

RESVERATROL ATTENUATES OXIDATIVE STRESS IN CHEMOTHERAPY INDUCED ACUTE KIDNEY INJURY: AN EXPERIMENTAL RAT MODEL

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ABSTRACT

OBJECTIVE: To investigate the anti-oxidative and anti-nephrotoxic effects of resveratrol in cisplatin induced nephrotoxic albino Wistar rats.

METHODS: This quasi-experimental study was performed at Isra University, Hyderabad, Pakistan. Thirty male albino Wistar rats were divided into three groups: group-A (control), group-B (cisplatin) and group-C (cisplatin+resveratrol). Biochemical [serum urea, creatinine and glutathione peroxidase (GPX)] and renal histomorphology was performed in all groups after 21 days of treatment.

RESULTS: Difference in mean pre- and post-experimental body weight was observed in all three groups. Mean body weight decreased from 241.7 ± 8.5 gm to 196.50 ± 9.34 gm and from 237 ± 7.4 gm to 207.2 ± 6.56 gm in group-B and group-C respectively. In group-A; mean serum urea was 22.7 ± 2.66 mg/dl, serum creatinine was 0.45 ± 0.05 mg/dl and serum GPX was 1.44 ± 0.13 ng/ml. In group-B; mean serum urea level was 51 ± 3.65 mg/dl, mean serum creatinine was 0.78 ± 0.05 mg/dl and serum GPX was 0.85 ± 0.11 ng/ml. In group-C, mean serum urea level was 32.8 ± 1.45 mg/dl, serum creatinine level was 0.41 ± 0.09 mg/dl and serum GPX was 1.53 ± 0.08 ng/ml. In group-A, renal structure was intact, marked changes were observed in renal histology of group-B while group-C displayed less glomerular damage. The mean distance between visceral and parietal layers of Bowman's capsule was 69.34 ± 0.87 μ m in group-A, 216.5 ± 1.32 μ m in group-B while 102.22 ± 1.65 μ m in group-C. Areas of peritubular fibrosis and congestion were observed in groups B and C but less prominent in group-C compared with group-B.

CONCLUSION: Resveratrol therapy is a potent anti-nephrotoxic regime showing promising results in chemotherapy induced nephrotoxicity and oxidative stress.

KEY WORDS: Resveratrol (MeSH); Cisplatin (MeSH); Oxidative Stress (MeSH); Antioxidants (MeSH); Glutathione Peroxidase (MeSH).

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urine output.² In addition, some other common clinical symptoms include reduced output of urine leading to a disturbance in the concentration of not only metabolic acids, but also in the levels of important ions such as phosphate and potassium.³

Although incidence rates vary from population to population, in United States of America, taking a population of 1 million people, more than 5000 cases/year are reported that do not require dialysis and approximately 295 cases/year that require this mode of treatment.⁴ AKI is highly prevalent in patients that are critically ill. The frequency of AKI in hospital inpatients is 1.9%,⁵ however this frequency is seen to rise almost above 40% when the patient is admitted to the ICU and is concomitantly suffering from sepsis.⁶ One out of 5 people suffer from AKI during their stay in the hospital which is directly related to an increased risk of morbidity and mortality (up to 30%), particularly when dialysis becomes necessary.⁷ Mild to moderate decrease in renal function is also related to longer stay in hospital, higher treatment expense and considerably higher chance of developing chronic renal disease.⁸

Although the underlying cause of AKI, in most cases, is multi-factorial such as hypoperfusion / ischemia, sepsis etc., data from recent epidemiological studies has shown that nephrotoxic drugs, such as chemotherapy agents like cisplatin, are responsible for 19-25% of AKI cases.⁹ Despite being one of the most efficacious anti-cancer drugs used against a wide variety of solid tumors, cisplatin therapy is limited owing to the fact that it is notoriously toxic to the kidneys, causing AKI in 25-35% of patients after administration of a single

INTRODUCTION

The new consensus term for acute renal failure (ARF) is acute kidney injury (AKI).¹ It is a multifactorial and multi-phasic clinical syndrome. AKI is a sudden occurrence of kidney damage or

failure that causes not only the reduction of renal function, but also the build-up of metabolic wastes, such as creatinine and urea in the blood. This makes it hard for kidneys to keep the right balance of fluid within a body and can thus cause abnormal or reduced

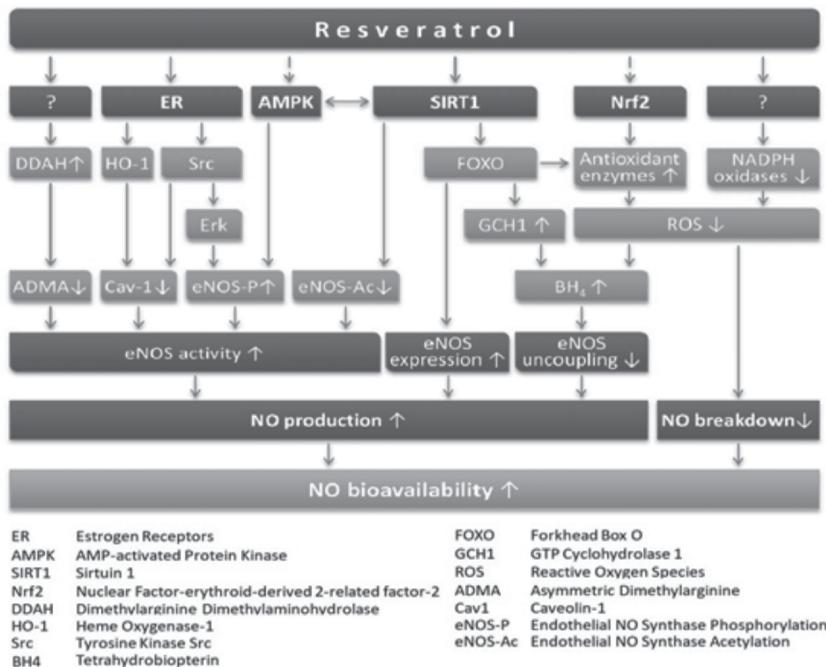


Figure 1: Targets and actions of resveratrol¹⁵

dose.¹⁰ The nephrotoxic nature of cisplatin is due to its reactive oxygen species (ROS) generating capability that causes depletion of glutathione levels and inhibition of renal antioxidant enzymes, causing AKI even with plausible reno-protective strategies such as co-administration with hydration.¹¹ Therefore, the prediction and prevention of cisplatin induced AKI remains a challenge.

Resveratrol (trans-3,4',5-trihydroxystilbene) (RSV) is a polyhydroxyphenol plant toxin (phytoalexin) present in several edible plants, including grapes, raspberries and plums etc.¹² Due to its potent anti-inflammatory as well as antioxidant potential, in recent years, there has been a general consensus regarding the positive effects of RSV, leading the usage of this drug for the prevention and treatment of various disease conditions.¹³ Endothelial nitric oxide synthase (eNOS) has been proposed as one of the possible targets responsible for the actions of RSV.¹⁴ RSV has also been found to simultaneously restrain production of ROS. The targets and actions of RSV are summarized in Figure 1.

The main objective of present study was

to determine the anti-nephrotoxic and cyto-protective effects of resveratrol on renal-cell physiology in cisplatin induced nephrotoxic rat model.

METHODS

Thirty healthy male albino Wistar rats were selected by purposive sampling. The inclusion criteria was set as, rats having body weight of 200 to 300gm, free from any co-morbidities and male rats, whereas exclusion criteria was set as rats having body weight less than 200 or more than 300 gm, female rats and moribund rats. After allowing the animals to acclimatize for one week, they were divided into 3 groups (10 rats per group) by simple random method; Group A (control rats), Group B (single dose of cisplatin 20mg/kg body weight intraperitoneally), Group C (single dose of cisplatin 20mg/kg body weight intraperitoneally + resveratrol 10 mg/kg body weight orally), and kept in stainless steel cages under standard conditions of illumination with a 12-hours light-dark cycle at $25 \pm 1^\circ\text{C}$. They were provided with normal chow diet and water *ad libitum*. Nephrotoxicity was induced by single intraperitoneal dose of cisplatin 20mg/kg body weight. RSV was crushed into powder and

mixed into the diet which was fed to the animals orally for 21 days (3 weeks). The level of the orally administered dosage of RSV (10 mg/kg body weight) and intraperitoneal dose of cisplatin (cisplatin 20mg/kg body weight intraperitoneally) was based on the previous works.^{16,17} Experimental work was conducted as per rules maintained by Ethical Committee of Isra University, Hyderabad, Pakistan. Body weights of all group rats were recorded before and after the experiment.

The experimental animals were anesthetized by placing them in an inverted glass jar containing cotton pieces soaked in chloroform. Blood samples were collected by cardiac puncture and transferred to gel tubes where they were allowed to stand for half an hour and then centrifuged at 3000 rpm for 15 min at 4°C to separate serum which was stored for the different biochemical measurements. The animals of all groups were sacrificed by cervical dislocation. Skin incision was given from suprasternal notch to the lower half of abdomen. The kidneys were dissected out and fixed immediately in 10% neutral formalin. Tissue was embedded in paraffin wax and 4-micron thick sections were obtained by microtome and stained with Hematoxylin and Eosin (H & E) staining for histopathological analysis.

Serum urea and creatinine were analyzed on Hitachi Roche automated analyzer (Cobas c311) whereas serum glutathione peroxidase (GPX) was analyzed using bioassay technology ELISA kit (catalogue no: E1242Ra) on DiaSource ELISA plate reader (RT0931117DIA) at Diagnostic Laboratory, Isra University, Hyderabad.

SPSS (Statistical Packages for Social Sciences) version 22.0 was used for data analysis. The statistical analysis of the differences of various quantitative variables between the experimental and control groups was evaluated by one way analysis of variance (ANOVA) followed by post hoc Tukey's test. P value ≤ 0.05 was taken as statistically significant.

RESULTS

Pre-experimental body weight

TABLE I: DIFFERENCE IN BODY WEIGHT BETWEEN EXPERIMENTAL GROUPS BY POST-HOC TUKEY TEST

Groups		Mean diff.	P-Value
Group A	Group B	-52.8	0.0001
	Group C	-42.1	0.0001
Group B	Group C	10.7	0.001

TABLE II: MULTIPLE COMPARISON OF SERUM UREA AND SERUM CREATININE LEVELS BETWEEN GROUPS BY POST-HOC TUKEY TEST

Parameter	Groups		Mean diff.	P-Value
Serum Urea	Group A	Group B	28.3	0.005
		Group C	10.1	0.0001
	Group B	Group C	18.2	0.0001
Serum Creatinine	Group A	Group B	0.33	0.0001
		Group C	0.04	0.502
	Group B	Group C	0.37	0.0001

TABLE III: MULTIPLE COMPARISON OF SERUM GLUTATHIONE PEROXIDASE BETWEEN GROUPS BY POST-HOC TUKEY TEST

Groups		Mean diff.	P-Value
Group A	Group B	0.59	0.0001
	Group C	0.09	0.172
Group B	Group C	0.68	0.0001

(mean±SD) of group A, B and C was 239.3±5.7 gm, 241.7±8.5 gm and 237±7.4 gm respectively. At the end of the study duration, the body weights of group A, B and C were again measured. Significant difference in mean post experimental body weight was observed in all three groups i.e. in group A there was a rise in body weight (249.30±6.27 gm) while in group B significant decline in mean body weight (196.50±9.34 gm) and in group C a similar decline was also observed (207.2±6.56 gm), however, the weight loss was not as much as seen in group B. There was a statistically significant difference (p-value <0.05) between the experimental groups (Table I).

Post experimental biochemical analysis of all three groups was performed. In group A the mean level of serum urea was 22.7±2.66 mg/dl, serum creatinine was 0.45±0.05 mg/dl and serum GPX was 1.44±0.13ng/ml. In group B the mean serum urea level was 51±3.65mg/dl, mean serum creatinine level was 0.78±0.05mg/dl and serum GPX was 0.85±0.11ng/ml. In group C the mean serum urea level was 32.8±1.45 mg/dl, mean serum

creatinine level was 0.41±0.09 and serum GPX was 1.53±0.08ng/ml. Post hoc analysis revealed that serum urea and creatinine levels were significantly high in cisplatin group (group B) compared to control (group A) and resveratrol group (group C) (Table II). Similarly, statistically significant decline in serum GPX levels in group B compared to group A and C (Table III).

Histopathological alterations in renal sections are shown in Figure 2. The distances between the visceral and parietal layers of Bowman's capsule were observed. In control group (group A), renal structure was intact and no change in renal histology was found. The mean distance between visceral and parietal layers of Bowman's capsule was 69.34±0.87µm whereas; in group B (cisplatin) marked changes in renal histology were observed. Glomerular structure shrinkage was prominent with the mean distance between the visceral and parietal layers of Bowman's capsule 216.5±1.32µm. However, resveratrol receiving (group C) displayed less glomerular damage with the mean distance between the visceral and parietal layers of Bowman's capsule

102.22±1.65µm as compared with cisplatin group (group B).

In addition, areas of peritubular fibrosis and congestion were observed in B and C group but less prominent in group-C compared with group-B.

DISCUSSION

The present study sought to investigate whether resveratrol exerted a reno-protective effect on cisplatin-induced AKI in a rat model by investigating various blood and histopathological parameters associated with renal function of nephrotoxic and treated rats. Our results revealed considerable decline in renal function as well as alterations in renal histological architecture after cisplatin administration. However, resveratrol co-treatment was associated with marked improvement of renal function causing significant reduction in serum creatinine and urea levels, increased glutathione peroxidase levels as well as improving the renal histological architecture. These findings show that resveratrol caused amelioration of nephrotoxicity produced by cisplatin administration by enhancing plasma anti-oxidant levels.

Being the waste disposal plants of the body, the kidneys are exposed to high quantities of toxic substances that make them prone to release substantial amounts of ROS. These free radicals ultimately lead to oxidative stress that has been attributed to the pathogenesis of renal damage.¹⁸ Cisplatin is known to deplete plasma level of endogenous antioxidants such as cysteine-rich proteins, glutathione and/or methionine, weakening the body's antioxidant defense machinery and ultimately leading to oxidative stress.¹⁹

As a polyphenolic phytoalexin, resveratrol has been reported to be beneficial for the prevention of numerous types of kidney diseases such as those caused by diabetes²⁰, various harmful drugs, sepsis²¹ and ischemia-reperfusion renal injuries.^{22,23} Consistent with these observations, the results of this current study show the positive effects of resveratrol in the prevention of renal damage and dysfunction as

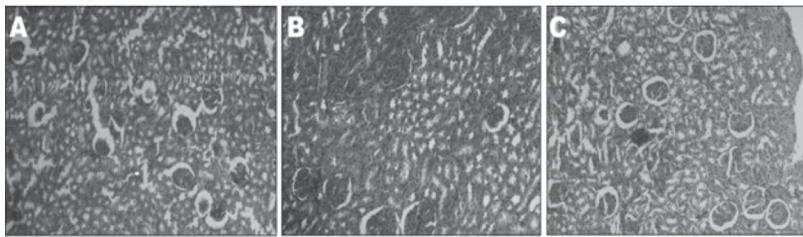


Figure 2: Evaluation of renal damage in experimental animals (100x Magnification) A - Control group showing normal glomerular and tubular architecture. B - Nephrotoxic group showing shrinkage of glomeruli, dilatation of bowmans slit and congestion. C - Resveratrol group showing near normal glomerular structure and comparatively much less apparent renal congestion.

concluded from decreased blood urea levels, decreased serum creatinine levels and increased anti-oxidant levels.

The results of this study are in accordance with the findings of various previous studies, which have proven that resveratrol is a potent anti-oxidant as well as a scavenger of ROS such as peroxides, and hydroxyl radicals.²³ Similar findings were also reported in a recent study by Kitada, et al.¹⁶ in which resveratrol was found to cause marked reduction in urinary levels of albumin, histological mesangial expansion as well as renal levels of oxidative and inflammatory markers through the scavenging of ROS and normalizing manganese superoxide dismutase (Mn-SOD) function.

Similarly, like previous studies by Osman, et al.¹⁹ and Al Dera, et al.¹⁴, resveratrol showed to have protective effects on renal histology as resveratrol group showed near normal renal architecture. In addition, Chen, et al.²⁴ reported that RSV therapy was able to alleviate symptoms of diabetic nephropathy induced by Streptozocin as it caused reduction in albuminuria, overall hypertrophy of the glomeruli as well as thickness of the glomerular basement membrane. It caused lowered transforming growth factor and nephrin expression in the renal tissues. Moreover, Wu, et al.²⁵ also confirmed that RSV has a nephro-protective effect by regulating the Sirtuin-1/Forkhead Box O (SIRT1/FOXO1) pathway causing increased expression of eNOS and leading to increase in Nitric oxide (NO) bioavailability.

With strengths, there were certain limitations in our study. There was limited time and funds availability due to

which certain laboratory tests such as urine analysis, plasma albumin levels and plasma levels of inflammatory markers could not be performed. Therefore, further studies are recommended to assess the various effects of resveratrol on other blood and urine parameters as well as on other organs and to explore the effects of resveratrol in combination with other anti-nephrotoxic agents such as L-arginine and ascorbic acid etc.

CONCLUSION

This study showed a protective effect of resveratrol against cisplatin induced renal damage. Reduction of oxidative stress may be considered as one of the main pathways of action.

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AUTHORS' CONTRIBUTIONS

Following authors have made substantial contributions to the manuscript as under:

KAM: Study design, acquisition, analysis and interpretation of data, drafting the manuscript, final approval of the version to be published

RAT, SN: Acquisition of data, drafting the manuscript, final approval of the version to be published

AAU, YMN: Analysis and interpretation of data, drafting the manuscript, final approval of the version to be published

NK: Conception, acquisition of data, critical review, final approval of the version to be published

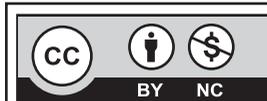
Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE

NIL



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