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1,2-Diazole prevents cisplatin-induced nephrotoxicity in experimental rats

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Abstract:

Background: Cisplatin (a platinum-compound) is a anti-neoplastic drug used in the treatment of various cancers but eventually results in severe adverse effects namely nephrotoxicity or renal disorder through generation of reactive oxygen species (ROS). This biochemical measurements and histopathology analysis investigated a possible protective effect of 1,2-diazole with regards to cisplatin-induced nephrotoxicity in experimental animals.

Methods: Animals were divided into four groups of six mice each. Group A: normal control, vehicle (1% (w/v) gum acacia in phosphate buffer saline (PBS)). Group B: cisplatin group, vehicle + cisplatin (7.5 mg/kg). Group C: 1,2-diazole (10 mg/kg) + cisplatin and Group D: silymarin (50 mg/kg) + cisplatin. Each vehicle/drug treatment was given daily *via* intraperitoneal (*ip*) injection for 10 consecutive days starting from day 1. On group B, C and D cisplatin was given in single dose only on day 5 one hour post drug administration. Animals were allowed till 10^{th} day and on day 11 all four groups animals were anesthetized. Blood samples were collected and serum was isolated for biochemical measurements. The rats were then euthanized by cervical dislocation and their kidney was recovered and then prepared for biochemical measurements and histopathology analyses.

Results: Pretreatment with 1,2-diazole prevented nephrotoxicity induced by cisplatin through a protective mechanism that involved reduction of increased oxidative stress by significantly increasing the enzymatic and non enzymatic antioxidant enzymes such as glutathione peroxidase (GPx), glutathione (GSH) and diminishing the lipid peroxidation (LPO). The pretreatment with 1,2-diazole does not affect superoxide dismutase (SOD), catalase (CAT), serum urea and creatinine level during nephrotoxicity when compared to cisplatin-induced group. Moreover, the 1,2-diazole animals shown significant decrease in urine volume and kidney weight when compared with cisplatin-induced group. Histopathological findings reveals the protective efficacy of 1,2-diazole that restores histopathological changes against nephrotoxicity.

Conclusion: These analysis will provide a critical evidence that 1,2-diazole could provide a new protective strategy against cisplatin-induced nephrotoxicity.

Key words:
1,2-diazole, pyrazole, cisplatin, nephrotoxicity, antioxidant, renal disorder, *Rhizophora apiculata*, mangroves

Introduction

Kidneys are the major elimination pathway for many anti-neoplastic drugs and their metabolites [33]. Chemotherapeutic drugs are excreted in the urine and after 24 hours, the renal toxicity concentration of platinum in the renal cortex elevates to higher level than in the other organs. Most of the drugs including anti-neoplastic drugs were found to induce nephrotoxicity by altering tubular cell toxicity, intraglomerular hemodynamics, crystal nephropathy, thrombotic microangiopathy and rhabdomyolysis [19, 30, 36, 39].

Cisplatin is a major anti-neoplastic drug commonly used as front-line therapy for cancers such as: small cell lung cancer, gut cancer, bladder cancer, stomach cancer, ovarian cancer and germ cell tumors. The compounds are a class of platinum containing anti-cancer drugs which induce apoptosis through caspase-9-dependent pathway. Cisplatin-DNA crosslinks cause cytotoxic lesions in tumor cells and other regenerative cells. Cisplatin is toxic to proximal tubule cells that results in renal damage [38]. Cisplatin gets accumulated in mitochondria of renal tubular epithelial cells with reactive oxygen species (ROS) (mediators exerting toxic effects) resulting in cisplatin-induced nephrotoxicity [16]. Cisplatin also targets primarily at the S3 segment of the proximal tubule that reduces glomerular filtration rate resulting in renal failure. A single dose of cisplatin may cause acute renal dysfunction [20, 34]. Mitochondria continuously scavenge ROS through the action of antioxidant enzymes such as superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx) and catalase (CAT). The anti-neoplastic drug cisplatin induces ROS in renal epithelial cells and reduces the antioxidant enzyme activity by decreasing the intracellular concentrations of GSH [6, 28].

Traditional medicines and natural products provide protection to adverse effects of conventional therapy in preventing nephrotoxic disorders. Natural products have long been known as an important source of therapeutic and effective medicines with no or less adverse effects. Silymarin is a mixture of flavonolignans extracted from milk thistle plant (*Silybum marianum*) which is a strong antioxidant compound competent of scavenging both free radicals and ROS. The name "milk thistle" is derived from the milky white sap that comes from the leaves and stems when crushed or broken. Silymarin has high importance in herbal medicine for its activity in the treatment and protection against variety of diseases including nephrotoxicity. Silymarin adverse effects like gastrointestinal disturbances and allergic skin rashes have been published [11].

Marine mangrove plants are widely used in medicine to treat variety of diseases. Few mangrove plants such as *Rhizophora mangle* and *Rhizophora mucronata* are known to exhibit antiviral, antibacterial and anti-inflammatory properties [2, 27]. Mangroves are extensively used in traditional medicine but only fewest have been determined for its medicinal activities. Our recent study showed that *Rhizophora apiculata* (*R. apiculata*) has predominant content of pyrazole in the crude methanolic extract which has wide range of medicinal properties [25]. We also reported that 1,2-diazole (pyrazole) possesses anti-inflammatory activity [26].

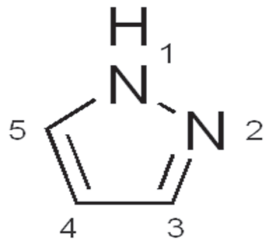

Fig. 1. Structure of 1,2-diazole – a plant alkaloid

The 1,2-diazoles (Fig. 1) are a class of simple aromatic ring organic compounds characterized by a ring structure of 3 carbon atoms and 2 nitrogen atoms that are in adjacent positions also in the unsubstituted parent compound, that are available very rarely in nature. The 1,2-diazoles were first isolated from the seeds of watermelon and are classified as alkaloids (occuring confined in few plants) [25] which is the largest groups of chemical arsenals delivered by plants with highly reactive substances with biological activity in low doses that contain nitrogen – usually derived from an amino acid. In the present study, we tried to evaluate the protective effect of 1,2-diazole as a nephroprotectant.

Materials and Methods

Animals

Male Wistar rats (130–150 g) were purchased from the Pasteur Institute of India, Coonoor, Tamil Nadu, India. All rats were kept in a pathogen-free air-controlled room maintained at 24°C with a ~50% relative humidity and 12-h light/dark cycle, and provided *ad libitum* access to normal rat chow (Sai Feeds, Bangalore, India) and filtered water. All animal experiments were performed according to the rules and regulations of the Institutional Animal Ethics Committee, (IAEC/KU/BT/12/015), Government of India.

Chemicals and kits

1,2-Diazole was purchased from Sigma (St. Louis, MO, USA). Cisplatin was purchased from VHD-MEDI Sciences Ltd., Uttarakhand, India. Silymarin drug was purchased from Microlabs Tamil Nadu, India. Urea and creatinine diagnostic kits were purchased from Span Diagnostics Ltd., Surat, India. All chemicals used in these studies were of analytical or reagent grade.

Grouping of animals

Animals were divided into four groups of six mice each (n = 24): Group A: served as normal control and received the vehicle (1%, w/v gum acacia with phosphate buffer saline (PBS)); Group B: served as cisplatin group, received only vehicle along with the cisplatin (7.5 mg/kg) [7]. Group C: rats received 1,2-diazole (10 mg/kg) + cisplatin (7.5 mg/kg) [26] and Group D: rats received standard drug silymarin (50 mg/kg) + cisplatin (7.5 mg/kg) [11]. Each vehicle/drug treatment was given daily *via* intraperitoneal (*ip*) injection for 10 consecutive days starting from day 1. For group B, C and D cisplatin was given as single dose only on day 5 one hour after drug administration. Animals were allowed till 10^{th} day, when all animals were housed separately on metabolic cages, to collect 24 h urine. On day 11, all four groups' animals were anesthetized with ether and blood was collected into non-coated tubes for isolation of serum for analysis. The rats were then euthanized by cervical dislocation and their kidneys were recovered, washed gently with saline and relative kidney weight was recorded. Samples were then prepared for biochemical measurements and histopathology analyses.

Biochemical measurements

Biochemical measurements were performed on kidney tissues of the rats. A portion of isolated kidney tissue from each rat was homogenized in 10% (w/v) Tris-HCl buffer (pH 7.0) are used for the measurement of SOD, GPx, CAT, GSH and total lipid peroxidation (LPO). Collected serum samples were used to determine creatinine and urea level.

Measurement of SOD activity

SOD activity in kidney tissues was determined based on the ability of the enzyme to inhibit nitroblue tetrazolium (NBT) reduction by superoxide [18]. In brief, into an incubation medium containing 0.1 ml of test sample, 2.55 ml of phosphate buffer, 0.2 ml EDTA/NaCN, 0.1 ml NBT, and 0.05 ml riboflavin (to a total volume of 3 ml) were added. The tubes then received uniform illumination for 15 min and the optical density was then measured at 560 nm in spectrophotometer (ELICO, Hyderabad, India). One unit of enzyme activity was defined as the amount of enzyme giving 50% inhibition of reduction of NBT and expressed as U/mg protein. Enzymatic activity was calculated from inhibition of reduction of NBT using standard curve constructed by varying amount of the test samples.

Determination of GPx activity

The activity of GPx in kidney tissue was determined based on the utilization of reduced GSH by the enzyme [29]. The kidney tissue homogenate (100 µl) was treated with 100 µl of GSH, 2.1 µl of buffer, 100 µl of sodium azide and 1.2 mM hydrogen peroxide (100 µl). The mixture was incubated at 37°C for 6 min and 2 ml of phosphoric acid (1.67%) were added and centrifuged. To the supernatant (2 ml), 1 ml of disodium hydrogen phosphate and 1 ml of DTNB were added and incubated at 37°C for 10 min. The absorbance was read at 412 nm.

Determination of CAT activity

The CAT activity was determined by method of Aebi [1]. In these assays, 10 µl of absolute ethanol is added to 100 µl of tissue extract (20–30 µg protein) and placed in an ice bath for 30 min. Nine volumes of 1% Triton X100 (900 µl) was added to the mixture and then homogenized. The sample (100 µl) was then mixed with 500 µl of 66 mM of hydrogen peroxide and 400 µl of 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA and the absorbance was monitored at 240 nm. One unit of CAT activity was defined as 1 nmol of hydrogen peroxide degraded/minute/mg protein.

Determination of GSH content

GSH levels in kidney tissue were determined by the method of Beutler and Kelly [4]. GSH was measured

by its reaction with 5,5'-dithionitrobenzoic acid (DTNB). For the reactions, 0.125 ml of 25% (w/v) trichloroacetic acid solution (TCA) was added to 0.5 ml of kidney tissue homogenate. The tubes were placed on ice for 5 min and then further diluted with 0.6 ml of 5% TCA. Each sample was then centrifuged (5000 rpm, 4°C, 10 min) and resultant supernatant was taken for GSH estimation. A volume of aliquot (0.3 ml) was combined with 0.7 ml of 0.2 M phosphate buffer, and then 2 ml of 0.6 mM DTNB was added to the tubes and the intensity of the resulting yellow color was measured at 412 nm. Values are expressed as nmol/mg protein.

Measurement of LPO level

The kidney tissue homogenate (0.1 ml) was treated with 200 µl of sodium dodecyl sulfate (SDS-8%) and 1.5 ml thiobarbutric acid (TBA). The mixture was kept in water bath for 1 h at room temperature and cooled by adding 1 ml of distilled water followed by 5 ml of a mixture of *n*-butanol and pyridine (15 : 1, v/v) and centrifuged. The supernatant was taken and the optimal intensity at 532 nm was measured. The levels of LPO were expressed as nmol/mg protein. The protein level was estimated by the Lowry method [21, 24].

Determination of serum urea and creatinine

Serum urea was measured manually with urease using Berthelot procedure (Span Diagnostics kit, Surat, India). The concentration of serum creatinine was measured by commercial kit (Span Diagnostics Ltd., Surat, India) as per manufacturers instructions.

Histopathological analyses

A portion of kidney tissue specimen from each rats (n = 6) was fixed in 10% formalin, cut into 4-µm thin sections, stained using H&E (hematoxylin and eosin) and then examined for any histopathological changes. Renal histology was examined in a blinded fashion. The stained sections of kidney were examined for cell lysis, loss of brush border, cast formation and other nephrotoxic damages.

Statistical analyses

Results are expressed as the mean ± SD. Statistical evaluation was performed using a one-way analysis of variance (ANOVA) followed by Dunnett's test using Graph Pad Instat (Version 3.0 for Windows 95; Graph Pad Software, San Diego, CA, USA). A p value < 0.05 was considered statistically significant.

Results

Effect of 1,2-diazole on kidney SOD activity measured in cisplatin-induced nephrotoxicity in rats

The effect of 1,2-diazole on kidney SOD activity measured in cisplatin-induced nephrotoxicity in rats is presented in Figure 2A. Treatments with 1,2-diazole do not affect the kidney tissue SOD activity level (10.11 ± 0.54 U/mg protein) (Group C) when compared to level of cisplatin-induced group (8.13 ± 1.94 U/mg protein) (Group B). The treatment with silymarin does not affect the kidney tissue SOD level (11.74 ± 1.38 U/mg protein) (Group D) when compared to cisplatin-induced group (Group B). The kidney tissue SOD level of the normal control group was found to be (13.53 ± 1.97 U/mg proteins) (Group A).

Effect of 1,2-diazole on kidney GPx activity measured in cisplatin-induced nephrotoxicity in rats

The effect of 1,2-diazole on kidney GPx activity measured in cisplatin-induced nephrotoxicity in rats is presented in Figure 2B. Treatment with 1,2-diazole significantly ($p < 0.05$) increased the kidney tissue GPx level (13.39 ± 0.21 U/mg protein) (Group C) when compared to the level of cisplatin-induced group (7.54 ± 1.24 U/mg protein) (Group B). The silymarin treated hosts significantly ($p < 0.01$) increased in kidney tissue GPx level (15.40 ± 0.71 U/mg protein) (Group D) compared to cisplatin-induced group (Group B). The kidney tissue GPx level of the normal control groups was found to be 22.63 ± 3.30 U/mg protein (Group A). Silymarin treatment could alleviate the GPx level in a much effective manner than 1,2-diazole.

Effect of 1,2-diazole on kidney CAT activity measured in cisplatin-induced nephrotoxicity in rats

The effect of 1,2-diazole on kidney CAT activity measured in cisplatin-induced nephrotoxicity in rats is

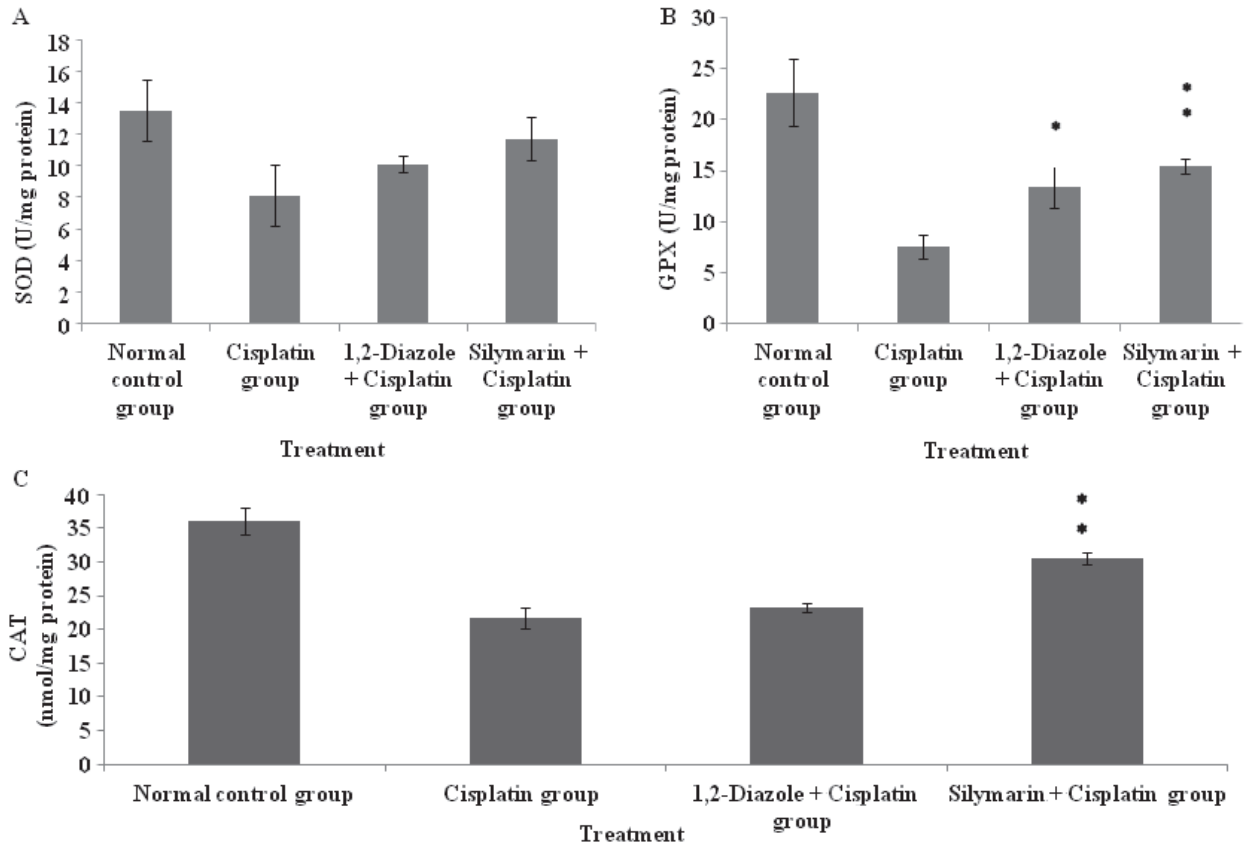

Fig. 2. Effect of 1,2-diazole on kidney SOD (**A**), Gpx (**B**) and CAT (**C**) activity. Animals are treated as follows: Group A: Normal control, Group B: Cisplatin group, Group C: 1,2-Diazole + Cisplatin group and Group D: Silymarin + Cisplatin group. Values are expressed as the mean ± SD (n = 24). * $p < 0.05$, ** $p < 0.01$ are considered statistically significant

presented in Figure 2C. Treatment with 1,2-diazole does not affect the level of kidney tissue CAT level (23.20 ± 0.70 nmol/mg protein) (Group C) when compared to the level of cisplatin-induced group (21.73 ± 1.51 nmol/mg protein) (Group B). The silymarin-treated hosts significantly ($p < 0.01$) increased in the kidney tissue CAT level (30.56 ± 0.90 nmol/mg protein) (Group D) compared to cisplatin-induced group (Group B). The kidney tissue CAT level of the normal group was found to be (35.98 ± 1.98 nmol/mg protein) (Group A). Silymarin treatment could alleviate the CAT level in a much effective manner with 1,2-diazole.

Effect of 1,2-diazole on kidney GSH activity measured in cisplatin-induced nephrotoxicity in rat

The effect of 1,2-diazole on kidney GSH activity measured in cisplatin-induced nephrotoxicity in rats is presented in Figure 3. Treatment with 1,2-diazole significantly ($p < 0.05$) increased the level of GSH of kidney tissue (32.58 ± 2.34 nmol/mg protein) (Group C) when compared to the level of cisplatin-induced group (25.65 ± 1.42 nmol/mg protein) (Group B). The silymarin-treated hosts also displayed significant ($p < 0.05$) increase in kidney tissue GSH level (32.10 ± 1.60 nmol/mg protein) (Group D) compared to cisplatin-induced group (Group B). The kidney tissue GSH level of the normal control was found to be (40.30 ± 2.05 nmol/mg protein) (Group A). Silymarin treatment could not alleviate the GSH level in effective manner than 1,2-diazole.

Effect of 1,2-diazole on kidney LPO activity measured in cisplatin-induced nephrotoxicity in rats

The effect of 1,2-diazole on kidney LPO activity measured in cisplatin-induced nephrotoxicity is pre-

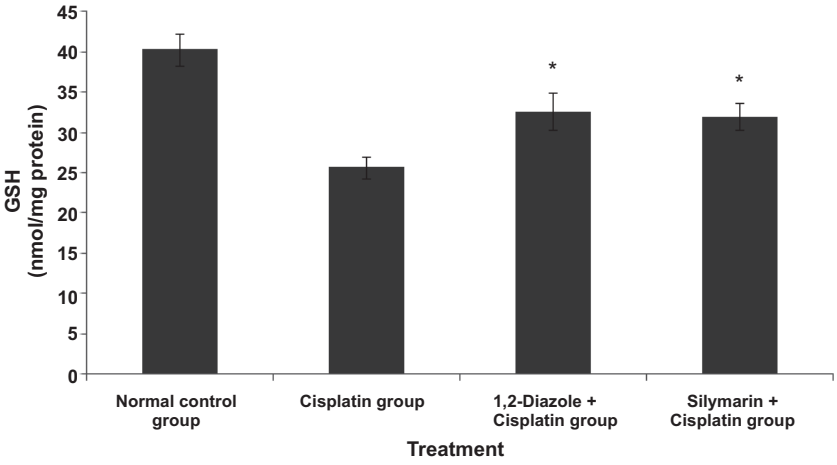

Fig. 3. Effect of 1,2-diazole on kidney GSH activity. Animals are treated as follows: Group A: Normal control, Group B: Cisplatin group, Group C: 1,2-Diazole + Cisplatin group and Group D: Silymarin + Cisplatin group. Values are expressed as the mean ± SD (n = 24). * $p < 0.05$ is considered statistically significant

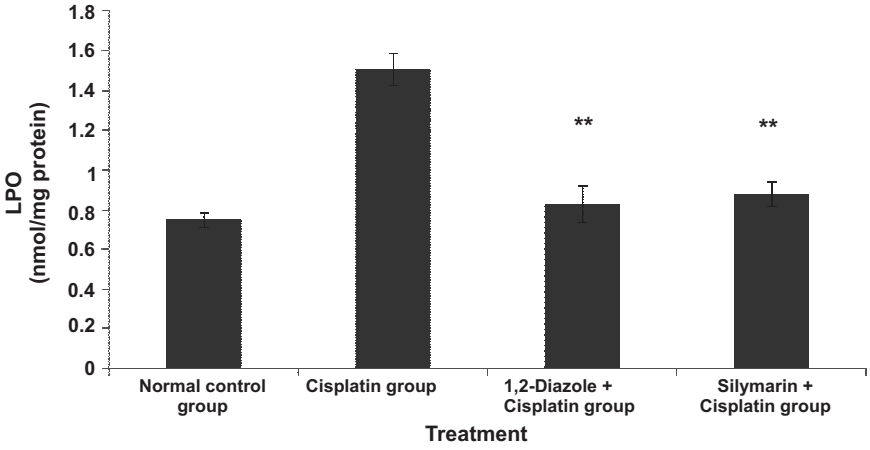

Fig. 4. Effect of 1,2-diazole on kidney LPO activity. Animals are treated as follows: Group A: Normal control, Group B: Cisplatin group, Group C: 1,2-Diazole + Cisplatin group and Group D: Silymarin + Cisplatin group. Values are expressed as the mean ± SD (n = 24). ** $p < 0.01$ is considered statistically significant

sented in Figure 4. Treatment with 1,2-diazole significantly ($p < 0.01$) decreased the level of kidney tissue LPO level (0.83 ± 0.09 nmol/mg protein) (Group C) when compared to the level of cisplatin-induced group (1.51 ± 0.08 nmol/mg protein) (Group B). The silymarin-treated hosts also significantly ($p < 0.01$) decreased the kidney tissue LPO level (0.88 ± 0.06 nmol/mg protein) (Group D) compared to cisplatin-induced group (Group B). The kidney tissue LPO level of the normal control was found to be (0.75 ± 0.04 nmol/mg protein) (Group A).

Effect of 1,2-diazole on serum urea, creatinine, urine volume, body weight and kidney weight measured in cisplatin-induced nephrotoxicity in rats

The effect of 1,2-diazole on serum urea, creatinine, urine volume, body weight and kidney weight measured in cisplatin-induced nephrotoxicity is shown in Table 1. Treatment with 1,2-diazole does not affect the serum urea level (89.70 ± 7.11 mg/dl) (Group C) when compared to serum urea level of cisplatin-induced group (96.11 ± 4.69 mg/dl) (Group B). The silymarin-treated hosts significantly ($p < 0.05$) decreased the level of serum urea level (75.20 ± 5.30 mg/dl) (Group D) when compared to cisplatin-induced group (Group B). The serum urea level of the normal group was found to be (69.64 ± 6.12 mg/dl) (Group A).

Treatment with 1,2-diazole, does not affect the serum creatinine level (1.11 ± 0.62 mg/dl) (Group C) when compared to the serum creatinine level of cisplatin-induced group (1.69 ± 0.14 mg/dl) (Group B). The treatment with silymarin drug also does not affect the serum creatinine level (0.85 ± 0.08 mg/dl) (Group D) when compared to cisplatin-induced group (Group B). The serum creatinine level of the normal group was found to be (0.54 ± 0.03 mg/dl) (Group A).

The treatment with 1,2-diazole significantly ($p < 0.01$) decreased the volume of the urine collected 24 h

Tab. 1. Effect of 1,2-diazole on serum urea, creatinine, urine volume, body weight and kidney weight. Animals were treated as follows: Group A: Normal control, Group B: Cisplatin group, Group C: 1,2-Diazole + Cisplatin group and Group D: Silymarin + Cisplatin group. Values are expressed as the mean ± SD (n = 24). (* $p < 0.05$, ** $p < 0.01$) are considered statistically significant

Groups	Serum urea (mg/dl)	Serum creatinine (mg/dl)	Urine volume (ml/24 h)	Body weight (g) Day 11	Kidney weight (g)
Normal control group	69.64 ± 6.12	0.54 ± 0.03	6.8 ± 1.5	140.6 ± 1.3	0.82 ± 0.06
Cisplatin group	96.11 ± 4.69	1.69 ± 0.14	21.8 ± 3.2	137.6 ± 1.1	1.20 ± 0.13
1,2-Diazole + Cisplatin group	89.70 ± 7.11	1.11 ± 0.62	10.0 ± 1.9**	142.5 ± 1.8*	0.86 ± 0.07*
Silymarin + Cisplatin group	75.20 ± 5.30*	0.85 ± 0.08	9.2 ± 1.7**	143.6 ± 1.0*	0.84 ± 0.08*

(10.0 ± 1.9 ml/24 h) (Group C) when compared to cisplatin-induced group (21.8 ± 3.2 ml/24 h) (Group B). The silymarin-treated hosts also significantly ($p < 0.01$) decreased the urine volume collected for 24 h (9.2 ±1.7 ml/24 h) (Group D) compared to cisplatin-induced group (Group B). The 24 h urine volume of the normal group was found to be (6.8 ± 1.5 ml/24 h) (Group A).

The treatment with 1,2-diazole, significantly ($p < 0.05$) increased the body weight of the animals on 11th day (142.5 ± 1.8 g) (Group C) when compared to the cisplatin-induced group (137.6 ± 1.1 g) (Group B). The silymarin-treated hosts also significantly ($p < 0.05$) increased the body weight of the animals on 11th day (143.6 ± 1.0 g) (Group D) when compared to the cisplatin-induced group (Group B). The body weight of the normal group animals on the same day was found to be (140.6 ± 1.3 g) (Group A).

The 1,2-diazole treatment significantly ($p < 0.05$) reduced the relative kidney weight (0.86 ± 0.07 g) (Group C) when compared to the cisplatin-induced group (1.20 ± 0.13 g) (Group B). The silymarin-treated host also significantly ($p < 0.05$) reduced the relative kidney weight (0.84 ± 0.08 g) (Group D) compared to cisplatin-induced group (Group B). The relative kidney weight of the normal group was found to be (0.82 ± 0.06 g) (Group A).

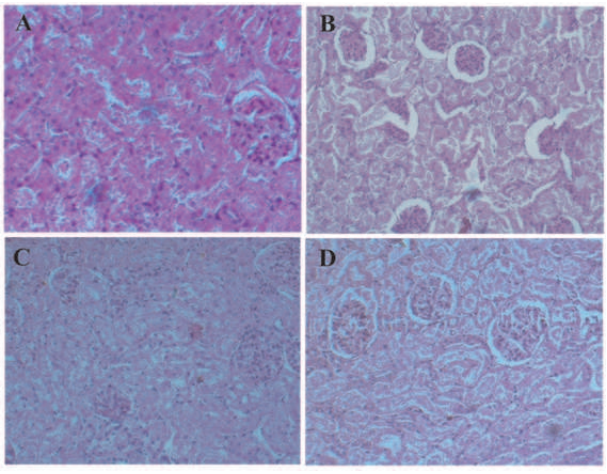

Fig. 5. Effect of 1,2-diazole on cisplatin-induced histopathological changes in rats' kidney. Figure **5A** represents normal kidney histology from normal control group. Figure **5B** represents kidney histology of cisplatin-induced group and shows more inflammatory cells infiltration at glomerular and in outer medulla. Figure **5C** represents kidney histology of 1,2-diazole treated group rat. Figure **5D** represents kidney histology of standard silymarin-treated group rat

Effect of 1,2-diazole on cisplatin-induced histopathological changes in rats kidney

The effect of 1,2-diazole on cisplatin-induced histopathological changes in rats kidney is shown in Figure 5. Kidney histopathological changes reveal abnormalities in the regions involving inner cortical and outer medullary areas of kidney, which are the target sites of cisplatin nephrotoxicity; after single dose of cisplatin administration, the 1,2-diazole drug treatment prevents the nephrotoxic damage. Figure 5A shows normal kidney histology from (Group A). In Figure 5B, cisplatin-induced group shows many cortical convoluted tubules revisited by necrotic epithelial cells, glomeruli exhibited swelling with reduction of Bowman's capsular space with more inflammatory cells infiltration at glomerular and in outer medulla (Group B). Figure 5C shows that the administration of 1,2-diazole prevented the nephrotoxic damage induced by cisplatin (Group C). In Figure 5D, standard drug silymarin shows minimal damage from nephrotoxic damage (Group D).

Discussion

Nephrotoxic injury is a damage to one or both of the kidneys and results from exposure to a toxic material that can occur in many different ways, depending upon the type of agent. Toxin has direct effect on the glomerulus or the renal tubules and leads to destruction of cells. Most drugs are found to cause nephrotoxicity or toxic effects that include altered inflammation, tubular cell toxicity, intraglomerular hemodynamics, crystal nephropathy, thrombotic microangiopathy and rhabdomyolysis [30, 39]. In recent days, the incidence of drug-induced nephrotoxicity is elevated to 66% [19]. Compared with 30 years ago, patients in the present day suffer from higher incidence of diabetes and cardiovascular diseases where they are prescribed with "chronic medication" of multitask drugs and are exposed to severe adverse effects including nephrotoxic injury or acute renal failure [19, 36]. Most cancer patients suffer from the adverse effects of chemotherapeutic drugs that include paraneoplastic glomerulonephritis, tumor lysis syndrome, obstructive uropathy and nephrotoxicity with renal failure and electrolyte disturbances [17].

Cisplatin is a chemotherapeutic drug often used to treat testicular, lung, bladder, ovarian and stomach cancer. The disproportionate accumulation of cisplatin in kidney tissue may result in cisplatin-induced nephrotoxicity [3]. Cisplatin may cause acute renal failure even after a single dose [20, 34]. In tumor cells and rapid dividing cells, the cisplatin-DNA intrastrand crosslinks lead to cytotoxicity and arrests of cancer cell proliferation [12]. Around 50% of cisplatin drugs are excreted in the urine during first 24 h and the concentration of platinum achieved in the renal cortex is several folds greater than that in plasma and other organs [14]. Cisplatin targets primarily at the S3 segment of the proximal tubule that leads to decrease in the glomerular filtration rate [13, 38]. Cisplatin initially triggers oxidative stress in the mitochondria of kidney proximal tubular and endothelial cells, followed by a secondary wave of ROS/RNS (reactive nitrogen species) generation, deterioration of mitochondrial structure and function, an intense inflammatory response and renal damage.

During drug induced nephrotoxcity, ROS produced in the course of metabolism are restrained by the natural antioxidant system that protects the functional and structural molecules against ROS-mediated modifications, thereby preventing cytotoxicity. The natural antioxidant system contains a series of antioxidant enzymes as well as numerous endogenous and dietary antioxidant compounds which are capable of reacting with and inactivating ROS. Superoxide is the primary ROS produced in the course of oxygen metabolism which is highly reactive, cytotoxic ROS. Superoxide is converted to a far less reactive product, hydrogen peroxide by a family of metalloenzymes known as SOD which constitute a front line of defense against ROS-mediated injury [35]. Oxidative stress is the major cause for the development of chronic renal failure [35]. In the present study, the pretreatment with 1,2-diazole and silymarin drug does not affect the kidney tissue SOD level compared to cisplatin-induced group. The cisplatin-induced animals show a decrease in tissue SOD level, which may be due to the depletion of copper and zinc in the kidney which are essential for the activity of enzymes.

GPx is the most important antioxidant enzyme in humans which is highly expressed in the kidney, involved in scavenging and inactivating hydrogen and lipid peroxides, providing protection to the body against oxidative stress and also removes peroxides and peroxynitrite that can cause renal damage [10]. GPx with other selenoproteins containing selenocysteine play an important role in the GSH dependent defense against peroxynitrite-mediated oxidations by serving as a peroxynitrite reductase [32]. The pretreatment with 1,2-diazole and standard drug silymarin significantly increased GPx level in kidney tissue which reveals their anti-oxidant efficacy against oxidative stress induced by cisplatin.

GSH is an important antioxidant tripeptide in the cells, preventing damage to important cellular components caused by ROS such as free radical and peroxides [37]. GSH can detoxify many endogenous toxins, including cisplatin, through the formation of GSH adducts to protect cells from the potential nephrotoxicity [23]. In this study, 1,2-diazole and standard drug silymarin prevented renal damage by significant increase in the kidney GSH level which effectively protects renal cells from the exposure to free radicals and peroxidase induced by cisplatin.

CAT is a common enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen [7]. CAT is highly effective in inhibiting various ROS-mediated injuries and could protect the kidney from cisplatin-induced nephrotoxicity [22]. The pretreatment with 1,2-diazole does not affect the kidney

tissue CAT level compared to cisplatin-induced group. Pretreatment with silymarin drug significantly increased the kidney tissue CAT level that shows its anti-oxidant activity during nephrotoxicity. LPO is generated naturally in small amounts in the body mainly by the effect of several ROS i.e., hydroxyl radical and hydrogen peroxide. An increase in the concentration of end products of LPO is the evidence for the involvement of free radicals in human disease [15]. Oxidative stress can damage proteins and DNA that are more significant targets of injury than lipids and LPO which often occur late in the injury process [15]. It is reported that cisplatin-mediated renal tissue injury increased kidney tissue LPO level due to release of free radicals, which is directly interrelated with an increase in MPO level during nephrotoxic condition [31]. The pretreatment with 1,2-diazole significantly reduced the kidney LPO level and counteracted the formation of free radicals induced by cisplatin-mediated nephrotoxicity that displayed its protective role in the prevention of renal damage. The standard drug silymarin also exhibited similar effect during nephrotoxicity. In this study, cisplatin-induced animals showed increased LPO level in kidney comparable to the measurement of previous reports [31].

Serum creatinine is an essential indicator of renal health since it is easily measured by product of muscle metabolism. Creatinine is primarily filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). If the filtering of the kidney is damaged, serum creatinine level increases. Experimental studies report that administration of cisplatin increased the serum creatinine level to the maximum on fifth day. Cisplatin toxicity induced severe renal dysfunction that allows the secretion of creatinine from the proximal tubules. The serum creatinine levels during cisplatin toxicity is a result of irreversible renal tubular injury [9]. Serum creatinine is probably the most widely used indirect measure of glomerular filtration rate. Renal function can also be determined by interpreting the serum urea level. During the metabolism of protein in the body, the liver generates ammonia, which is transformed into a by-product called urea. Kidneys completely filter an excess of urea into the urine but due to renal dysfunction, urea is released into the bloodstream as serum urea. Therefore, higher serum urea level is directly proportional to severity of renal damage [9]. Pretreatment with 1,2-diazole and silymarin drug does not affect the serum creatinine level compared to cisplatin-induced nephrotoxicity animals. Pretreatment with silymarin significantly decreased serum urea level and moreover, treatment with 1,2 diazole didn't affect the serum urea level during cisplatin-induced nephrotoxicity. Cisplatin-induced animals showed increased level of serum creatinine and urea level which are proportional to the degree of renal injury. These observations are comparative to the results to the measurements of earlier reports [5, 31].

In conclusion, the present study reveals that pretreatment with 1,2-diazole (alkaloid) significantly attenuated cisplatin-induced nephrotoxicity. The biochemical studies such as determinations of kidney GPx, GSH and LPO levels as well as urine volume, kidney weight, body weight and histopathological studies validate that the 1,2-diazole (10 mg/kg) has nephroprotective activity. Our results show comparatively similar results with the standard drug silymarin (50 mg/kg). The overall results suggest that 1,2-diazole can be used as a nephroprotectant in pair with silymarin.

Our previous findings report that marine mangrove *R. apiculata* has higher level of 1,2-diazole (pyrazole) compound which has potent anti-inflammatory properties [25, 26]. Therefore, the high presence of this compound in *R. apiculata* extract (available rarely in nature) added advantage for naturally available plant alkaloid with potent and efficient pharmacological role in protecting cisplatin-induced renal damage.

Declaration of interest:
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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