Global Veterinaria 9 (2): 225-231, 2012
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2012.9.2.6527

Protective Role of Melatonin against Chromium-Induced Nephrotoxicity in Male Rabbits

[1]Gamal H. Abdel-Rahman, [2]El-Sayed K. Abdel-Hady and [3]Mohammad S. Al-Harbi

[1]Zoology Department, Faculty of Science, Assiut University, Assiut, Egypt
Current address: Biology Department, Faculty of Science, Taif University, Taif, KSA
[2]Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt,
Current address: Biology Department, Faculty of Science, Taif University, Taif, KSA
[3]Biology Department, Faculty of Science, Taif University, Taif, KSA

Abstract: The present study was designed to investigate the potential protective effects of melatonin on potassium dichromate ($K_2Cr_2O_7$)(hexavalent chromium) induced nephrotoxicity in adult male rabbits. Animals were randomly divided into four experimental groups. The first group received saline solution daily for 30 days. The second group received melatonin at a dose of 10 mg/kg b.w./day. The third group was treated with 0.4 mg/kg b.w. $K_2Cr_2O_7$. The fourth group was treated with 0.4 mg/kg b.w. $K_2Cr_2O_7$ plus 10 mg/kg b.w. melatonin. Results showed normal kidney tissue and normal kidney functions in the first two groups. Histopathological observations of the third group showed structural abnormalities in the renal cortex including glomeruli and tubules. Histomorphometric study in the third group revealed significant decreased of the mean values of glomerulus area and glomerulus diameter, while, the mean percentage of the glomerular affection and tubules lumen area showed significant increased. Biochemical results of the third group showed significant increased of the levels of serum urea and creatinine. Melatonin co-treatment to the chromium-administered rabbits attenuated the increase of urea and creatinine and also improved the histopathological and histomorphometrical changes in the kidneys. The present study suggests that melatonin may be useful in ameliorate kidney damage due to chromium toxicity.

Key words: Potassium dichromate · Melatonin · Kidney · Rabbit · Histopathology · Histomorphometry

INTRODUCTION

Chromium (Cr) is a heavy metal that occurs predominantly in two valence states-hexavalent chromium Cr (VI) and trivalent chromium (Cr III) which they are the most common types in the environment [1]. Chromium compounds are abundant in the earth's crust and are extensively used by humans in stainless steel manufacturing, leather tanning, wood treatment, chrome plating, paints, metal finishes, photography, etc., which leads to environmental pollution[2,]. Chromium is a strong oxidizing agent, causes dermatotoxicity, immunotoxicity, neurotoxicity, genotoxicity, cytotoxicity, mutagenesis and carcinogenicity [3]. Chromium can mainly causes nephropathy in rats [4] and humans [5]. Hexavalent chromium Cr (VI) is stable and the most common heavy metal pollutants in the ambient environment and known to cause toxicity in humans and animals [1]. In aqueous solution, Cr (VI) predominately exists as chromate ion, which easily penetrates biological membranes and causes cellular damage by inducing oxidative stress [6,7]. Many researchers have suggested that Cr(VI) is more toxic than Cr (III) [2,8,9]. The kidney is the main route of Cr excretion and it has been reported that acute exposure with $K_2Cr_2O_7$ induces an increase in Cr kidney content in rats [10]. [2,11,12] demonstrated that Potassium dichromate is recognized as a human carcinogenic.

Melatonin, a hormone (N-acetyl-5-methoxytryptamine) produced especially at night time from the pineal gland [13]. It is powerful antioxidant and its antioxidative actions protect from oxidative stress [14] and anti-inflammatory functions [15]. Melatonin can easily cross cell membranes and the blood brain barrier [16]. Many authors reported the protective role of melatonin on

Corresponding Author: Gamal H. Abdel-Rahman, Zoology Department, Faculty of Science, Assiut University, Assiut, Egypt. Current address: Biology Department, Faculty of Science, Taif University, Taif, KSA.

the liver and kidney exposed to different toxic substances [17-19]. Melatonin acts to stabilize cell membranes, thereby making them more resistant to free oxygen radical attacks [20,21]. Several investigators believe that melatonin's antioxidant properties are due to its ability to scavenge ROS and increase cellular antioxidants [22,23]. After its release, melatonin exerts its functions in the extracellular space, where it acts as a free radical scavenger [24].

MATERIALS AND METHODS

Animals: Twenty-four adult male New Zealand White rabbits (2.20 ± 0.07 kg) were obtained from a farm in Taif city, KSA. Animals were kept at standard housing facilities (24±1 °C, 45±5% humidity and 12 h light/dark cycle). They were supplied with standard laboratory food and water *ad-libitum* and left to acclimatize for 1 week before the experiments. The experimental protocol was approved by the Local Animal Care Committee and the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals. Animals were randomly divided into four equal groups (n=6, each):

Chemicals: Potassium dichromate ($K_2Cr_2O_7$) was obtained from Merck (Darmstadt, Germany). Melatonin was purchased from Sigma Chemical Company, St. Louis, MO, USA.

Experimental Protocol: The rabbits were divided into four groups, 6 individual for each.

Group I: served as controls and received a subcutaneous injection of physiological saline.

Group II: served as melatonin group: the animals were daily injected with freshly prepared melatonin at dose of 10 mg/kg b.w. / day 2 h before lights off.

Group III: rabbits received i.p. injection of potassium dichromate ($K_2Cr_2O_7$) (hexavalent chromium salt) dissolved in sterile distilled water at a dose of 0.4 mg/kg b.w. [25].

Group IV: rabbits were injected i.p. with potassium dichromate (0.4 mg/kg b.w./day) which was preceded, 30 min earlier, by a subcutaneous melatonin injection with a dose of 10 mg/kg body weight 2 h before lights off. All the injections were repeated daily for 30 days.

Histopathology: Animals were sacrificed under ether anaesthesia. Kidneys were carefully separated and immediately fixed in neutral buffer formalin. Paraffin sections of (5 µm thickness) were prepared for histopathological and morphometrical examinations. Sections were stained with hematoxylin and eosin (H and E) using the standard techniques [26] and then examined under light microscope.

Histomorphometry: For morphometrical study, 5 slides from kidney of H and E stained sections of each individual (6 sections per slide) were measured. 10 non overlapping microscopic fields were studied. The number of the affected glomeruli was expressed as a percentage in relation to the total number of the glomeruli in each field. Kidney measurements included glomerulus area, glomerulus diameter and proximal and distal convoluted tubules lumen areas. Sigma Scan Pro (version 4.0, Jendel Scientific, SPSS Inc., Chicago, USA) was used for image analysis and morphometrical data acquisition.

Biochemical Determinations: Blood samples were collected from the marginal ear vein of the rabbits and were placed into plain Vacutainer® silicone- coated tubes then, allowed to clot at room temperature. The blood samples were centrifuged at 3000 rpm for 15 minutes. Serum samples were frozen immediately at- 20°C and stored until required for analysis. The levels of urea and creatinine in serum were estimated spectrophotometrically using commercial diagnostic kits according to [27].

Statistical Analysis: For statistical analysis, quantitative results were expressed as means ± S.D. Differences between means were tested by univariate Analysis of Variance followed by Mann-Whitney Rank Sum Test. The values were considered significantly when $P<0.05$. All statistical analyses were performed SPSS (version 12).

RESULTS

Histopathological Observations: The histological examination of the H and E-stained kidney of control and melatonin groups showed normal structure of renal tubules and glomeruli (Figs. 1 and 2). Kidney sections of the $K_2Cr_2O_7$-treated group showed remarkable changes in the renal cortex versus control animals (Fig. 3). These changes include shrinkage of the glomeruli, tubular epithelial cells degeneration and tubular swelling were observed. Furthermore, presence of hyaline cast in renal tubular lumens was seen (Fig.4). Lymphocytic infiltration and congestion were also observed (Fig.5).

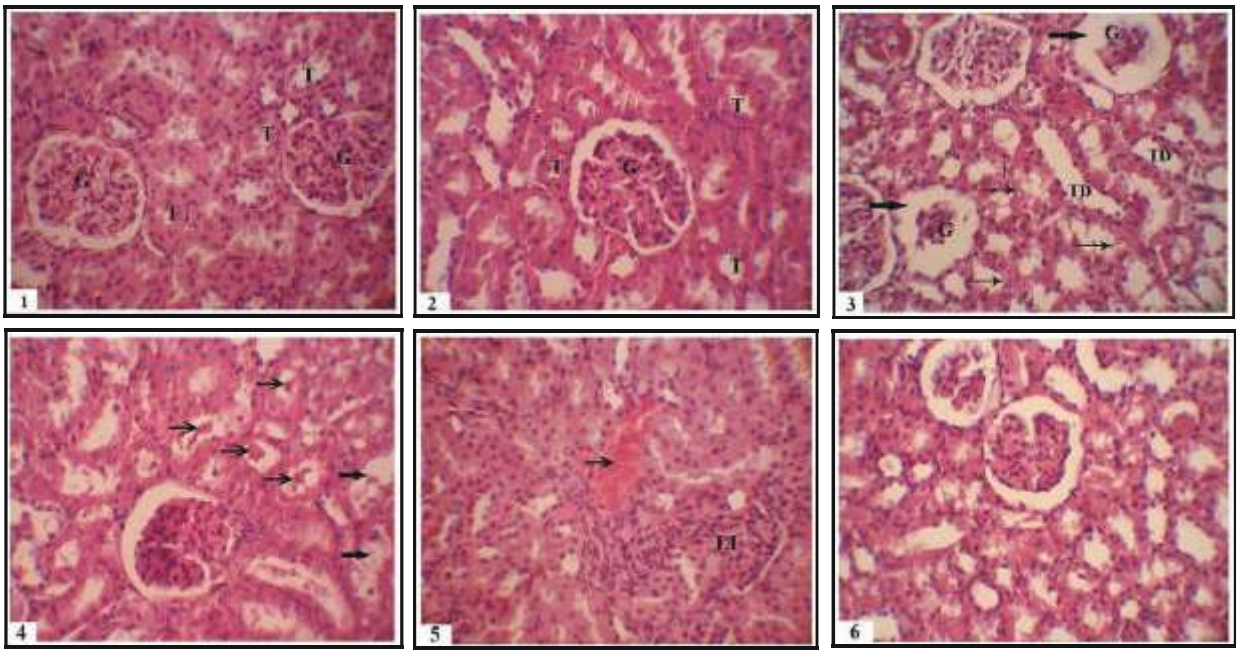

Fig. 1: Photomicrograph of the kidney of control rabbits showing normal architecture of renal tubules and glomeruli. (H and E, X 400)

Fig. 2: Photomicrograph of the kidney of melatonin-treated rabbits showing normal renal parenchyma with well-defined glomeruli and tubules. (H and E, X 400)

Fig. 3: Photomicrograph of the kidney of rabbits treated with Potassium dichromate for 30 days illustrating glomerular shrinkage (thick arrow), tubular epithelial cells degeneration (thin arrow) and tubular dilatation (TD). (H and E, X 400)

Fig. 4: Photomicrograph of the kidney of rabbits treated with Potassium dichromate for 30 days showing presence of hyaline cast in renal tubular lumen (thin arrow) as well as, tubular damage(thick arrow). (H and E., X 400)

Fig. 5: Photomicrograph of the kidney of rabbits treated with Potassium dichromate for 30 days displaying lymphocytic infiltration (LI) and congestion (arrow). (H and E., X 400)

Fig. 6: Photomicrograph of the kidney of rabbits treated with Potassium dichromate plus melatonin for 30 days illustrating improvement in the glomeruli and renal tubules compared to that of Potassium dichromate treated group. (H and E., X 400)

When melatonin was given to $K_2Cr_2O_7$-administered-rabbits, some morphological changes were reduced and glomeruli and renal tubules cells affected become few (Fig. 6).

Biochemical Results: The results showed that the creatinine and urea levels of $K_2Cr_2O_7$ treated group were, respectively, higher (+0.89 and +72%) and (+15.5 and +43%) in comparison with the control group. Administration of melatonin decreased the creatinine and urea levels (- 0.36 and- 17%) and (-6.86 and- 13%) versus those that measured in $K_2Cr_2O_7$ group (Table 1).

Morphometry: The percentage measurements of the affected glomeruli of $K_2Cr_2O_7$ and $K_2Cr_2O_7$ + melatonin groups (Table 2) showed statistically significant increase in their values in comparison with the corresponding control group. While, $K_2Cr_2O_7$+melatonin group showed a significant decrease in comparison with $K_2Cr_2O_7$ group.

Data obtained from kidney histomorphometrical measurements (Table 3) demonstrated statistically significant decreased ($P<0.05$) of the mean values of glomerulus area, glomerulus diameter of $K_2Cr_2O_7$- treated rabbits in comparison with their control group (-4784 and-16.5%) and (-28.93 and- 10 %) respectively. While the mean values of glomerulus area, glomerulus diameter were statistically significant increased ($P<0.05$) when rabbits treated with melatonin (+2178 and +9 %) and (+ 10.63 and +4 %) respectively. Otherwise, proximal and distal convoluted tubules lumen area showed statistically significant increased ($P<0.05$) of $K_2Cr_2O_7$- treated rabbits in comparison with the control group (+357 and + 9.6 %).

Table 1: Kidney functions of rabbits of the different groups. Data presented as means ± Standard deviation, n = 6 animals

	Groups Parameter	
	Creatinine (mg/dl)	Urea (mg/dl)
Control	1.23 ± 0.05	35.61 ± 1.14
Melatonin	1.25 ± 0.06	36.51 ± 1.01
$K_2Cr_2O_7$	2.12 ± 0.16 *	51.11 ± 1.45 *
% higher than control	72%	43%
$K_2Cr_2O_7$ +Melatonin	1.76 ± 0.11 *	44.25 ± 1.59 *
% lower than $K_2Cr_2O_7$	17%	13%

* Significant difference at $P<0.05$ compared with control group.

Table 2: The mean percentage of the number of affected glomeruli in the experimental groups. Data presented as means ± Standard deviation, n = 6 animals

Groups	Mean ± SD
Control	11.43 ± 3.78
Melatonin	12.27 ± 4.46
$K_2Cr_2O_7$	33.82 ± 9.84*
$K_2Cr_2O_7$ +Melatonin	20.67 ± 6.34*

Table 3: Histomorphometrical measurements of the glomeruli. Data presented as means ± Standard deviation, n = 6 animals

Groups	Glomerulus area (sq pixel)	Glomerulus diameter(pixel)
Control group	28967 ± 495	289.60 ± 18.15
Melatonin group	28994 ± 508	293.54 ± 19.82
$K_2Cr_2O_7$ group	24183 ± 312*	260.67±14.87*
% Reduction vs. control	%16.5	% 10
$K_2Cr_2O_7$ and melatonin group	26361 ± 323*	271.30 ± 11.6*
% Stimulation vs $K_2Cr_2O_7$- treated	% 9	% 4

* Significant difference at *P<0.05* compared with control group.

Table 4: Histomorphometrical measurements of the tubules lumen area. Data presented as means ± Standard deviation, n = 6 animals

Groups	Tubules lumen area (sq pixel)
Control group	3696 ± 14.48
Melatonin group	3645 ± 13.52
$K_2Cr_2O_7$ group	4053 ± 11.87*
% increased vs control	% 9.6
$K_2Cr_2O_7$ and melatonin group	3865 ± 12.95*
% Reduction vs. $K_2Cr_2O_7$- treated group	% 4.6

* Significant difference at *P<0.05* compared with control group.

Tubules lumen area of $K_2Cr_2O_7$ + melatonin treated rabbits (Table 4) showed statistically significant decreased ($P<0.05$) in comparison with $K_2Cr_2O_7$ treated rabbits (-188 and- 4.6 %).

DISCUSSION

The kidney is the main route of Cr excretion. It has been reported that acute exposure to $K_2Cr_2O_7$ in rats induced an increase in Cr kidney content [11]. Exposure to Cr (VI) compounds can lead to nephrotoxicity in humans and experimental animals [28]. The present work showed nephrotoxicity in rabbits when exposed to chromium. Histopathological changes due to chromium administration are in agreement with those of many authors [29-32]. Results of the present study are consistent with the previous reports that the dichromate causes acute tubular necrosis and glomerulonephritis [5,33,34]. Renal tubules showed dilatation, degeneration and epithelial cells damage. These observations are consistent with previous report [11]. Degeneration of tubular epithelial cells and tubular damage which observed when exposure to Cr (VI) may be due to the accumulation of inflammatory cells associated with chromium toxicity [35]. Studies of the of effects of chromium on histomorphometry of the kidney of rabbits are very rare. In the present work, Cr (VI) has been shown to cause shrinkage of glomeruli (expansion of spaces inside the Bowman's capsule). This is in accordance with [36-39] in several studies on fishes.

It is known that melatonin was found to be an antioxidant and free radical scavenger. It detoxifies a variety of free radicals and reactive oxygen intermediates, [14]. In the current study, melatonin pretreatment significantly increased the glomerular area, glomerular diameter and decreased the tubules lumen area. Results of the present study showed increased serum creatinine level after $K_2Cr_2O_7$ administration, this may be due to toxic injuries of tubules and tubular obstruction by cell debris [40]. Data obtained from the present work show that increased levels of kidney function markers in serum (urea and creatinine) after $K_2Cr_2O_7$-administration may be due to dysfunction of cell membrane permeability and loss of functional integrity in the kidney. In contrast, pretreatment with melatonin had improved the increase of kidney function markers caused by $K_2Cr_2O_7$ administration.

The role of melatonin in this work to ameliorate toxicity of $K_2Cr_2O_7$ is in agreement with several studies. In both *in vitro* and *in vivo* experiments, melatonin has been found to protect cells, tissues and organs against oxidative damage induced by a variety of free-radical-generating agents [41,42]. Calvo *et al*. [43] indicated that melatonin is effective on inhibiting kidney and liver damage.

It is known that, besides the antioxidative properties of melatonin, which serve to protect lipids from oxidative abuse due to detoxification of free radicals, it also enhances the activity of antioxidative enzymes. Mealtonin was shown to prevent the loss of important dietary antioxidants including Vitamins C and E [44], bind iron and participate in maintaining iron pool at appropriate level resulting in control of iron haemostasis [45], thereby providing tissue protection.

CONCLUSION

It can be concluded that $K_2Cr_2O_7$- administration caused impairments of renal function markers, histopathological and histomorphometrical changes in both renal tubules and glomeruli of rabbits kidney. Pre-treatment with melatonin led to a significant attenuation in all of these parameters. This work suggest that melatonin might be a useful against $K_2Cr_2O_7$-induced nephrotoxicity.

REFERENCES

1. Cohen, M.D., B. Kargacin, C.B. Klein and M. Costa, 1993. Mechanisms of chromium carcinogenicity and toxicity. Crit. Rev. Toxicol., 23: 255-281.
2. Bagchi, D., S. Stohs, B. Downs, M. Bagchi and H.G. Preuss, 2002. Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicol., 180: 5-22.
3. Bagchi, D., E.A. Hassoun, M. Bagchi and S.J. Stohs, 1995. Chromium-induced excretion of urinary lipid metabolites, DNA damage, nitric oxide production and generation of reactive oxygen species in Sprague-Dawley rats. Comp. Biochem. Physiol. C: Pharmacol. Toxicol. Endocrinol., 110: 177-187.
4. Appenroth, D.S., K.H. Gambaryan, H. Fries and D. Braunlich, 1990. Influence of metyrapon and phenobarbital on sodium dichromate nephrotoxicity in developing rats. J. Appl. Toxicol., 10: 227-232.
5. Wang, X., Q. Qin, X. Xu, J. Xu, J. Wang, J. Zhou, S. Huang, W. Zhai, H. Zhou and J. Chen, 1994. Chromium-induced early changes in renal function among ferrochromium producing workers. Toxicol., 90: 93-101.
6. Irwin, R.J., M. Van Mouwerik, L. Stevens, M.D. Seese and W. Basham, 1997. Chromium VI (hexavalent chromium). Environmental contaminants encyclopedia. Fort Collins, Colorado: National Park Service, Water Resources Division.
7. Begum, G., J. Venkateswara Rao and K. Srikanth, 2006. Oxidative stress and changes in locomotor behavior and gill morphology of Gambusia affinis exposed to chromium. Toxicol. Environ. Chem., 88: 355-65.
8. Blasiak, J. and J. Kowalik, 2000. A comparison of the *in vitro* genotoxicity of tri- and hexavalent chromium. Mutat. Res., 469: 135-145.
9. Valko, M., H. Morris and M.T. Cronin, 2005. Metals, toxicity and oxidative stress. Curr. Med. Chem., 12: 1161-1208.
10. Pedraza-Chaverri, J., D. Barrera, O.N. Medina-Campos, R.C. Carvajal, R. Hernandez-Pando, N.A. Macias-Ruvalcaba, P.D. Maldonado, M.I. Salcedo, M.I. Tapia, E. Saldivar, L. Castilla and M.E. Ibarra-Rubio, 2005. Time course study of oxidative and nitrosative stress and antioxidant enzymes in $K_2Cr_2O_7$- induced nephrotoxicity. BMC Nephrol., 26: 40.
11. Travacio, M., J.M. Polo and S. Llesuy, 2005. Chromium (VI) induces oxidative stress in the mouse brain, Toxicol., 162: 139-148.
12. Liu, K.L. and X. Shi, 2001. *In vivo* reduction of chromium(VI) and its related free radical generation. Mol, Cell, Biochem., 222: 41-47.
13. Tan, D.X., R.J. Reiter, L.C. Manchester, M.T. Yan, M. EL-Sawi, R.M. Sainz, J.C. Mayo, R. Kohen, M. Allegra and R. Hardeland, 2002. Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. Curr. To.p Med. Chem., 2: 181-197.
14. Hardeland, R., I. Balzer, B. Poeggeler, B. Fuhrberg, H. Uria, G. Bahrmann, R. Wolf, T.J. Meyer and R. Reiter, 1995. On the primary functions of melatonin in: mediation of photoperiodic signals in unicell, photoxidation and scavenging of free radicals. J. Pineal Res., 18: 104-111.
15. Cuzzocrea, S. and R.J. Reiter, 2002. Pharmacological action of melatonin in shock, inflammation and ischemia/reperfusion injury. Eur. J. Pharmacol., 426: 1-10.
16. Hardeland, R., 2005. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. Endocrine., 27(2): 119-130.
17. El-Sokkary, G.H., 2008. Melatonin and vitamin C administration ameliorate diazepam-induced oxidative stress and cell proliferation in the liver of rats. Cell Proliferation, 41: 168-176.

18. El-Sokkary, G.H., G.H. Abdel-Rahman and E.S. Kamel, 2005. Melatonin protects against lead-induced hepatic and renal toxicity in male rats. Toxicol., 213: 25-33.
19. El-Sokkary, G.H., S. Cuzzocrea and R.J. Reiter, 2007. Effect of chronic nicotine administration on the rat lung and liver: beneficial role of melatonin. Toxicol., 239: 60-67.
20. Reiter, R.J., D.X. Tan, L.C. Manchester, W.Q. Manchester, M. Karbownik and J. Carlo, 2000. Pharmacology and physiology of melatonin in the reduction of oxidative stress *in vivo*. Biol. Signals Recept, 9: 160-71.
21. Reiter, R.J., 2002. Beneficial neurobiological effect of melatonin under condition of increased oxidative stress. Curr. Med. Chem., 4: 45-58.
22. Okatani, Y., A. Wakatsuki, R.J. Reiter, H. Enzan and Y. Miyahara, 2003. Protective effect of melatonin against mitochondrial injury induced by ischemia and reperfusion of rat liver. Eur. J. Pharmacol., 469: 145-147.
23. Reiter, R.J., D.X. Tan, L.C. Manchester and W. Qi, 2001. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species. A review of the evidence. Cell Biochem. Biophys., 34: 237-256.
24. Tan, D.X., L.C. Manchester, M.P. Terron, L.J. Flores and R.J. Reiter, 2007. One molecule, many derivatives: a never ending interaction of melatonin with reactive oxygen and nitrogen species. J. Pineal Res., 42: 28-42.
25. Amar, K.C., C. Aparajita, G. Rituparna and S. Mahitosh, 2007. Effect of curcumin on chromium-induced oxidative damage in male reproductive system. Environ. Toxicol. and Pharmacol., 24: 160-166.
26. Clayden, E.C., 1971. Practical section cutting and staining. Churchill Livingstone. Edinburgh and London.
27. Charrel, M., Ure´e, *et al*, 1991. creatinine in: Semiologiebiochimique. [Urea and creatinin]. Ellipses Publisher Paris, pp: 124-128.
28. Fatima, S., N.A. Arivarasu, A.A. Banday, A.N. Yusufi and R. Mahmood, 2005. Effect of potassium dichromate on renal brush border membrane enzymes and phosphate transport in rats. Human. Exp. Toxicol., 24: 631-8.
29. Barrera, D., P.D. Maldonado, O.N. Medina-Campos, R. Hernandez- Pando, M.E. Ibarra-Rubio and J. Pedraza-Chaverri, 2003. Protective effect of $SnCl_2$ on K2Cr2O7- induced nephrotoxicity in rats: the indispensability of HO-1 preinduction and lack of association with some antioxidant enzymes. Life Sci., 73: 3027-3041.
30. Fatima, S. and R. Mahmood, 2007. Vitamin C attenuates potassium dichromate-induced nephrotoxicity and alterations in renal brush border membrane enzymes and phosphate transport in rats, Clin. Chim. Acta, 386: 94-99.
31. Yam-Canul, P., Y.I. Chirino, D.J. Sanchez-Gonzalez, C.M. Martinez-Martinez, C. Cruz, C. Villanueva and J. Pedraza-Chaverri, 2008. Nordihydroguaiaretic acid attenuates potassium dichromate-induced oxidative stress and nephrotoxicity. Food Chem. Toxicol., 46: 1089-1096.
32. Pedraza-Chaverri, J., P. Yam-Canul, Y.I. Chirino, D.J. Sanchez-Gonzalez, C.M. Martinez-Martinez, C. Cruz and O.N. Medina-Campos, 2008. Protective effects of garlic powder against potassium dichromate-induced oxidative stress and nephrotoxicity. Food Chem. Toxicol., 46: 619- 627.
33. Kirschbaum, B.B., F.M. Sprinkel and D.E. Oken, 1981. Proximal tubule brush border alterations during the course of chromate nephropathy. Toxicol. Appl. Pharmacol., 58: 19-30.
34. Wedeen, R.P. and L.F. Qian, 1991. Chromium-induced kidney disease, Environ. Health Perspect, 92: 71-74.
35. Kurtov, I.B. and C.E. Teskered, 2008. Histological comparison of spleen and kidney tissue from farmed and wild European sea bass (*Dicentrarchus labrax L.)*. Acta Adriat., 49: 147-154.
36. Ahmad, I., V.L. Maria, M. Oliveira, M. Pacheco and M.A. Santos, 2006. Oxidative stress and genotoxic effects in gill and kidney of *Anguilla anguilla* exposed to chromium with or without pre-exposure to β- naphthoflavone. Mut. Res., 608: 16-28.
37. Ashish, K.M. and M. Banalata, 2008. Acute toxicity impact of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the fresh water fish *Channa puntatus*. Environ. Toxicol. Pharmacol., 26: 136-141.
38. Lushchak, O.V., O.I. Kubrak, M.Z. Nykorak, K.B. Storey and V.I. Lushchak, 2008. The effect of potassium dichromate on free radical processes in goldfish: possible protective role of glutathione. Aquat. Toxicol., 87: 108-114.

39. Mishra, K. and B. Mohanty, 2009. Chronic exposure to sublethal hexavalent chromium affects organ histopathology and serum cortisol profile of a teleost, *Channa punctatus* (Bloch). Sci. Total Environ., 407: 5031-5038.
40. Arreola-Mendoza, L., J.L. Reyes, E. Melendez, D. Martin, M.C. Namorado, E. Sanchez and L.M. Del Raz, 2006. Alpha-tocopherol protects against the renal damage caused by potassium dichromate. Toxicol., 218: 237-246.
41. Marchiafava, P.L. and B. Longoni, 1999. Melatonin as an antioxidant in retinal photoreceptors. J. Pineal Res., 26: 184-189.
42. Khaksary, M., H. Mahabady, R. Najafzadeh Varzi, Ranjbar, and S. Mehrzadi, 2011. Melatonin and Vitamin E Protects Against Sodium Arsenite-InducedSkeletal Malformations in Rats. American-Eurasian Journal of Toxicological Sciences, 3(3): 184-189.
43. Calvo, J.R., R.J. Reiter, J.J. Garcia, G.G. Ortiz, D.X. Tan and M. Karbownik, 2001. Characterization of the protective effects of melatonin and related indoles against alpha-naphthylisothiocyanate-induced liver injury in rats. J. Cell. Biochem., 80: 461-470.
44. Susa, N., S. Ueno, Y. Furukawa, J. Ueda and M. Sugiyama, 1997. Potent protective effect of melatonin on chromium(VI)-induced DNA single-strand breaks, cytotoxicity and lipid peroxidation in primary cultures of rat hepatocytes. Toxicol. Appl. Pharmacol., 144: 377-384.
45. Maharaj, D.S. and J.L. Limson, 2003. Day S. 6-Hydroxymelatonin converts Fe(III) to Fe(II) and reduces iron-induced lipid peroxidation. Life Sci., 12: 1367-1375.