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Protective Effects of Exogenous Melatonin on Cisplatin- Induced Acute Nephrotoxicity in Rats

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Abstract

This study was planned to investigate the consequences of exogenous administration of melatonin on the amelioration of some biochemical variables in cisplatin (CP)-induced acute nephrotoxicity in rats. Twenty-four male rats weighing 300-350g were used. Housing of the animals and the experiment were achieved and the rats were -housed in the Department of Biology /College of Science/ Sulaimani University. The Animals were divided into three groups (n=5); control group (receiving prepared standard chow for rats and water ad libitum); model group(The animals administered a single intraperitoneal (i.p) dose of CP (8 mg/kg BW.); and the third group in addition to CP injection the animals were supplemented with melatonin (180 mg/kg food) at the same day of CP-injection for five days. Finally, the blood specimen was taken using cardiac puncture. The studied parameters include Malondialdehyde (MDA), serum creatinine, serum urea, serum total bilirubin, serum albumin, serum electrolytes (Ca+2, Na+ and K+), as well as some hematological parameters (WBC, RBC, HCT, HGB). Our results showed significant ($P<0.05$) elevation in the serum level of MDA,, creatinine, urea and total bilirubin in model group. On the other hand, the WBC count was also increased significantly ($P<0.01$), whereas there was no significant alterations in the level of calcium, sodium, and potassium. The outcome of our study indicated that the melatonin administration in injected rats for five days significantly ($P<0.01$) improved the elevated serum levels of MDA, creatinine, urea, total bilirubin and WBC count. In conclusion, this study confirmed that melatonin has protective effects of ameliorating the nephrotoxicity induced by cisplatin in rat.

Introduction

Cisplatin or cis-Diaminedichloroplatinum (CP) is a platinum complex, which is composed of a platinum molecule in the center surrounded by a chlorine and ammonia molecules in the cis situation. This complex is applied in the medical care as an antineoplastic agent with an extensive therapeutic potentiality, especially against solid tumors [1]. Unfortunately, the powerful cisplatin is related to many adverse pharmaceutical reactions, such as renal failure, gastrointestinal dysfunction, and nephrotoxicity, which is considered as a major side effect and a dose-dependent effect of CP treatment [2].

The essential target of CP is the proximal and distal tubules of the kidney, where it gather and stimulate the promotion of tissue damage by integrating numerous actions including distraction of DNA, apoptosis (programmed cell death), and inflammation [3, 4]. The mode of action of CP is still not fully understood, but it is thought to depend on hydrolytic reactions that believed to be the active radical [5-7]. The free radicals formation effects of CP are perhaps due to the reduction of the enzymatic or non-enzymatic antioxidant mechanisms [3].

Melatonin is considered as a major secretory product of the pineal body, originated from tryptophan, predominantly the serum level of this hormone rises and increases at night and darkness, and the continuation of high melatonin concentration is proportional to the darkness duration [8]. It is crucial for the regulation and management of circadian rhythm and seasonal change in a contrasting aspect of physiology

andandand neuroendocrine purpose [8-9]. Melatonin was found to be a straight scavenger for free radicals in 1993 (ref). Since then,, many investigations have confirmed the protective effects of melatonin in various models of oxidative stress [8, 10, 11]. This ability for quickly scavenging hydroxyl radicals has encouraged several studies about radical detoxification and antioxidative protection [10, 12].

Beside its capability to directly neutralizean amount of free radicals and ROS, melatonin induces a number of antioxidant enzymes [13], including; glucose-6-phosphate dehydrogenase (G6PD) superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRd) [10, 14]. Melatonin, as an electron-rich molecule, may interact with free radicals via an additive reaction to form several stable end-products which are excreted in the urine. From this point of view, it can be considered as a terminal antioxidant [47].

Several investigations have shown that melatonin prevents renal tubular injury and kidney failure by its antioxidative and free radical scavenging activities [15]. Accordingly, the current study was planned to determine the protective role of melatonin on some biochemical parameters of experimentally cisplatin-induced acute nephrotoxicity in rats.

Material and Methods

Animals and Housing

Twenty-four male rats weighing 300-350g were acclimated for one week (we depend on males to keep away from the hormonal and physiological modifications related to the estrous cycle of females) [16]. The experiment was performed in Biology Department / Faculty of Science and Science Education / University of Sulaimani. Animals were housed in plastic cages (n=5), bedded with wooden chips. During the experimental period, climate controlled conditions were maintained and the temperature was set as 23±2 ℃, and 12:12 light/dark photoperiod (LD) was followed using an automated light-switching devise [17]. The animals had ad libitum access to water and diet.

A. Study Design

The animals were allocated into three groups randomly, and the work was started with eight animals. Unfortunately, some of these animals died during the experiment, probably due to the effect of the CP or any other factor, thus, they were reduced to five animals for each group as follows:

Group 1: Control: Rats of this group (n = 5) were receiving prepared standard chow for rats and water *ad libitum*

Group 2: cisplatin(CP)-induced acute nephrotoxicity (Model): Intraperitoneal (i.p.) injection of CP (8 mg/kg BW.) in one dose [18]. Cisplatin was from Vitane pharmaceutical inc., USA.

Group 3: A combination between CP Induced-acute nephrotoxicity (model) a Melatonin.

The rats were injected with CP (8 mg/kg BW .i.p.) then melatonin (Merck gènèriques, France) was supplemented (180 mg/kg food) to the standard rat chow which started at the same day of cisplatin injection for 5 days. At the end of the experiments, all rats were fasted overnight and the blood specimen was taken using cardiac puncture, then centrifugation (3000 rpm for 15 min at + 4 C°) followed by serum separation.

B. Biochemical Parameters measurement:

Some biochemical parameters were determined including Malondialdehyde measured by spectrophotometer with a thiobarbituric acid (TBA) solution:

We used 150µl of serum sample to 1ml trichloroacetic acid (TCA) 17.5 % and 1ml of 0.66 % TBA), then blended. The mixture was then blended well by vortex, incubated invortexing, incubated in a water bath (boiled) for 15 minutes and then cooled Later, we added 1ml of 70 % TCA, and the mixture was left to stand at 25 C° for 20 minutes. after that, the mixture was centrifuged at 2000 rpm for 15 minutes to separate the supernatant was taken out for final step of reading by a spectrophotometer [19].

The concentration of MDA was calculated by this equation:

MDA (µmol/L) = Absorbance at 532 nm x D/L x $E_{\circ}$

Where

L: light bath (1 cm)

$E_{\circ}$: Extinction coefficient 1.56 x 10^5 $M^{-1.}$ Cm^{-1}

D: Dilution factor = 1 ml Vol. used in ref./0.15 = 6.7

The serum creatinine, urea, total bilirubin, and albumin were determined by using standard kits for each test by Automatic biochemistry analyzer (KENZA 240 TX, BIOLABO; France). The serum calcium (Ca^{+2}) was determined by a colorimetric method using RX daytona™ automated clinical chemistry analyzer. The sodium (Na^{+}) and potassium (K^{+}) were determined by means of an electrolyte analyzer (i-Smart 30 electrolyte analyzer/ i-SENS, Korea), using). Weused 60 µl of sample for measuring automatically. Finally, some hematological parameters like WBC, RBC, HCT, and HGB were determined.

C. Statistical Analysis

The data analysis was achieved by using SPSS (Version 18.0), and the results were expressed as mean ± standard error (mean ± SE). The multiple comparisons between the means were determined by Duncan's test after using analysis of variance (ANOVA).

Results

Our results revealed that the serum MDA in CP-injected rats (3.88±0.072 µmol/L) was elevated significantly (*P<0.05)* compared to the control group (1.753± 0.067 µmol/L). The administration of melatonin significantly (*P<0.05)* prevented this increasing (2.676 ±0.097 µmol/L) (Figure: 1).

The rats with CP-induced nephrotoxicity were associated with notable rise (*P<0.01*) in the concentration of creatinine (12.020 ± 0.719 mg/dl) when compared to the control group (0.822 ± 0.040 mg/dl). Melatonin supplementation of the CP-injected rats resulted in the significant (*P<0.01*) decrease in serum creatinine level (0.748 ± 0.047mg/dl) (Figure: 2). The same results were also obtained for the level of serum urea, in which CP injection resulted in a significant increase (*P<0.01*) in the level of serum urea (577.760 ± 40.716 mg/dl) when compared to the control group (41.120 ± 2.621mg/dl), while melatonin treatment caused a significant (*P<0.01*) decrease in serum urea level (57.700 ± 7.579 mg/dl) (Figure: 3).

The model group revealed that the total bilirubin level (0.706 ± 0.024 mg/dl) was significantly higher (*P<0.01*) in comparison with the control group (0.390 ± 0.023 mg/dl). Melatonin supplementation significantly (*P<0.01*) lowered the level of serum total bilirubin (0.284 ± 0.032 mg/dl). (Figure: 4).

The rats with induced nephrotoxicity were associated with a significant decrease (*P<0.01)* in serum albumin level (2.952 ± 0.091 mg/dl) in comparison with the control group (3.604 ± 0.102 mg/dl). Melatonin co-treatment with cisplatin-injection significantly (*P<0.01*) upgraded the serum albumin level (3.186 ± 0.034 mg/dl) similar to the control group (Figure: 5).

According to our results (Figure: 6), calcium level didn't show any significant changes (*P>0.01*) neither in nephrotoxic group (8.862 ± 0.522 mg/dl), nor in CP + melatonin group (8.150 ± 0.542 mg/dl) equivalent to that in the control group (8.646 ± 0.462 mg/dl). Also, there was no significant change in the serum sodium level (77.400 ±7.724 mEq/L) when compared to the control group (70.400 ± 5.372 mEq/L). The administration of melatonin with CP significantly (*P<0.01*) induced the increase of serum sodium level (131.800 ±1.356 mEq/L) when compared to the control group (Figure: 7).

In rats with induced-acute nephrotoxicity, the value of serum potassium exhibited non-significant (*P>0.01*) alteration (3.320±0.314 mEq/L) when compared with control group (2.860±0.359 mEq/L). Further significant (*P<0.01*) increase in serum potassium level was obtained after melatonin treatment (4.640±0.414 mEq/L) compared to the control group (Figure: 8).

Regarding the hematological analysis, in CP-injected rats the level of RBC counting (4.59±0.29* 10^{12}/L), HCT% (26.18±0.97) and Hemoglobin concentration (HB) (8.54±1.2 g/dL) were dropped statistically (P<0.05) when compared with the control group(7.68±0.23* 10^{12}/L), (41.08±1.01) and (13.86± 0.21 g/dL), respectively. However, melatonin supplementation didn't seem to decrease their levels (4.98± 0.75* 10^{12}/L), (29.13±0.78) and (9.35±0.49 g/dL) respectively (Figures: 9, 10, and 11).

On the other hand, the WBC counts significantly (P<0.05) elevated in the induced nephrotoxicity group (6.62±0.12*109/L) when compared with the control group (3.8±0.31*109/l),L) and the melatonin co-treatment with CP (5.33±0.24*109/l) significantly (P<0.05) improved the WBC elevation (Figure: 12).

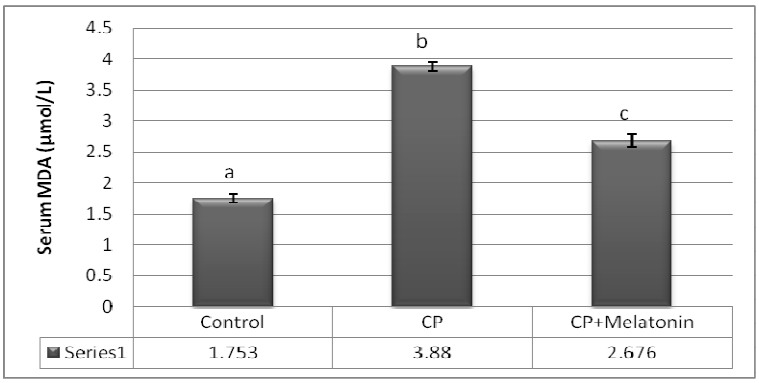

Figure 1: Effects of melatonin on serum MDA in CP-injected rats (*P<0.05)*. The different letters indicate significant differences.

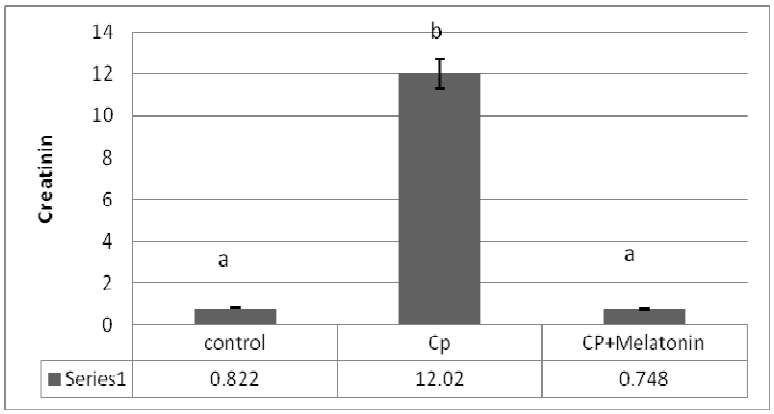

Figure 2: Effects of melatonin on serum Creatinin (mg/dl) in CP-injected rats (*P<0.01)*. The different letters indicate significant differences.

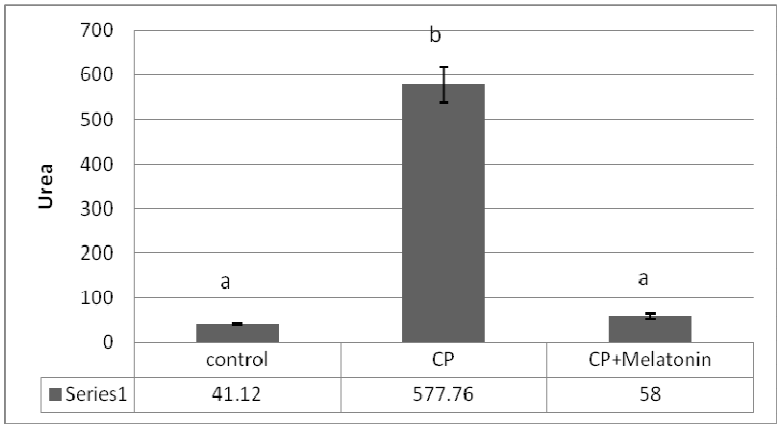

Figure 3: Effects of melatonin on serum Urea (mg/dl) in CP-injected rats (*P<0.01)*. The different letters indicate significant differences.

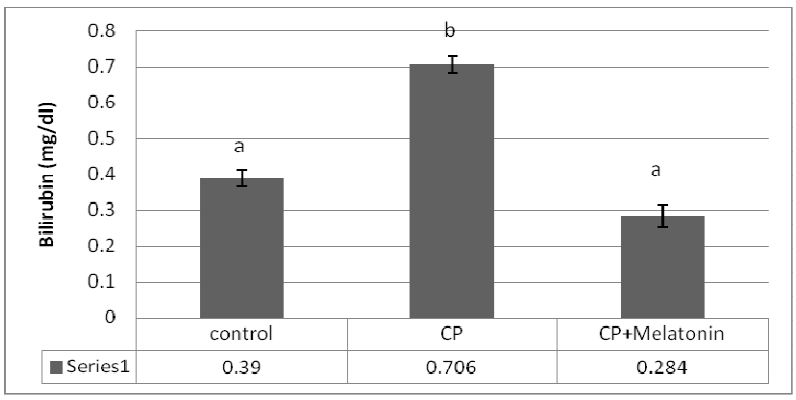

Figure 4: Effects of melatonin on serum total Bilirubin (mg/dl) in CP-injected rats (*P<0.01)*. The different letters indicate significant differences**.**

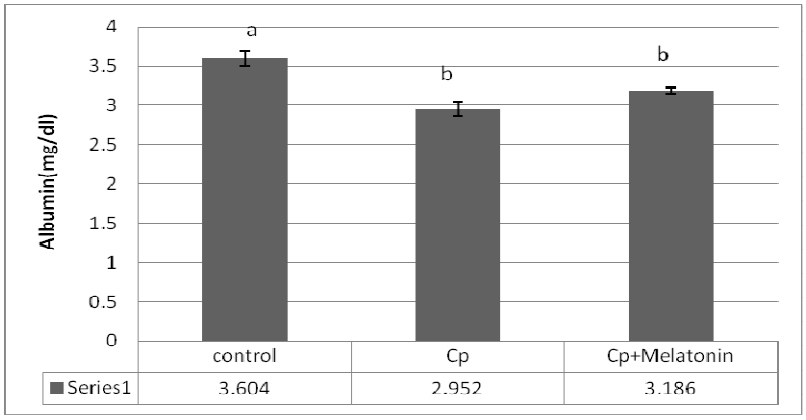

Figure 5: Effects of melatonin on serum Albumin (mg/dl) in CP-injected rats (*P<0.01)*. The different letters indicate significant differences.

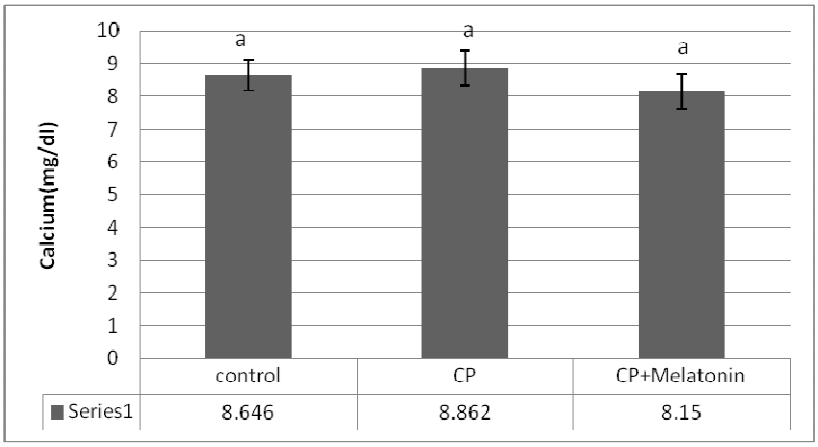

Figure 6: Effects of melatonin on serum Calcium (mg/dl) in CP-injected rats (*P<0.01)*. The different letters indicate significant differences.

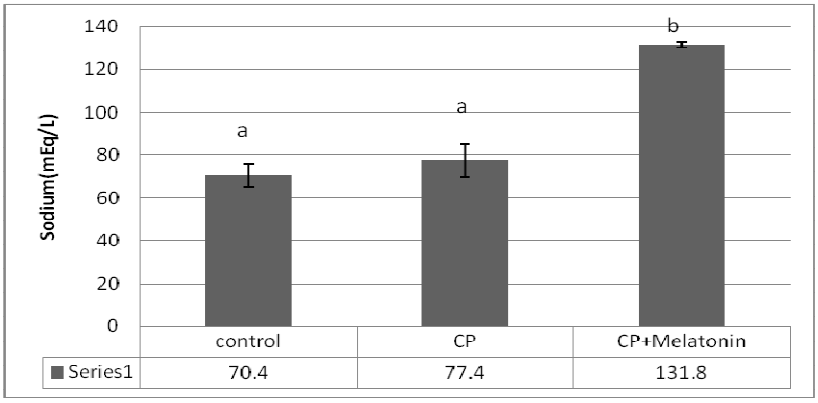

Figure 7: Effects of melatonin on serum Sodium (mEq/L) in CP-injected rats (*P<0.01)*. The different letters indicate significant differences**.**

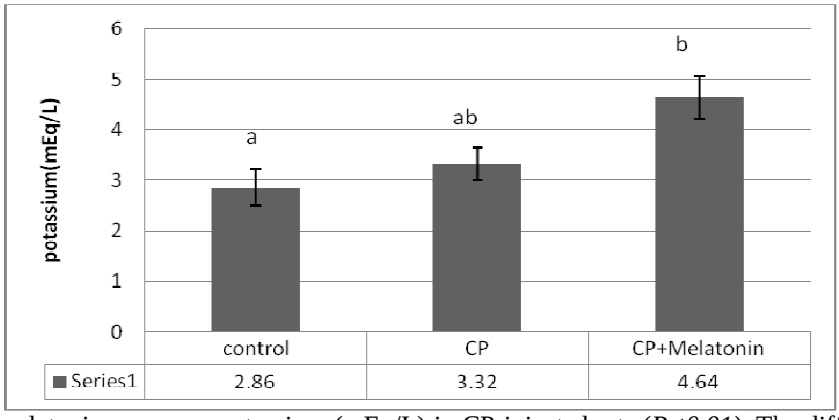

Figure 8: Effects of melatonin on serum potassium (mEq/L) in CP-injected rats (*P<0.01)*. The different letters indicate significant differences.

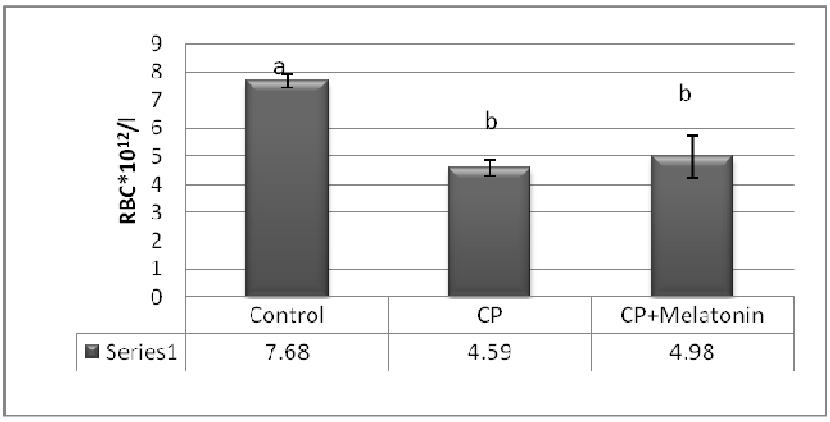

Figure 9: Effects of melatonin on RBC count in CP-injected rats (*P<0.05)*. The different letters indicate significant differences.

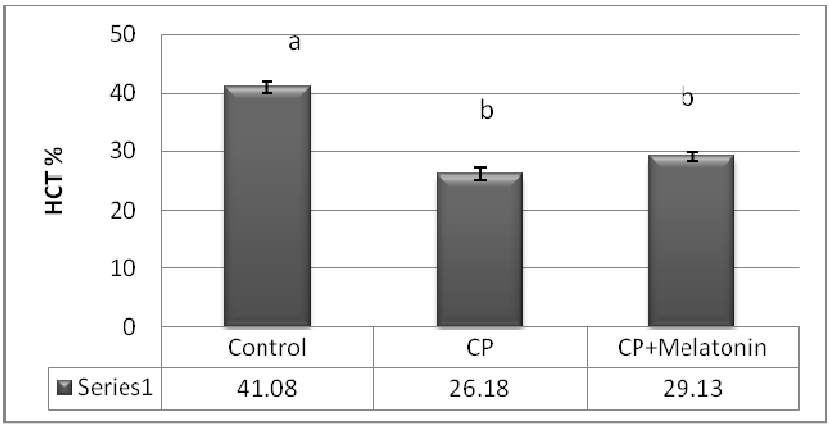

Figure 10: Effects of melatonin on HCT% in CP-injected rats (*P<0.05)*. The different letters indicate significant differences.

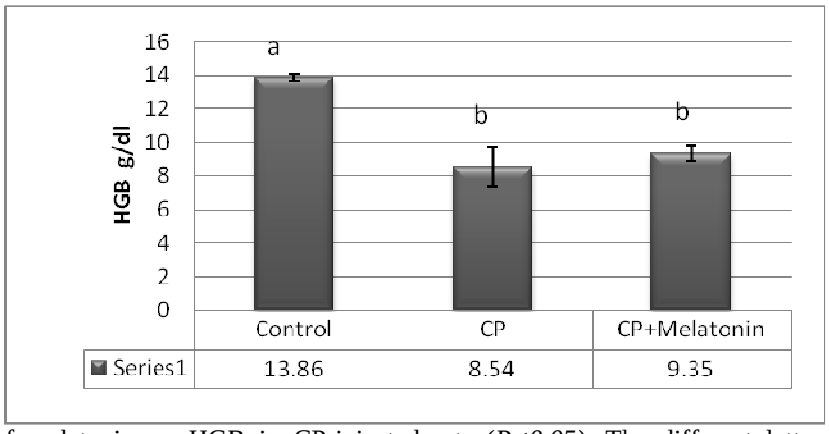

Figure 11: Effects of melatonin on HGB in CP-injected rats (*P<0.05)*. The different letters indicate significant differences.

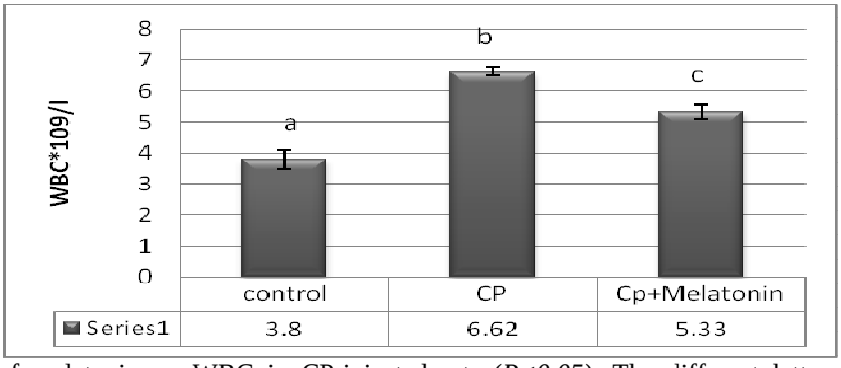

Figure 12: Effects of melatonin on WBC in CP-injected rats (*P<0.05)*. The different letters indicate significant differences.

Discussion

The complex actions of CP-induced nephrotoxicity and necrosis in proximal tubule cells, are incorporated with oxidative stress, inflammation, fibrogenesis and apoptosis [20]. Thereby, the ROS instantly affect cellular constituents, such as membrane lipids, proteins, and nucleic acid, and damage their construction [48]. Lipid peroxidation, hydrolysis reactions and ROS generation are the major mode of actions of cisplatin in kidney [3].

Predominantly, the main by-product of lipid peroxidation is malondialdehyde which is considered as a well-known marker of the degree of ROS production [21, 22]. In the current study, the level of malondialdehyde was elevated significantly ($P<0.05$) in CP-injected rats. The powerful antioxidant and free radical scavenging property of the melatonin may have prevented lipid peroxidation and improved MDA concentration in the serum [23, 24].

The identification of renal toxicity is essentially decided by raising degree of nitrogenous-based waste products, such as creatinine (a sign of dropping the rate of glomerular filtration, hence kidney failure) or blood urea nitrogen (a marker of disability of kidneys for their excretory function and/or multiplication of catabolism) [3, 4, 25].

In our results, elevation in the concentration of creatinine and urea in the serum samples confirmed the induction of nephrotoxicity by CP-injection [20, 26]. A previous study [27] has observed that CP significantly ($P < 0.05$) raises the concentration of serum urea and creatinine to about 128% and 170%, respectively. Treatment with CP causes renal dysfunction associated with polyuria, thus, the serum creatinine concentration was elevated along with a drop in its renal clearance [28]. The administration of cisplatin led to suppressing the activities of some enzymatic antioxidants in the kidney such as superoxide dismutase, glutathione peroxidase, and catalase [3, 20].

The supplementation of melatonin significantly improved the concentration of altered serum creatinine and urea which occurred by CP induction. The mechanism of improvement may be in part due to the melatonin's free radical scavenger and antioxidative effects that prevent tissue damages. Another study [26] has indicated that both serum creatinine and urea nitrogen increased significantly after CP treatment alone; these values decline significantly with melatonin co-treatment of CP-treated rats. In another study, melatonin markedly reduces CP-induced cytotoxicity and DNA fragmentation by directly scavenging hydroxyl radical [29].

The results of the current study clearly exhibit a crucial elevation of total bilirubin in response to CP administration consistent with previous studies [30, 31]. The CP chemotherapy most commonly causes anemia. Approximately 40% of patients develop anemia during CP utilization [32].

Increased red cell destruction releases a large amount of heme into the cells of the reticulo endothelial system which is later altered into bilirubin [49]. Another explanation is that cisplatin may lead to hepatotoxicity or obstruction in the hepatic ducts that may cause elevation of serum bilirubin [33]. Melatonin administration significantly improved the elevated serum total bilirubin level. The melatonin may promote functional and morphologic tubular regeneration and glomerular filtration, the antioxidative activity of melatonin may prevent shortening survival of RBCs and their destruction, as well as, melatonin may prevent hepatotoxicity.

A Recent investigation [34] has demonstrated that the value of serum albumin was decreased significantly during renal damage. This was in agreement with our study, and melatonin administration failed to prevent this elevation. One of the most considerable and abundant studied protein in the urine is albumin, the mechanism of decreasing serum albumin levels seems to be multifactorial; including acute renal failure and lowered the synthesis of albumin due to liver dysfunction [28].

In the results of this study, there was no significant alteration of serum calcium level neither in models nor in melatonin-treated rats in comparison to the control group. However, [35] our results showedCP administration resulted in a significant decrease in serum calcium level. In contrast, Lishner [36] has indicated that the administration of CP may lead to the elevation of serum calcium level; this may be due to the blocking of calcium excretion due to a defect in glomerular filtration. In another way, CP may increase bone breakdown to liberate free calcium.

Abdelghani [37] has demonstrated that CP decreases serum sodium level due to salt wasting accompanied by renal dysfunction and failure in contrast to our results in which there was no significant alteration in serum sodium level in nephrotoxic-induced rats. This may be due to that CP did not have affect the main sites of sodium reabsorption, including sodium potassium chloride co-transporter (NKCCT) (loop of Henle) and sodium chloride cotransporter (NCCT) (distal convoluted tubule), which may need longer duration and

higher dose of cisplatin [50]. Melatonin may increase sodium reabsorption by stimulating the juxtaglomerular cells to activate the renin-angiotensin system [38].

Glover [39] has indicated that CP may lead to decreasing of serum potassium level. This result wascontrary to our result. The high frequency of hypokalemia may be caused by a proximal or thick ascending segment of Henle loop dysfunction [40].

The value of RBC, HCT%, and HGB significantly reduced in CP-injected animals. One of the most common obtained resutls of anemia is the shortening of RBC life span which can be due to abnormalities in the plasma membrane, the cytoplasmic enzyme system and/or in hemoglobin [49]. Horl [41] has demonstrated that free radicals cause membrane lipid peroxidation in erythrocytes and subsequent impaired RBC deformability and splenic sequestration thought to be involved in aging and lysis of red blood cells. Thus, oxidative stress contributes to the shortened survival of RBCs. These defects make the mature erythrocytes more susceptible to damage during their passage through the circulation and thus to the development of a hemolytic anemia.

Although the anemia of renal failure results from multifactors, including depletion of marrow activity and blood loss, an inappropriately low level of erythropoietin is central in its pathogenesis [42].

According to our results, the WBC counting significantly reduced by cisplatin administration. It has been recorded that CP induces not only leukocytopenia and thrombocytopenia but also severe anemia when given repeatedly [43]. Recent investigations have confirmed a significant role of inflammatory processes moderating risk factors of CP-induced nephrotoxicity through the recruitment of inflammatory cells, such as white blood cells, that contribute to their destruction by cisplatin [44, 45]. Furthermore, the ROS and apoptosis mediated by CP may cause tissue necrosis and injury which lead to inflammation and WBC production. The administration of melatonin significantly improved the WBC count. Melatonin may attenuate cell damage, necrosis, and inflammation by scavenging the free radicals and activating the antioxidant enzymes [11, 29, 46]. In conclusion, this study verifies that melatonin has protective effects of improving the nephrotoxicity induced by cisplatin in rat model.

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