

# Comparative study of the renoprotective effects of captopril and aminophylline against cisplatin – induced nephrotoxicity in rats

\*Adel .H .Sheeh .

## Abstract

**Background:** Cisplatin is one of the most commonly used anti-cancer drugs , but its clinical use was limited by its nephrotoxicity .

**Methods:** In this study we try to investigate the renoprotective effect of captopril and aminophylline against cisplatin induced nephrotoxicity .For this purpose a 36 Sprague Dawley rats was divided randomly to 6 groups , each group consist of 6 rats. The first group given normal saline and act as control group, while the other 5 groups given cisplatin ( 7.5 mg/kg ) , captopril ( 60 mg/kg ) , aminophylline ( 24 mg/kg ) , captopril with cisplatin and aminophylline with cisplatin respectively. All drugs are given as single dose through intraperitoneal route. After 6 days blood urea and serum creatinine, malondialdehyde and glutathione are measured and compared with control group.

**Results:** The data show that both captopril and aminophylline posses renoprotective effect against cisplatin – induced nephrotoxicity, also the data show that captopril renoprotective effect is more than that produced by aminophylline.

**Conclusions:** This data can help in increase the dose of cisplatin in clinical uses together with use of renoprotective agent , specially if the patient already need such renoprotective drugs for treatment of disease a way from cisplatin as hypertension .Also more clinical studies required for more assessment of the clinical pattern of this renoprotective effect

**Key words:** captopril, aminophylline, cisplatin, cisplatin-induced nephrotoxicity.

*Al - Kindy Col Med J 2010 ; Vol .6 . No. (1) p*

## Introduction

Cisplatin is a potent anti-tumor agent currently used in treatment of solid malignant disease <sup>(1)</sup>, but the dose of administrated cisplatin is limited by its nephrotoxicity <sup>(2)</sup>. This nephrotoxicity which can be observed in human as well as in animals is a dose dependent <sup>(3)</sup>. Several possible pathways by which cisplatin can induce its nephrotoxicity , but it is differ from that pathway by which cisplatin kills tumor cells . <sup>(4)</sup>

The mechanisms that contribute to cisplatin -induced renal dysfunction may includes :

1-vasoconstriction in renal vasculature: through modulation of renal hemodynamics by adenosine <sup>(5)</sup>, which is a potent vasoconstrictor in the renal vasculature. <sup>(6)</sup>

2- cellular toxicity: by activation of cisplatin in the kidney to toxic metabolite through a platinum- glutathione conjugate which is further processed to cysteine conjugate that is a metabolically reactive thiol <sup>(7)</sup>. Moreover, many evidence have been accumulated that this side effect is closely related to reactive oxygen species , which cause mitochondrial damage <sup>(8)</sup> , inhibition of membranous transport proteins <sup>(9)</sup> and lipid peroxidation . <sup>(10)</sup>

The aim of this study is to investigate the renoprotective effect of captopril and aminophylline against cisplatin induced nephrotoxicity , so compare between renoprotective effect produced by captopril and aminophylline

## **Methods:**

### **Materials**

A 36 adult healthy males Sprague- Dawley rats with weight ranging between 210-250 gm. The rats were kept in cages in animal house , College of Medicine , University of AL-Qadysia, the study was conducted between 10-9-2008-to 12-12-2008 ,each cage contained 6 rats , The animals were left one week in animal house for adaptation , during this period they were fed with standard rodent chew diet and tap water.

### **Drugs :**

1- Cisplatin (cisplatyl 50, laboratoire Roger Bellon, France ) were dissolved in normal saline and given intraperitoneal in dose of 7.5 mg/kg body weight . ( 11 )

2- Captopril ( E.I.P.I.CO. Egypt ) were dissolved in normal saline and given intraperitoneal in dose of 60 mg/kg body weight. ( 12 )

3- Aminophylline ( Dar-AL-Dawa company ) given intraperitoneal in dose of 24 mg/kg body weight . ( 13 )

The animals randomly divided to 6 groups , each group contain 6 animals.

Group 1: given 0.5 ml normal saline , I.P , and act as control group.

Group 2 : given Cisplatin ( 7.5 mg/kg ) single dose , I.P.

Group 3: given captopril ( 60 mg/kg ) single dose , I.P.

Group 4 : given aminophylline ( 24 mg/kg ) single dose , I.P.

Group 5 : given captopril ( 60 mg/kg ) single dose , I.P. , 1 hour before Cisplatin ( 7.5 mg /kg ) single dose I.P.

Group 6 : given aminophylline ( 24 mg/kg ) single dose , I.P, 1 hour before Cisplatin ( 7.5 mg/kg ) single dose I.P.

For purpose of evaluation of nephrotoxicity in this study , nephrotoxicity was defined as increase in

the serum creatinine concentration by 0.5 mg/dl or more over the baseline levels , this reflected a mean drop of 56 % ( 31.4 – 80 % ) in the glomerular filtration rate ( GFR ). (14)

Before administration of drugs , blood sample ( 0.5 ml ) was withdrawn from the caudal artery for measurement blood urea and serum creatinine 6 days after giving the drugs , all animals are anesthetized with diethyl-ether and sacrificed , 0.5 ml of blood taken from the heart .

For measurement of oxidative stress done by determination of total glutathione by measurement of absorbance according to Burtis and ashwood , while malondialdehyde by thiobuteric acid according to albro et al (15) . measurement blood urea and serum creatinine which carried out by using kit method which obtained from biomerieux (16 ) and randox laboratories, . (17)

### **Statistical analysis:**

The data was expressed as a mean  $\pm$  SEM unless otherwise stated , ANOVA and pair wise comparisons done and the significant difference was accepted at 0.05 level.

### **Results :**

The results show that Cisplatin ( 7.5 mg / kg ) single dose I.P. caused significant increase bloods urea by 440 % after 6 days from treatment as compared with control group ( table 1). On the other side pretreatment of animals with captopril ( 60 mg / kg ) single dose , I.P. and aminophylline ( 24 mg / kg ) also single dose I.P. significantly reduced the elevated level of blood urea by 71.80 % , and 63.07% respectively as compared with Cisplatin – treated group, and return to near normal value especially in captopril group.

**Table 1: Effect of Cisplatin , captopril and aminophylline on blood urea in rats after single intraperitoneal dose.**

drugs	Blood urea( mg/dl )
Control (0.5 ml normal saline )	47 ± 0.57
Cisplatin ( 7.5 mg/kg )	206 ± 2.79 *
Captopril ( 60 mg / kg )	43 ± 1.21
Aminophylline ( 24 mg / kg )	44 ± 1.15
Captopril( 60 mg /kg )+ Cisplatin ( 7.5 mg / kg )	62 ± 0.73≠
Aminophylline ( 24 mg/kg )+ Cisplatin ( 7.5mg/kg )	86 ± 1.74≠

\* significantly difference from control group at  $p \leq 0.05$ .

≠ significantly difference from Cisplatin treated group at  $p \leq 0.05$ .

The results show that Cisplatin ( 7.5 mg / kg ) single dose I.P. caused significant increase serum creatinine by 637 % after 6 days from treatment as compared with control group (table 2 ), on the other side pretreatment of animals with captopril ( 60 mg / kg ) single dose , I.P. and aminophylline ( 24 mg / kg ) also single dose I.P. significantly reduced the elevated level of serum creatinine by 75.6% and 62.8 % respectively , as compared with Cisplatin – treated group, and return to

near normal value especially in captopril group.

Single dose Cisplatin I.P (7.5 mg/kg ) significantly increase serum malondialdehyde as compared with control group, (Table:3) ,which significantly reduced by addition of captopril to cisplatin.(Table:3). Glutathione level significantly reduced after administration of cisplatin ,while pretreatment with captopril show high limitation of this reduction (Table: 3).

**Table 2: Effect of Cisplatin , captopril and aminophylline on serum creatinine in rats after single intraperitoneal dose.**

drugs	Serum creatinine ( mg/dl )
Control (0.5 ml normal saline )	0.44±0.12
Cisplatin ( 7.5 mg/kg )	2.80 ± 0.63 *
Captopril ( 60 mg / kg )	0.44 ± 0.11
Aminophylline ( 24 mg / kg )	0.42 ± 0.11
Captopril( 60 mg /kg )+ Cisplatin ( 7.5 mg / kg )	0.68 ± 0.10≠
Aminophylline ( 24 mg/kg )+ Cisplatin ( 7.5mg/kg )	1.02 ± 0.07≠

\* significantly difference from control group at  $p \leq 0.05$ .

≠ significantly difference from Cisplatin treated group at  $p \leq 0.05$ .

**Table 3: The effects of some drugs on serum level of malondialdehyde( MDA ) and glutathione.**

drugs	N.S	Cis.	Cap.+Cis.	Ami+Cis.
Malondialdehyde. nmol/ml	3.44± 0.11	5.12±0.73*	3.51±0.10#	5.00±0.08
Glutathione mmol/L	22.41±0.58	15.48±0.79*	20.79±0.51#	16.85±0.54

\* significantly difference from control group at  $p \leq 0.05$ .

≠ significantly difference from Cisplatin treated group at  $p \leq 0.05$ .

### **Discussion:**

Our study show that single dose of cisplatin ( 7.5 mg / kg ) in rats resulted in nephrotoxicity and deterioration of renal function which reflected by elevation in the level of blood urea and serum creatinine, this finding are in agreement with Jones et al ( 1992 )<sup>(18)</sup>, Miyaji et al<sup>(19)</sup> ( 2001 )<sup>(20)</sup> and Behling et al ( 2006 ).

Our data revealed that blood urea and serum creatinine reduced and return to approximately near normal specially after use of captopril 1 hour before cisplatin administration , although , both blood urea and serum m creatinine also reduced in high percentage with the use of aminophylline1 hour before cisplatin , but this reduction still less than that produced by captopril ,.

As we mention before , there are several mechanisms contribute to nephrotoxicity induced by cisplatin such as vasoconstriction or cellular toxicity , regarding captopril our results are in agreement with kalia et al ( 2007 )<sup>(21)</sup> and mansour et al ( 1999 )<sup>(12)</sup> , the possible explanation that a moderate increase in the malondialdehyde (MDA ) concentration was observed in rat treated with Cisplatin alone Baliga et al ( 1998 )<sup>(9)</sup> , Bohling et al<sup>(20)</sup> ( 2006 )<sup>(22)</sup> and Cetin et al ( 2006 )

that indicate an important role of reactive oxygen species in the pathogenesis of nephrotoxicity induced by cisplatin that causing mitochondrial damage , inhibition of membranous transport protein s and lipid peroxidation. Therefore kuhlmann et al ( 1997 )<sup>(8)</sup> and matsushima et al ( 1998 )<sup>(10)</sup> mention that cisplatin induce free radical production that causing oxidative damage .

According to this point ,Weickert<sup>(23)</sup>

Jacobsen et al ( 1999 ) mention that various free radicals scavengers have been shown to be effective as renoprotective agents against cisplatin induced nephrotoxicity .captopril significantly reduce the increase of MDA concentration .this probably due to free radicals scavenging and antioxidant properties which are sulf-hydral dependent<sup>(24)</sup>

. Chopra et al ( 1992 )<sup>(25)</sup> , specially when Decavanaghet et al( 1997 ) notice that

captopril was found to increase antioxidant enzymes and non- enzymatic antioxidant defenses in mouse .

Glutathione it is the enzyme of the antioxidants defense system , which participate in regulation the lipid peroxidation ,and act as scavenger of ROS

including hydroxyl radicals , nitric oxide<sup>(26)</sup> and peroxynitrite

a decrease in the activity of this enzyme may predispose tissue to free radicals damage, our results show that captopril enhance glutathione which in agreement<sup>(27)</sup> with Decavanaghet et al (2000).

Regarding aminophylline , our data are in agreement with Heidemann et al ( 1989 )<sup>(28)</sup> and Peter Benoehr ( 2005 )<sup>(13)</sup> , and disagreement with Franzke et al ( 2000 )<sup>(29)</sup>

our possible explanation for our data is that adenosine had been proposed to exert important regulatory function in the kidney ,affecting renal blood flow , regulate filtration rate , tubular water and electrolyte transport and secretion of<sup>(30)</sup>

rennin ( Osswald et al ( 1997 ) In contrast to other vascular bed s ( e.g. brain , heart ), vessels in the kidney responded to exogenous or endogenous adenosine with vasoconstriction of the afferent arterioles . this vasoconstriction is mediated by adenosine A-1 receptors and can blocked by the non-selective adenosine receptors antagonist<sup>(31)</sup> thiophylline ,Yao ,k ,et al (1994)<sup>(32)</sup> and Winston ( 1985 ) show that in animal experimental studies xanthine derivatives ( adenosine receptors antagonist like thiophylline ) prevent or reduce the severity nephrotoxicity induced by drugs including cisplatin .<sup>(33)</sup>

In addition to that , a Baht et al ( 2002 ) studies demonstrate an up-regulation of adenosine A1- receptors in the rat kidney induced by cisplatin indicating an increased sensitivity to adenosine in case of application of cisplatin .

So, according to our data we demonstrate a renoprotective effect of aminophylline in rats exposed to nephrotoxicity induced by Cisplatin .

Lastly ,our idea from this study is to compare the renoprotective effect against Cisplatin – induced nephrotoxicity caused by prevention or reduction of cellular toxicity ( as in captopril ) with that renoprotection caused by prevention or reduction of vasoconstriction in the renal

vasculature ( as in aminophylline ) , our study show that there is significant difference in renoprotection of captopril as compared with that of aminophylline as we can say between interference with cellular toxicity as compared with interference with vasoconstriction of renal vasculature.

## **Conclusions**

Our work has shown that :

- 1-cisplatin –induced nephrotoxicity can be prevented or greatly reduced by using captopril or aminophylline
- 2- captopril renoprotection is higher than that of aminophylline. due to difference in mechanism of renoprotection .

## **Acknowledgments :**

1- this work was supported and it is a part of scientific plain for department of Pharmacology and Therapeutics , College of Medicine , University of Al-Qadysia for 2008-2009.

2- my sincere thanks to Dr. Hayder Jehaam , department of Community Medicine , College of Medicine, University of Al-Qadysia . for his help in the performance of the statistical analysis.

## **References:**

- 1-Giaccone ,G: clinical perspective on platinum resistance . Drugs : 59 [ suppl. 4 ]: 9- 17.2000
- 2- Pinzaniv , Berssolle F, Haug IJ , Galtier M, Blayac JP : Cisplatin induced renal toxicity and toxicity modulating strategies . A review cancer chemother. Pharmacol. 35 : 1-9 , 1994 .
- 3- Ward JM, Grabin ,ME, .Lerosy AF, Young DM.: modification of renal toxicity of cisdichlorodiamine- platinum (TT) with frusemide in male F344 rats . Cancer Treatment Rep. 61 :375-379. 1997.
- 4-Townsend DM, Deng M ,Zhang l, Lapus MG, and Hangian MH, :metabolism of Cisplatin to a nephrotoxic in proximal tubule cells . J. Am. Soc.. Nephrol.14 ( 1 ) : 1- 10 : 2003.

- 5- Osswald H, Habakowski ,G, Herms ,H, : adenosine as a possible mediator of metabolic control of glomerular filtration rate . *Int. J. Biochem.* 12 , 263- 267. 1980 .
- 6- Thurau , K, :renal hemodynamics . *Am. J. Med.* 36 : 689- 719. 1964.
- 7-Salahadeen A, Poovala, Parry W, Pande Roberts J: Cisplatin induced N-acetylcysteine suppressible F2- isoprostane production and injury in renal tubular epithelial cells . *J. Am .Soc. Nephrol.* 9 ( 8 ) : 1448-1455 : ( 1998 ) .
- 8- Kuhlmann MK , Burkhardt G, and Kohler H: insight in to potential cellular mechanisms of Cisplatin nephrotoxicity and their clinical application. *Nephrol. Dial. Transplant* : 12 (12) 2478- 2480. 1997.
- 9- Baliga R, Zhang , Baliga ML: in-vitro and in-vivo evidence suggesting a role for iron in Cisplatin –induced nephrotoxicity . *Kidney Int.* 53 : 394-400. 1998.
- 10- Matsushima H, Yonemura K, Ohishik and Hishida.: the role of oxygen free radicals in Cisplatin induced acute renal failure in rats . *J.Lab.Clin.Med.*131: 518-526 .1998.
- 11-Mansour MA, Hafez HF and Fahmy NM: silymarin modulates Cisplatin – induced oxidative stress and hepatotoxicity in rats. *J. Biochem. Mol. Biol.* 39 ( 6 ) : 656-661. 2006.
- 12- Mansour MA, EL-Kashef HA, and AL-Shabana OA,; effect of captopril on doxorubicin – induced nephrotoxicity in normal rats .*Pharmacol.Res.*39 ( 3 ) :233-237. 1999.
- 13- H. TH .Heidemann , St .Muller,L.Mertins G .Stepan ,K, Hoffmann and the late E.E. Ohnhaus : effect of aminophylline on Cisplatin nephrotoxicity in rat. *Br. J. Pharmacol.* 97,313-318. 1989.
- 14-Kayser,Caglar,CanKinalp,Mustafa Turan :Cumulative prior dose of Cisplatin as a cause of nephrotoxicity of high -dose chemotherapy fallowed by autologus stem cell transplantation. *Nephrol. Dial. Transplant* 17 : 1931-1935 . 2002 .
- 15-AlbropW, Corbelt JT et al : application of thiobarbiturate assay to measurement of lipid products. *Chem. .Biol .Interact.* 86:185-194. 1986.
- 16-Barham and Trinder .*analyst.*:97-142. 1972
- 17-Randox laboratories Ltd., antrum. BT 29,4QY,UK,manual procedures 4<sup>th</sup> edition .:89-126. 1996
- 18- Jones MM, Basinger MA and Hoslcher MA : control of nephrotoxicity of Cisplatin by clinically used sulfur-containing compounds . *Fundam. Appl.Toxicol.*18 : 181-188. . 1992.
- 19- Miyaji T, Kato, A, Yasuda H, Fujigaki Y, and Hishida A, ) : role of the increase in P21 in Cisplatin –induced acute renal failure in rats .*J. Am. Soc. Nephrol,* 12 : 900-908. 2001.
- 20- Behling EB Sendao. MC. Francescato HDC, Antunes LMG, Costa RS and Bianchi MP : comparative study of multiple dosages of quercetin against Cisplatin – induced nephrotoxicity and oxidative stress in rat kidney . *Pharmacol. Rep.* 58 : 526- 532. 2006.
- 21- Kalia K, Narula GD, Kannan GM, and Flora SJ. : effects of combined administration of captopril and DMSA on arsenite induced oxidative stress and blood and tissue arsenic concentration in rats . *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 144 ( 4 ) : 372 – 379. 2007.
- 22-Cetin R, devrim E, Kilicogula ,B, Avci A ,Candir O and Durak I. : Cisplatin impairs antioxidant system and causes oxidation in rat kidney tissues: possible protective roles of natural antioxidant foods .*J. Appl. Toxicol.* , 26 ( 1 ) :42-46. 2006.
- 23- Weichert –Jacobsen KJ ,Bannowski A, Kupperts F, Loch T, and Stockle M, : direct amifostine effect on renal tubule cells in rats . *Cancer Res.* 59: 3451- 3453. 1999 .
- 24- Chopra, M, Beswick H, Clapperton M, Dargie HJ, Smith. WE, and McMurray J : antioxidant effect of angiotensin - converting (ACE) inhibitors : free radicals and antioxidant scavenging are sulf-hydral dependent but lipid peroxidation is inhibited by both sulf-hydral and non-sulf-hydral containing ACE-inhibitors . *J. Cardiovasc. Pharmacol.* 19: 330- 340. 1992.
- 25- deCavanagh EM, Fraga CG, Freder L, and Inserra F, : Enalapril and captopril enhancing antioxidant defense in mouse tissues . *Am. J. Physiol. Regul. Integr. Comp. Physiol.* , 272 : R514-R518.

- 26-Halliwell B, and Gutteridge JM(1989): protection against oxidant in biological system. the super- oxide theory .of oxygen toxicity. free radicals in biology and medicine (2nd ed.),oxford , U.K .PP87-187. 1997.
- 27- DeCavanagh EM, Inserra, : Enalapril and Captopril enhance glutathione dependent antioxidant defense in mouse .Am. J .Physiol .Regul .Integ .Comp. physiol.. 278: R572-R577. 2000.
- 28- Peter Benoehr , Patricia Krueth , Carsten Bokemeyer, Almut Grenz , Hartmut Osswald and jorg T, Hartmann : nephroprotection by thiophylline in patient with Cisplatin chemotherapy : A randomized , single –blinded , placebo – controlled trial .J. Am. Soc. Nephrol. 16:452-458. 2005.
- 29- Franzke A, Knop, s, Renn M, Maess. et al : comparative study of the acute nephrotoxicity from standard dose Cisplatin+/\_ ifosfamide and high dose chemotherapy with carboplatin and ifosfamide .Anticancer Res.20 : 3767-3773. 2000.
- 30- Osswald H, Habakowski G, et AL : Adenosine and tubuloglomerular feedback .Blood Purif. 15 :243-252. 1997.
- 31- Yao, K, Kusaka, H, Sano,J, Sato K.Karasawa A :diuretic effects of KW-3902, a novel adenosine A1- receptors antagonist in various models of acute renal failure in rats . JPN. Pharmacol. 64: 281-288 , 1994.
- 32- Winston JA, Safirstein R: Reduced renal blood flow in early Cisplatin – induced acute renal failure in the rat. Am. J. Physiol. 249:F490-F496, 1985.
- 33- Bhat SG, Mishra S, MeiY, NIE , Z, Whitworyth CA. Rybak LP. Ramkumar V: Cisplatin up-regulates the adenosine A1- receptors in the rat kidney . Euro. J. Pharmacol. 442: 251- 264 . 2002.