein showed reno-protective effect as previously reported in different models of renal injuries. The mechanisms of nephroprotective effect include reduction of in the pro-inflammatory mediators like prostaglandin E2, IL-1β, TNFα and IL-12 at post-transcriptional or post-translational level. In addition, diacerein succeed to be a nephroprotective drug against acetaminophen induced nephrotoxicity[6, 20,21].

CONCLUSION

It is concluded that pretreatment of cisplatin-treated rats with either diacerein (50 mg kg^{-1}) or diacerein (100 mg kg^{-1}) protect against cisplatin-induced nephrotoxicity. The nephroprotective effects of the doses of diacerein were accompanied with attenuation of cisplatin-induced alteration in oxidative stress.

SIGNIFICANT STATEMENTS

This study discovers the renoprotective effect of diacerein against cisplatin-induced nephrotoxicity in rats shedding light on its antioxidant and anti-inflammatory properties. This study suggests diacerein for investigation as rescue therapy with other strategies like hydration to prevent nephrotoxicity in cancer patients receiving cisplatin.

ACKNOWLEDGMENT

Authors like to thank Eva pharmaceutical company, Egypt for providing diacerein powder as a gift from the company.

REFERENCES

1. Karasawa, T. and P.S. Steyger, 2015. An integrated view of cisplatin-induced nephrotoxicity and ototoxicity. Toxicol. Lett., 237: 219-227.
2. Kilic, U., E. Kilic, Z. Tuzcu, M. Tuzcu and I.H. Ozercan *et al*., 2013. Melatonin suppresses cisplatin-induced nephrotoxicity via activation of Nrf-2/HO-1 pathway. Nutr. Metab., Vol. 10. 10.1186/1743-7075-10-7.
3. Pezeshki, Z., M. Nematbakhsh, H. Nasri, A. Talebi and A.A. Pilehvarian *et al*., 2013. Evidence against protective role of sex hormone estrogen in cisplatin-induced nephrotoxicity in ovarectomized rat model. Toxicol. Int., 2: 43-47.
4. Crona, D.J., A. Faso, T.F. Nishijima, K.A. McGraw, M.D. Galsky and M.I. Milowsky, 2017. A systematic review of strategies to prevent cisplatin induced nephrotoxicity. Oncologist, 22: 609-619.
5. Martel-Pelletier, J. and J.P. Pelletier, 2010. Effects of diacerein at the molecular level in the osteoarthritis disease process. Ther. Adv. Musculoskelet. Dis., 2: 95-104.
6. Sun, H., G. Luo, D. Chen and Z. Xiang, 2016. A comprehensive and system review for the pharmacological mechanism of action of Rhein, an active anthraquinone ingredient. Front. Pharmacol., Vol. 7. 10.3389/fphar.2016.00247.
7. Sheng-Nan, P., Z. Hui-Hong, F.U. Ai-Xiang, C. Xiao-Wen and Z. Qing-Xian, 2013. Protection of rhein on IgA nephropathy mediated by inhibition of fibronectin expression in rats. Indian J. Pharmacol., 45: 174-179.
8. El Sayed, E.S.M., O.M. Abd El Raouf, M. Ola, H.M. Fawzy and M.F. Manie, 2013. Comparative study of the possible protective effects of cinnamic acid and cinnamaldehyde on cisplatin induced nephrotoxicity in rats. J. Biochem. Mol. Toxicol., 27: 508-514.
9. Cockcroft, D.W. and M.H. Gault, 1976. Prediction of creatinine clearance from serum creatinine. Nephron, 16: 31-41.
10. Geddawy, A., M. Hussian, M.Y. Kamel, R. Kamal and M.A. Ibrahim, 2017. Effects of liraglutide and vitamin E in fructose-induced metabolic syndrome in rats. Pharmacology, 99: 48-56.
11. Hassan, H.A., G.M. Edrees, E.M. El-Gamel and E.A. El-Sayed, 2014. Amelioration of cisplatin-induced nephrotoxicity by grape seed extract and fish oil is mediated by lowering oxidative stress and DNA damage. Cytotechnology, 66: 419-429.
12. Miller, R.P., R.K. Tadagavadi, G. Ramesh and W.B. Reeves, 2010. Mechanisms of cisplatin nephrotoxicity. Toxins, 2: 2490-2518.
13. Nematbakhsh, M., V. Hajhashemi, A. Ghannadi, A. Talebi and M. Nikahd, 2013. Protective effects of the *Morus alba* L. leaf extracts on cisplatin-induced nephrotoxicity in rat. Res. Pharm. Sci., 8: 71-77.
14. Mika, D. and C. Guruvayoorappan, 2013. The effect of *Thespesia populnea* on cisplatin induced nephrotoxicity. J. Cancer Res. Ther., 9: 50-53.
15. Bulacio, R.P. and A.M. Torres, 2013. Organic anion transporter 5 (Oat5) renal expression and urinary excretion in rats treated with cisplatin: A potential biomarker of cisplatin-induced nephrotoxicity. Arch. Toxicol., 87: 1953-1962.
16. Ahangarpour, A., A.A. Fard, M.K. Gharibnaser, T. Jalali and I. Rashidi, 2014. Hydrogen sulfide ameliorates the kidney dysfunction and damage in cisplatin-induced nephrotoxicity in rat. Vet. Res. Forum, 5: 121-127.
17. Ashrafi, F., M. Nematbakhsh, H. Nasri, A. Talebi, S.M. Hosseini and M. Ashrafi, 2013. Vacuolization, dilatation, hyaline cast, debris or degeneration: Which one is the most correlated item to score the kidney damage pathologically in Cisplatin induced nephrotoxicity model? Nephrourol. Monthly, 5: 918-920.
18. Refaie, M.M.M., E.F. Amin, N.F. El-Tahawy and A.M. Abdelrahman, 2016. Possible protective effect of diacerein on doxorubicin-induced nephrotoxicity in rats. J. Toxicol., Vol. 2016. 10.1155/2016/9507563.
19. Banerjee, M. and M. Saxena, 2012. Interleukin-1 (IL-1) family of cytokines: Role in type 2 diabetes. Clin. Chim. Acta, 413: 1163-1170.
20. Meng, Z., Y. Yan, Z. Tang, C. Guo and N. Li *et a*., 2015. Anti-hyperuricemic and nephroprotective effects of rhein in hyperuricemic mice. Planta Med., 81: 279-285.
21. Zhao, Y.L., G.D. Zhou, H.B. Yang, J.B. Wang, L.M. Shan, R.S. Li and X.H. Xiao, 2011. Rhein protects against acetaminophen-induced hepatic and renal toxicity. Food Chem. Toxicol., 49: 1705-1710.