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Comparison between the Effect of Melatonin and Zinc sulfate in Prevention of cisplatin induced Nephrotoxicity in Rats

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الخلاصة:

السيسبلاتين هو علاج كيمياوي فعال يستخدم في معالجة طيف واسع من الاورام السرطانية لكن المشكلة في استخدامه تكمن في أن كمية الدواء الضرورية لإحداث تأثير علاجي عادة تؤدي الى حدوث تلف كلوي.

أجريت هذه الدراسة على الجرذان للمقارنة بين دور الميلاتونين وسلفات الزنك في حماية الكلى من التلف الذي يسببه دواء السيسبلاتين. تم استخدام 40 جرذ تتراوح أوزانهم بين (200–250غم) وتم تقسيمهم بالتساوي إلى أربع مجاميع: المجموعة الأولى أعطيت النورمال سلاين عن طريق البريتون بجرعة 0.2 مليلتر، المجموعة الثانية أعطيت السسبلاتين (كجرعة واحدة بمقدار 12 ملغم/كغم) عن طريق البريتون، المجموعة الثالثة أعطيت الميلاتونين حيث حقنت الجرذان بالميلاتونين قبل اعطاء السيسبلاتين بيوم واحد بجرعة (15 ملغم/كغم) عن طريق البريتون واستمر اعطاؤه لمدة ثلاث أيام، اما المجموعة الرابعة فقد حقنت الجرذان بسلفات الزنك قبل اعطاء السيسبلاتين بيوم واحد بجرعة (50 ملغم/كغم) عن طريق البريتون واستمر اعطاؤه لمدة ثلاث ايام ايضا.

بينت النتائج ان اعطاء السيسبلاتين نتج عنه زيادة ملحوظة في مستوى الكرياتنين واليوريا والمالونداياالديهايد وحصول نقصان ملحوظ في مستوى الكلوتاثيون، وقد اظهرت النتائج وجود فرق ملحوظ بين سلفات الزنك والميلاتونين في تقليل مستوى الكرياتنين واليوريا وعدم وجود اي فرق بينهما في تقليل مستوى المالونداياالديهايد وزيادة مستوى الكلوتاثيون في المصل، كما ان المقاطع النسيجية لكلى الجرذان اظهرت تلف نسيجي ناتج عن اعطاء السيسبلاتين، فضلاً عن ان اعطاء الميلاتونين قلل التلف الكلوي الذي يسببه السيسبلاتين بشكل ملحوظ واكبر من الحماية الناتجة عن سلفات الزنك.

يمكن الاستنتاج ان كل من الميلاتونين وسلفات الزنك ضمن الجرع المستخدمة لهما دور في حماية الكلى من التلف الذي يسسببه السيسبلاتين. وبالنسبة للحماية من التغيرات النسيجية التي يسببها السيسبلاتين فقد وجد ان الميلاتونين أكثر فعالية في حماية الكلى من سلفات الزنك.

Abstract

Cisplatin is an inorganic complex; the therapeutic effects of it are significantly improved by dose escalation. However high-dose therapy with cisplatin is limited by its cumulative nephrotoxicity and neurotoxicity.

The aim of study was to compare between the effect of melatonin and zinc sulfate in prevention of cisplatin induced-nephrotoxicity in rats.

Forty male albino rats weighing (200-250gm) were equally divided into four groups: normal group (0.2 ml isotonic saline group), control group "cisplatin group" (12 mg/kg cisplatin, single dose I.P.), melatonin group (15 mg/kg I.P.) prophylactically one day prior to cisplatin administration and continued for further 3 days, and zinc sulfate group (50 mg/ kg I.P.) prophylactically one day prior to cisplatin administration and continued for further 3 days.

There was significant difference ($p<0.05$) between zinc sulfate and melatonin in reducing serum creatinine and BUN (melatonin more effective in reducing these parameters), and there was no significant difference ($p>0.05$) between them in reducing serum malondialdhyde (MDA) and increasing serum glutathione (GSH).

As a conclusion, melatonin (15 mg/ kg I.P.) and zinc sulfate (50 mg/ kg I.P.) had protective effects against cisplatin- induced nephrotoxicity in rats. Melatonin was found to be more effective than zinc sulfate in its ability to reverse the abnormal renal function and preventing histopathological changes.

Key words: cisplatin, melatonin, zinc sulfate, nephrotoxicity

Introduction:

Cisplatin is a platinum anticancer drug approved by FDA in 1978 [1]. It is the queen of chemotherapy with applications in more than 50% of human cancers [2, 3]. It is a heavy metal compound [4] with therapeutic effects that significantly improved by dose escalation. However, high-dose therapy with cisplatin is limited by its cumulative nephrotoxicity and neurotoxicity [3]. It gets accumulated in the tubular epithelial cells of proximal kidney tubule, causing nephrotoxicity [5]. Cisplatin causes tubular injury through multiple mechanisms, including hypoxia, oxidative stress, inflammation, and apoptosis [6], production of tumor necrosis factor-α (TNF-α) by renal parenchymal cells, and generation of reactive oxygen species (ROS) [7]. However, the generation of free oxygen radicals in tubular cells has been proposed as an important pathogenic process [8]. Melatonin, a chief secretary product of the pineal gland. It plays a crucial role in regulating circadian rhythm and it is involved in immunomodulation, hematopoiesis, and antioxidative processes [9]. Melatonin antioxidant activity may be related to its ability to scavenge reactive oxygen species including hydroxyl, superoxide anion, peroxyl radicals, singlet oxygen, nitric oxide, peroxynitrite anion and hypochlorous acid[10] and its ability to stimulate antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase [11]. Zinc is an essential biological trace element [12]. Over 300 enzymes have been shown to contain zinc. Another large group of zinc containing proteins is transcription factors, many of which contain zinc fingers and similar structural

motives[13]. It is an essential nutrient required for the maintenance of cell growth, immune maturation, and reproduction, and is known to function as an antioxidant via induction of metallothioneins [14].

Materials and Methods:

Forty male rats weighing (200-250 gm) were selected. The animals were housed in the animal house of Institute of Embryo Researches and Infertility Treatment/ Al-Nahrain University under condition of controlled temperature, allowed free access to water and food. The rats were equally divided into four groups (10 rats/ group) as follow: Normal group (isotonic saline group) :rats were received 0.2 ml of isotonic saline intraperitoneally (I.P.) for four days and sacrificed one day after.

Control group (cisplatin- induced nephrotoxicity group): rats were received 0.2 ml of isotonic saline (I.P.) one day prior to cisplatin administration (single I.P. injection of 12 mg/kg of cisplatin) and continued for further three days.

Melatonin group: rats received melatonin (15 mg/ kg I.P.) prophylactically one day prior to cisplatin administration and continued for further three days.

Zinc sulfate group: rats received zinc sulfate (50 mg/ kg I.P.) prophylactically one day prior to cisplatin administration and continued for further three days. Then rats were sacrificed after 3 days of cisplatin administration in the last three groups mentioned above. Levels of serum creatinine [15], blood urea nitrogen (BUN) [16], serum glutathione (GSH) [17] and serum Malondialdehyde (MDA) [18] were measured, and histopathological study was achieved by staining with hematoxylin / eosin stain and examining under light microscope [19].

In this study, the obtained data were presented as mean ± standard error of mean (SEM). Student's (unpaired) t-test for independent data was used to test the significance of difference between the results of any two groups [20]. *P*-value of less than 0.05 was considered significant.

Results

Cisplatin administration resulted in a significant increase in serum creatinine ($P=1.923\times10^{-7}$) (Fig.1), BUN ($P=3.9\times10^{-9}$) (Fig.2) and serum MDA ($P=0.000339$) (Fig.3), while serum glutathione level significantly decreased ($P=5.314\times10^{-11}$) (Fig.4) when compared to that of normal group.

Section of the kidneys of rats treated by cisplatin showing degeneration and necrosis of the proximal and distal convoluted tubules (Fig.6) when compared with normal group (Fig.5).

Melatonin administration resulted in a significant decrease in serum creatinine ($p=3.29\times10^{-6}$**)** (Fig.1), BUN ($p=6.9\times10^{-8}$) (Fig.2) and serum MDA ($p=0.00015$) (Fig.), while serum glutathione level was significantly increased ($p=2.49\times10^{-9}$) (Fig.4) when compared to control group.

There were so minimal degenerative changes in kidney tissues of rats treated with melatonin (Fig.7) compared to that of control group (Fig.6). Zinc sulfate

administration resulted in a significant decrease in serum creatinine (p=0.02) (Fig.1), BUN (p=0.018) (Fig.2) and serum MDA (p=0.000158) (Fig.3), while serum glutathione level was significantly increased (p=4.63×10^{-6}) (Fig.4) compared to that of control group and there was a mild protective effect of zinc sulfate on kidney tissues (Fig.8).

There was a significant difference between the effect of zinc sulfate and melatonin (P=0.0098) on serum creatinine level [the reduction of this parameter level in melatonin group was higher than with zinc sulfate group (Fig.1)]. In addition**,** there was a significant difference between the effect of zinc sulfate and melatonin (P=0.00143) on BUN level [the reduction of this parameter level in melatonin group was higher than with zinc sulfate group (Fig.2)]. Regarding oxidative stress status, there were no significant difference between the effect of melatonin and zinc sulfate on serum MDA levels, which decreased and became comparable to that of normal group (Fig.3). In addition, there were no significant difference between the effect of melatonin and zinc sulfate on serum GSH levels, which elevated and became comparable to that of normal group (Fig.4).

Histopathological study showed that melatonin was more effective in protection against the damage of kidney tissues induced by cisplatin, represented by less degeneration and necrosis of the proximal and distal convoluted tubules (Figures 7, 8).

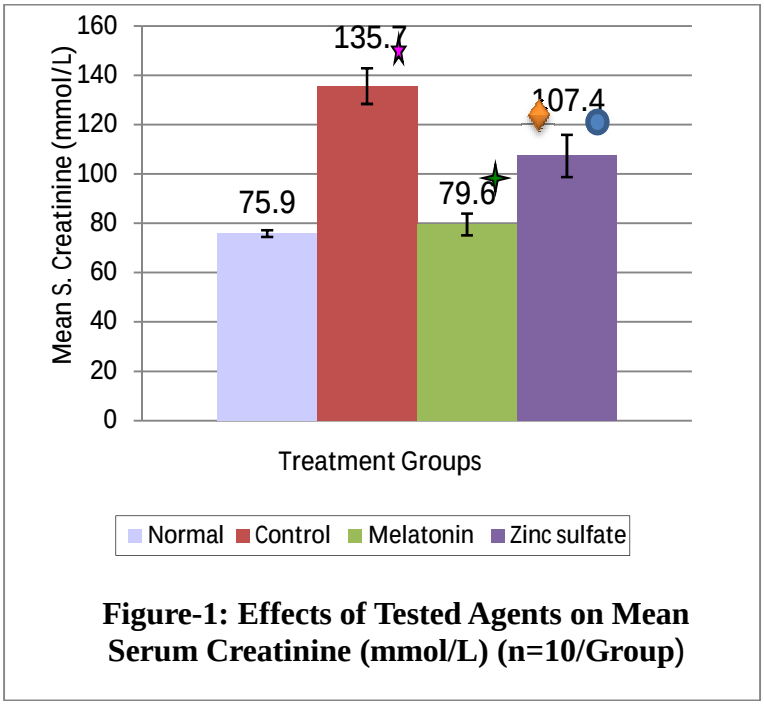

Figure-1: Effects of Tested Agents on Mean Serum Creatinine (mmol/L) (n=10/Group)

= Significant difference (P =1.923× 10^{-7}) when compared to corresponding value in normal group

=Significant difference (p =3.29× 10^{-6}) when compared to corresponding value in control group

= Significant difference (p = 0.02) when compared to corresponding value in control group

=Significant difference (p = 0.0098) when compared to corresponding value in melatonin group

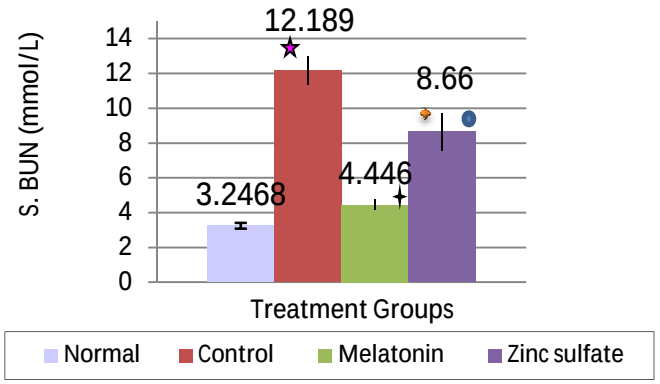

Figure -2: Effects of Tested Agents on Blood Urea Nitrogen BUN (mmol/L) (n= 10/Group)

=Significant difference (P =3.9× 10^{-9}) when compared to corresponding value in normal group.

= Significant difference (p =6.9× 10^{-8}) when compared to corresponding value in control group.

= Significant difference (p = 0.018) when compared to corresponding value in control group.

= Significant difference (p = 0.001) when compared to corresponding value in melatonin group.

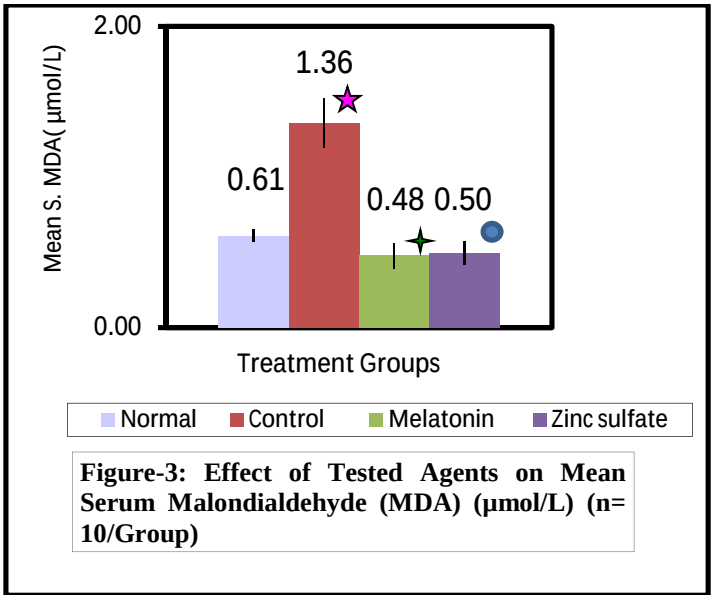

Figure-3: Effect of Tested Agents on Mean Serum Malondialdehyde (MDA) (µmol/L) (n= 10/Group)

★ = Significant difference (P =0.000339) when compared to corresponding value in normal group.

✦ =Significant difference (p = 0.00015) when compared to corresponding value in control group.

● = Significant difference (p = 0.000158) when compared to corresponding value in control group.

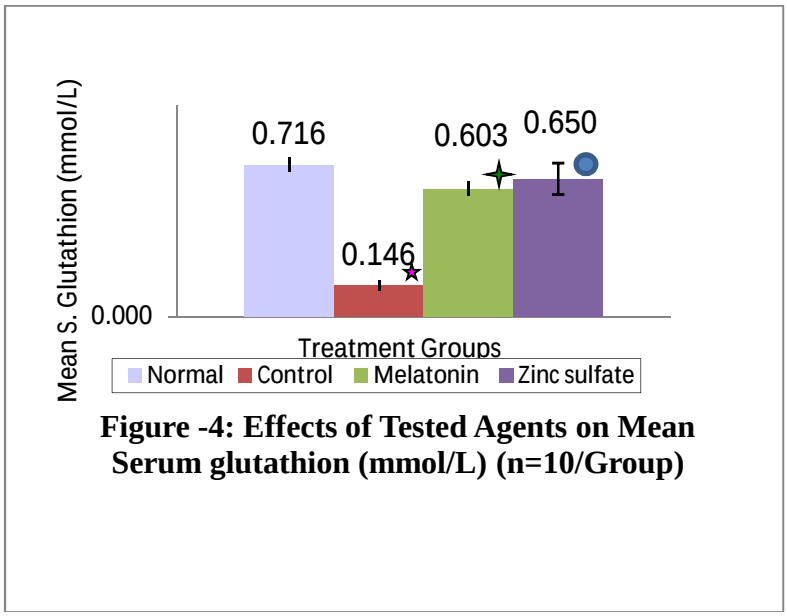

Figure -4: Effects of Tested Agents on Mean Serum glutathion (mmol/L) (n=10/Group)

=Significant difference (p =5.314× 10^{-11}) when compared to corresponding value in normal group.

= Significant difference (p = 2.49× 10^{-9}) when compared to corresponding value in control group.

= Significant difference (p = 4.6× 10^{-6}) when compared to corresponding value in control group.

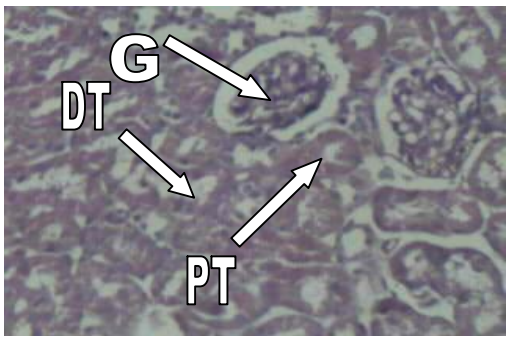

Figure-5: Normal appearance of glomeruli, proximal and distal convoluted tubules of rat's kidney.
(Magnification: 200 X, staining; haematoxylline and eosin)
G: glomerulus, PT: proximal tubule, DT: distal tubule

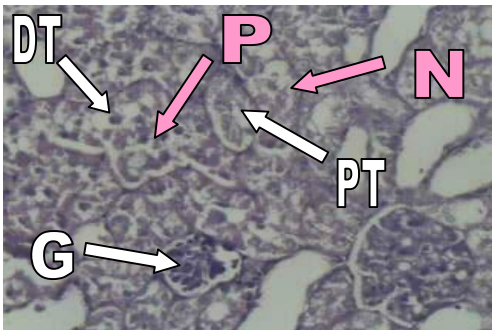

Figure-6: Nephrotoxic effect of cisplatin on rat's kidney.
(Magnification: 200 X, staining; haematoxylline and eosin)
G: glomerulus, PT: proximal tubule, DT: distal tubule N: Necrosis, P: Pyknosis

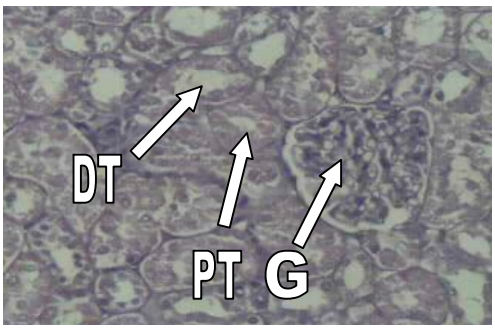

Figure-7: Protective effect of melatonin on cisplatin-induced nephrotoxicity in rat's kidney
(Magnification: 200 X, staining; haematoxylline and eosin)
G: glomerulus, PT: proximal tubule, DT: distal tubule

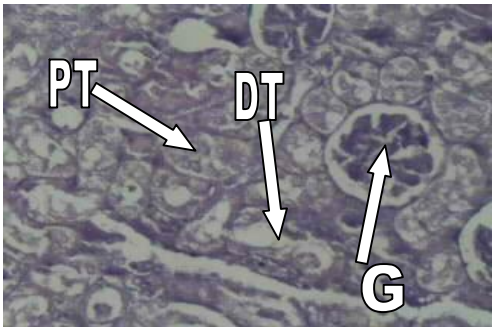

Figure-8: Protective effect of zinc sulfate on cisplatin-induced nephrotoxicity in rat's kidney
(Magnification: 200 X, staining; haematoxylline and eosin)
G: glomerulus, PT: proximal tubule, DT: distal tubule

Discussion:

Nephrotoxicity is a major complication and dose- limiting factor for cisplatin therapy and the usefulness of cisplatin is limited by its toxicity to normal tissues, including cells of the kidney proximal tubules [21]. It has been reported that cisplatin induced-nephrotoxicity was closely associated with an increase in lipid peroxidation in the kidney. In addition, cisplatin has been found to lower the activities of antioxidant enzymes and to induce depletion of GSH levels [5]. Recent

studies have shown increasing tissue contents of inflammatory mediators together with inflammatory cell infiltration, suggesting that inflammation plays an important role in cisplatin-induced renal injury. Marked attenuation of cisplatin-induced renal damage by inhibition of TNF-α indicates that TNF-α has a central role in mediating cisplatin-induced inflammatory renal injury [22].

In the present study, using an experimental model of cisplatin-induced nephrotoxicity in rats (single dose of 12 mg/kg I.P.) was characterized by alterations in renal function as a significant increase in serum creatinine and BUN levels compared to normal group (fig.1,2), and these results were compatible with those observed by many others[5,8,22]. Cisplatin in this study cause an elevation in MDA levels with glutathione depletion (Fig. 3, 4), which indicate severe kidney damage. Histological results of this study indicated that severe degenerations at the proximal tubular cells (Fig.6) could be correlated with the harmful effects of cisplatin parallel to high MDA and low GSH levels. The increase in the thickness of the glomeruli basement membrane could be a result of membrane disturbance due to cisplatin administration. Lipid peroxidation mediated by oxygen free radicals causes destruction and damage to cell membranes [23].

Melatonin (15 mg/kg I.P.) administration resulted in significant reduction of serum creatinine and BUN levels compared to control group (Fig.1, 2). There was significant reduction in serum MDA levels and significant elevation in serum GSH levels compared to that of control group (Fig.3, 4). Since it is known that free radicals are responsible for the lipid peroxidation caused by cisplatin, the inhibition of this effect by melatonin may be attributed to the free radical scavenging ability [24]. The histological damages caused by cisplatin had been reduced, represented by very mild degenerative changes of the proximal and distal tubule (Fig.7). This could be related to the fact that melatonin is an efficient free radical scavenger and antioxidant [25, 26]. The production of nitric oxide (NO•) is one of the mechanisms by which cisplatin causes tubular injury [7]. In 1998, Gilad *et al.* [27] reported that melatonin inhibits nitric oxide production from the inducible isoform of nitric oxide synthase (iNOS) and mentioned that melatonin exerts protective effects in septic and hemorrhagic shock and during inflammation. Oxidative stress resulted from cisplatin administration lead to activation of nuclear factor kappa B (NF-kB), which can promotes the production of TNF-α [28]. Melatonin inhibits NF-kB activation and reduces the levels of TNF-α [27,29].

Zinc sulfate (50 mg/kg I.P.) administration resulted in significant reduction of serum creatinine and BUN levels compared to control group (Fig.1, 2). There was significant reduction in serum MDA levels and significant elevation in serum GSH levels compared to that of control group (Fig.3, 4). Zinc has anti-inflammatory actions [30] and Molle *et al.* in 2007 reported the protective effect of zinc sulfate against TNF -induced lethal inflammation and mentioned that zinc has dose-responsive protection against TNF-induced hypothermia, systemic induction of interleukin-6, as well as against TNF-induced bowel cell death. [31]. Du and Yang [32] in 1994 found that zinc ameliorated gentamicin nephrotoxicity via scavenging reactive oxygen metabolites by induction of metallothionien synthesis. Many

studies have shown that metallothionein has antioxidant effects under various conditions of oxidative stress, including radiation exposure, toxicity of anticancer agents and other various chemicals [23,30]. Other study of the effect of zinc sulfate on intestinal injury in ethanol-administered rats showed that pretreatment with zinc sulfate caused decrease in histological damage, serum creatinine, BUN and lipid peroxidation level, but increase the glutathione contents [23], and these results were compatible with those of the present study. Comparing to zinc sulfate group, melatonin was more effective in lowering serum creatinine and BUN levels (Fig.1,2) at the same time, the effect of zinc sulfate in lowering MDA levels and elevating GSH levels was comparable to that of melatonin (Fig.3,4).The histopathological studies showed that melatonin had more protective effects on tissues of kidney rat's than that of zinc sulfate (Fig.7,8)

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