Antagonistic Effects of Melanin on Chromium-Induced Nephrotoxicity: Involvements of Inflammation, Oxidative Stress, and Mitochondrial Dysfunction

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Abstract

Nephrotoxicity is a kidney-specific condition in which excretion fails to take place smoothly because of the negative effects of drugs or toxic chemicals. In the present study, the researchers investigated the potential of renoprotective effect and underlying mechanisms of Lachnum melanin (LM) using a rat model of potassium dichromate-induced nephrotoxicity. The rationale for using LM is based on the fact that research and empirical evidence have identified it as metal chelator with antioxidant properties. Nephrotoxicity was induced by a single injection of $K_2Cr_2O_7$ (15 mg/Kg) and $K_2Cr_2O_7$-treated animals were pre-treated by LM (sc, single dose) at three different doses (100, 200 and 300 mg/kg) 30 min before $K_2Cr_2O_7$ administration. It was established that LM pre-treatment attenuated $K_2Cr_2O_7$-induced renal dysfunction evaluated by serum creatinine, blood urea nitrogen, proteinuria, serum glutathione peroxidase activity, and urinary excretion of N-acetyl-d-glycosaminidase. Furthermore, it was also observed that LM prevented the $K_2Cr_2O_7$-induced renal oxidant stress as well the decrease in the activity of the antioxidant. Moreover, $K_2Cr_2O_7$-induced inflammation was reduced by treatment of LM by suppression of NFkr activities. It was further established that LM decreases the renal Cr(VI) content. Consequently, this empirical finding prompted the researchers to evaluate the potential Cr(VI) chelating properties of this compound. The researchers reported that pretreatment with LM significantly removed the mitochondria dysfunction by resorting the mitochondrial respiratory enzyme and antioxidant activities and decreased the elevated oxidative stress to normal. The nephroprotective effect of LM against Cr(VI)-induced nephrotoxicity may be explained, at least partially, by the ability of DFO to chelate Cr(VI) and to attenuate renal Cr(VI) content.

Keywords: Melanin; Potassium dichromate; Oxidative stress; Inflammation; Nephrotoxicity; Mitochondria

1.0 Introduction

Chromium, according to Collins et al. (2010), is a naturally-occurring element that ubiquitously exists in different oxidation states. However, the trivalent [Cr (III)] and hexavalent [Cr (VI)] shapes of this element are most essential from both natural and mechanical viewpoints. The toxicity and carcinogenicity of the Cr compound largely depends on the oxidation state as well as the solubility of the Cr types. The Cr(III)-containing compounds are generally insoluble and poorly absorbed from

the gastrointestinal tract (Jannetto, Antholine, & Myers, 2001). In contrast, the Cr(VI)-containing compounds are highly water soluble and are easily transported across cell membranes by an anion carrier (Jannetto et al., 2001). Intracellular reduction of Cr (VI) to Cr(III) produce the massive amount of reactive oxygen species (ROS), which is an important characteristic of the Cr(VI) toxicity (Stohs, Bagchi, Hassoun & Bagchi, 2000). Potassium dichromate ($K_2Cr_2O_7$) is a Cr(VI) form of Cr, and research has been demonstrated that this compound can induce nephrotoxicity both humans and laboratory animals through an oxidative stress-mediated mechanism (Pedraza-Chaverrí et al., 2005).

Suarez (2010) describes melanins as polyphenolic amorphous polymers that are widespread in plants, animals, and microbes (Suarez, 2010). On the other hand, Solano (2014) defines melanin as a generic name that is often used to refer to presumably the most heterogeneous, resistant, and ubiquitous pigments found in nature (Solano, 2014). The researcher argues that from a structural point of view, melanins represent "a group of complex pigments with a structure relatively diverse and undefined. Melanins have been widely and traditionally used in various industrial fields, for example, food, cosmetics, and medicine for their antioxidant effect as well as anti-quorum and anti-radiation sensing functionalities (Sun, Zhang, Chen, Zhang, & Zhu, 2016). Tarangini and Mishra (2014) acknowledge that melanin has shown in different model systems that it has several health beneficial properties, for example, metal chelating; anti-HIV; anti-radiation and immunity regulation; liver injury protection; and anti-oxidant action. Lachnum Melanin (LM) is a category of melanin that is derived from saprophytic fungi, which is capable of yielding large amounts of melanins using submerged fermentation approach (Ye et al., 2014).

Kidneys are a critical organ needed by the body to carry out several important functions, including the effective maintenance of the homeostatic balance, regulation of the extracellular environment, for instance, detoxification and excretion of toxic drugs and metabolites (Ferguson, Vaidya, & Bonventre, 2008). Thus, the kidneys can be regarded as a key target organ for many exogenous toxic agents. According to Kim and Moon (2012), nephrotoxicity is a kidney problem that occurs when a toxin or drug causes damage to the kidney. In other words, it is a kidney-specific condition in which excretion fails to take place smoothly because of the negative effects of drugs or toxic chemicals. It is estimated that 20% of nephrotoxicity is caused by drugs (Kim & Moon, 2012). However, it has been reported that medication for the elderly population increases the frequency of this kidney condition up to 66% as the average lifespan increases. The available body of knowledge confirms that nephrotoxicity is a major reason why there is limited use of chemotherapy and anticancer medicine (Naughton, 2008; Nagai & Takano, 2010). The implication of this is that nephrotoxicity has indirectly and negatively affected the treatment and management of cancer. Due to their secretory mechanisms, the tubular proximal cells of kidneys are usually exposed to higher concentrations of toxic materials than those occurring in extracellular or plasma fluids (Reyes et al., 2013). Scientists add that kidneys also have mechanisms for absorbing and excreting xenobiotics. Research shows that due to the nature of their metabolic function in the body, specifically the secretion of toxic substances, kidneys may be exposed to some heavy metals (Thomas, Hodgson, Nieuwenhuijsen, & Jarup, 2009; Johri, Jacquillet, & Unwin, 2010). Reyes et al. (2013) explain that chromium ($K_2Cr_2O_7$) is one of the many heavy metals that accumulate in the kidneys and consequently may cause nephrotoxicity.

Incidentally, melanin refers to a generic name that is often used to refer to presumably the most heterogeneous, resistant, and ubiquitous pigments found in nature (Solano, 2014). Fundamentally, there are at least five major types of melanins: plant melanin, synthetic melanin, fungal melanin, animal melanin, and bacterial melanin. However, for the purpose of the scope of this paper, the discussion shall be limited to animal melanin as it relates to kidneys. Melanin is the major pigment that is responsible for the varied pigmentations found in human and animal eyes, skin, and hair (Solano, 2014). Most of the animal melanins are dark, from black to brown, whereas others can be

yellowish or reddish (Simon & Peles, 2010). Thus, animal melanins can be categorized into two main groups: eumelanin and pheomelanin.

Despite the extensive knowledge about the metal chelate and antioxidant properties of melanins, scholars have paid little attention to establishing the effects of this compound on chromium-induced nephrotoxicity. The primary focus of the present research is to fill this knowledge lacuna by investigating the effects of melanins on chromium-induced nephrotoxicity and in turn establish whether or not melanins possess therapeutic benefits for the treatment of chromium-induced nephrotoxicity. Specifically, the research demonstrates the effect of LM on biochemical alterations, oxidative stress, inflammation, mitochondrial respiratory enzyme following acute exposure to potassium dichromate in rats.

2. Methods

Enzyme-Linked Immunosorbent Assay (ELISA) kits were bought from Shanghai Yansheng Biotechnology Co. Ltd (Shanghai, China). On the other hand, potassium dichromate ($K_2Cr_2O_7$) and the rest of the reagents used in the present diagnostic review were acquired from Shanghai Zhenqi Compound Reagent Co., Ltd (Shanghai, China). Ye et al's (1995) strategy was used to extract and purify melanin from Lachnum YM-296 (LM).

Eight to nine-week-old Kunming male rats were acquired from the Experimental Animal Center of Anhui Medicinal College. The animals were isolated for 7 days and were housed under pathogen-free conditions with 12 h light/dark cycle at 25 ± 2°C and 55 ± 5% humidity. The animals were allowed standard pellet diet and drinking water ad libitum throughout the study. These investigations were assessed and affirmed by the Animal Use and Care Committee at the Hefei University of Technology. All the trials were carried out in strict conformity with the prescribed Rules of Experimental Animal Administration distributed by the State Committee of Science and Technology of People's Republic of China. Moreover, the researcher ensured that due diligence is applied during the carrying out of the experiment. In particular, all the procedures were strictly adhered to in order to reduce the risk of the mice being harmed during the experiment.

2.4. Study Design

The animals used in this experiment were acclimatized for 7 days and thereafter, separated into a total of five (5) experimental groups (4 animals per group) throughout the experiment. The first (1st) group served as a control group and received a subcutaneous injection of physiological saline. In other words, the animals in this experiment did not receive any treatment. The second (2nd) group, on the other hand, served as a toxicant group ($K_2Cr_2O_7$) and was pre-treated with saline, followed by a single subcutaneous injection of $K_2Cr_2O_7$ (15 mg/kg bw) (Yam-Canul et al., 2008). Incidentally, the third (3rd), fourth (4th), and fifth (5th) groups were pre-treated with LM (100) (LM, 100 mg/kg, bw), LM (200) (LM, 200 mg/kg, bw), and LM (300) (LM, 300 mg/kg, bw) respectively, followed by a single subcutaneous injection of K2Cr2O7 (15 mg/kg bw). The researcher in the present experiment compared the data collected from the 2nd group $K_2Cr_2O_7$ with the control group, the 1st group to be precise. On the other hand, the data derived from the 3rd, 4th, and 5th groups were compared with the data generated from the 2nd group.

2.5. Sample Collection and Tissue Preparation

Urine was collected for the last 24h of the study. Animals were dicected 48h after the final dose; blood was immediately collected for serum preparation and frozen at ?4 °C till investigation. The kidneys were immediately removed, washed and homogenized in cold phosphate-buffered saline (PBS) with a homogenizer machine (Scientz Biotechnology Co., Ltd., Ningbo, Zhejiang, China). The homogenates were centrifuged (19,000, 10 min, 4°C), and clear supernatants were collected

and stored for subsequent assays.

2.6. Analysis of Hematological Parameters

The blood tests were examined for hematological parameters, for example, RBC (red platelets), HGB (hemoglobin), WBC (white platelets), and HCT (hematocrit) were analyzed using auto hematology analyzer (Mindray BC-6900, Shenzhen, Guangdong, China).

2.7. Estimation of renal functional markers

The following markers were used to measure the $K_2Cr_2O_7$-induced renal injury, blood urea nitrogen and creatinine concentration in serum were estimated using assay kit, and employing with an autoanalyzer (Technicon RA-1000, Bayer Tarrytown, NY, USA). Protein data was estimated by a turbidimetric method (Yam-Canul et al., 2008) at 420 nm utilizing 12.5% TCA and data were expressed as mg/24h. Urinary NAG activity was analyzed at 405nm utilizing p-nitrophenyl-N-acetyld-glucosaminide as substrate and result were showed as units (U)/24 h (Yam-Canul et al., 2008). Serum GPx activity was estimated in serum at 340 nm utilizing GSH-reductase and NADPH in a coupled reaction.

2.8. Evaluation of Inflammatory Cytokines and NF?B

The levels of renal tumor necrosis factor alpha (TNF-?), interleukin (IL)-10, and IL-6 and nuclear factor kappa B (NF?B) activities were tested utilizing ELISA kits following maker's directions.

2.9. Estimation of Antioxidant Enzymes Activities and Oxidative Stress

The levels of ROS (Yam-Canul et al., 2008); Protein carbonyl (PCO) [13]; and lipid peroxidation (Jasso-Chávez et al., 2010), as well as the activities of antioxidant enzymes GSH (Fernández-Checa & Kaplowitz, 1990); CAT (Barrera et al., 2003); SOD (Barrera et al., 2003); GPx (Barrera et al., 2003), and GST were determined in kidney tissue homogenates of all animals in previously mentioned procedures.

2.10. Estimation of Cr(VI) Accumulation

The estimation of the Cr(VI) accumulation was carried out by first weighing 1 to 2g of the samples into a 250ml volumetric flask and adding 10ml of concentrated nitric acid. The solution was then allowed to pre-digest overnight at room temperature. The pre-digested tissue samples were gently boiled until the brown fumes disappeared completely. After the samples had cooled at room temperature, the amount of nitric acid was added to the samples and slowly boiled until white fumes evolved. The samples were once again allowed to cool at room temperature after which 10ml of distilled deionized water was added and the solution boiled to further release any present fumes. The solution was filtered through a 0.45-? millipore membrane filter paper and subsequently, 0.5ml of supernatant was mixed with 0.5ml of the diphenylcarbazide reagent and 5ml of 10% H2SO4 (Taras, Greenberg, Hoak, Rand, 1974) Furthermore, the absorbance of the mixture was measured at 540nm. A commercial 0.1% (w/v) Cr(VI) stock solution was used as a standard for calibration. The reaction was linear up to 50nmol of Cr(VI) (García-García et al., 2009).

2.11. Isolation of Renal Mitochondria

The kidney (~2 g total) was rapidly washed and maintained in cold isolation buffer (250mM sucrose, 10mM HEPES, 1mM EGTA, pH 7.3). The renal cortex was separated and utilized for mitochondria isolation. The same buffer was used to homogenize the tissue. For isolation of mitochondrial fraction, homogenates were centrifuged at 600g for 10 min and the supernatant

obtained was centrifuged at 14,000g for 8 minutes to pellet the mitochondria-enriched fraction. This pellet was resuspended in isolation buffer (without EGTA) and centrifuged once again at 10,000g for 10 min. The final pellet obtained was used as mitochondrial fraction and was resuspended in the storage buffer (10 mM Tris, 250 mM Sucrose, pH 7.2) (Johnson & Lardy, 1967). The whole isolation procedure was performed at 4°C and the protein content was estimated (Jasso-Chávez et al., 2010)

2.12. Enzymatic Activities of Mitochondria

The assay kits were used to determine the cytochrome c oxidase (COX) activity and previously described method was used to determine the activities of NADH dehydrogenase (NDH) and succinate dehydrogenase in isolated mitochondria. Mitochondrial redox activity was assessed as a measure of intact mitochondria (viable cells) based on the MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assay as described in the earlier literature (Liu et al., 1997). Mitochondrial oxidative stress as well as, antioxidant markers, MDA (Molin-Dijn et al., 2011) and GSH [12] SOD [13] CAT (Barrera et al., 2013) GPx (Barrera et al., 2003) and PCO (Reznick & Packer, 1994) levels were also estimated as described in earlier literature.

3. Results

3.1. Effect of LM on Hematological Parameters

The analysis of the collected data revealed that a single subcutaneous (s.c.) injection of $K_2Cr_2O_7$ (15mg/kg) did not yield any significant change to the hematological parameters, for example, WBC, HCT, RBC, and HGB levels when contrasted with those of control group ($p < 0.05$, Table 1).

3.2. LM Supplementation Attenuates $K_2Cr_2O_7$-Induced Renal Dysfunction

As shown in Table 1, the $K_2Cr_2O_7$ treatment alone led to a significant deterioration of the renal function, 48h after $K_2Cr_2O_7$ injection. The levels of renal damage biomarkers, such as proteinuria, serum creatinine, BUN, and NAG were obviously increased in $K_2Cr_2O_7$-treated animals compared to the control group ($p < 0.05$). On the other hand, LM (400 mg/kg) administration significantly attenuated the renal dysfunction (Table 1, vs. $K_2Cr_2O_7$, $p < 0.05$). Additionally, the pre-treatment of the $K_2Cr_2O_7$-induced rats with either 100 or 200 mg/kg of LM could not reduce the full renal dysfunction.

3.3. LM Impeded $K_2Cr_2O_7$-Induced Renal Oxidative Stress

$K_2Cr_2O_7$-induced renal oxidant stress was evident by increased (a) ROS, (b) lipid peroxidation, and (c) protein carbonyl content and decreased (d) GSH content as compared to control group ($p < 0.05$). LM pretreatment with 400mg/kg significantly attenuated the $K_2Cr_2O_7$- induced oxidant stress (Table 3) as compared to $K_2Cr_2O_7$ group ($p < 0.05$). Pretreatment with 100 mg/kg did not reduce the oxidative stress while and 200 mg/kg of LM prevented only in a partial way with oxidant stress; the increased lipid peroxidation and protein carbonyl content were not prevented with this dose (Table 2)

3.4. LM Up-Regulated the $K_2Cr_2O_7$-Induced Changes in Renal Antioxidant

As shown in Table 2, $K_2Cr_2O_7$ administration caused a reduction of the antioxidant defense. Essentially, this is confirmed by a decline in the activities of SOD, GPx, GST, and CAT (versus control group, $p < 0.05$). Meanwhile, compared to $K_2Cr_2O_7$ group ($p < 0.05$), the administration of LM (400 mg/kg) kept the status of these enzymes nearly close to normal. Pretreatment with 200 mg/kg significantly attenuated the $K_2Cr_2O_7$-induced decline in the activity of SOD GST, but not in that of CAT and GPx as compared to $K_2Cr_2O_7$ group. Compared to $K_2Cr_2O_7$ group, pretreatment

with 100mg/kg did not significantly attenuate the $K_2Cr_2O_7$-induced decrease in the activity of antioxidant enzymes (Table 2).

3.5. LM-1 mitigates $K_2Cr_2O_7$-induced Renal Inflammation

NF-kB acts as a key switch in the modulation of inflammatory cytokines and renal tissue injury. It has been demonstrated in a recent study that melanin treatment suppressed the renal inflammation. The present study further confirms this effect using the analysis of the renal NF-kB activation and measurement of proinflammatory cytokines (TNF-?, IL-10, and IL-6.) expression induced by $K_2Cr_2O_7$, by ELISA. As expected, the administration of $K_2Cr_2O_7$ produced a marked (P < 0.05) upregulation of NF-kB activation and levels of TNF-?, IL-10, and IL-6 about 1?%, 12%, 21%, and 33% respectively when compared with the control rats, as depicted in Table 2. Nevertheless, LM significantly reversed these abnormal cytokines (TNF-?, IL-10, and IL-6) expression in $K_2Cr_2O_7$-treated rats; effects were dose-dependent (p < 0.05). In addition, only 200 and 400mg LM significantly suppressed the NF-kB activation in the kidneys. These results indicate that LM could abate $K_2Cr_2O_7$-induced inflammation in the kidneys of $K_2Cr_2O_7$ rats. Three doses of LM significantly suppressed the TNF-?, IL-10, and IL-6 concentration in the kidneys. In regard to the NF-kB activation, only 200 and 400 mg significantly suppressed the NF-kB activation.

3.6. LM Pretreatment (400 mg/kg) Changes Renal Cr (VI) Content

As shown in figure 2, Cr(VI) content was negligible in renal homogenates from control-treated rats. Cr(VI) content in renal homogenates from $K_2Cr_2O_7$-treated rats was of 40±3.2nmol/mg protein. LM pretreatment with 400 mg/kg was able to decrease the amount of Cr(VI) that reaches the kidneys (20.4±2.1nmol/mg protein). It is concluded that LM pretreatment was able to affect Cr(VI) accumulation in the kidneys of $K_2Cr_2O_7$-treated rats indicating that LM exerts a protective effect of renal alterations. Among the three doses, only 400mg LM significantly decreased the Cr(VI) content.

3.5. Impacts of LM Pretreatment (400 mg/kg) on K2Cr2O7-Induced Diminished Action of Renal Mitochondrial Respiratory Enzymes

In comparison to the test results of the control group, enzymatic activities of mitochondrial respiratory enzymes such as NADH dehydrogenase (NDH), succinate dehydrogenase (SDH), and cytochrome-c-oxidase (COx) were pointedly decreased in $K_2Cr_2O_7$-treated rats. Besides, MTT reduction, as an index of mitochondrial redox activity to detect intact mitochondria, was also considerably (p < 0.05) decreased in mitochondrial fraction of kidney tissues from rats administered with $K_2Cr_2O_7$. Pretreatment with LM ameliorated the $K_2Cr_2O_7$-induced decrease in the activity of this respiratory enzyme and MTT as compared to $K_2Cr_2O_7$ group (p < 0.05). It is imperative to note that in this study, the researcher only used 400mg doses, and that for purposes of clarity, 200 and 100mg LM mitochondria were never used in any step of the experiment.

3.7. Effect of LM on Renal Mitochondrial Antioxidant and Oxidative Stress Markers

The researcher in the present study assessed the markers of the oxidative stress in the mitochondria derived from $K_2Cr_2O_7$-treated rats to determine whether oxidant stress can be observed in kidney homogenates (Figure 3). As observed in Figure 4, $K_2Cr_2O_7$-treated group increased lipid peroxidation, protein carbonyl content, and GSH, which was prevented by LM pretreatment (p < 0.05). GPx, GST, and SOD were mainly assessed to determine the influence of $K_2Cr_2O_7$ injection on the enzymatic activity of mitochondrial CAT. It is evident from Figure 5, that when compared to the control group, K2Cr2O7- treatment induced a significant decrease in the activity of CAT, GPx, GST, and SOD. These effects were prevented by LM in isolated mitochondria from $K_2Cr_2O_7$-treated rats (compared to $K_2Cr_2O_7$, p < 0.05). Accordingly, this set of data suggests

that there is a relationship between the decrease in antioxidant enzymes and the increase in oxidant stress markers.

4. Discussion

Multiple studies have reported that the *in vitro* and *in vivo* evidence suggests that oxidative stress (Khan et al., 2010) mitochondrial dysfunction (Molina-Jijón et al., 2011) and inflammation (Wang et al., 2010) play an important role in potassium dichromate-induced nephrotoxicity. The present study was designed taking into account the therapeutic potential of LM, in a well-established rat model of potassium dichromate-induced renal injury. The results of the present research provide sound evidence that animals that received antioxidant and chelate agent, together with chromium-treated rats, had less oxidative damage than those that received Cr(VI) alone. The research demonstrated that pre-treatment with LM of the group of rats that had been administered with potassium dichromate offered considerable protection against potassium dichromate-induced acute renal injury. In particular, the LM treatment attenuated renal dysfunction, oxidative stress, inflammation, and mitochondrial dysfunction.

Subsequently, the kidney dysfunction research in the present experiment was carried by estimating BUN. It is important to note that chromium-induced toxicity is a serious health issue. The administration of potassium dichromate induces significant renal dysfunction, which in the present research, was evident by proteinuria, increased serum creatinine, BUN, and urinary NAG excretion as well as decreased serum GPx activity which has previously been reported (Pedraza-Chaverrí et al., 2005; Molina-Jijón et al., 2011). Pretreatment with LM significantly reduced the severity of abnormalities. Furthermore, the different hematological parameters, for instance, RBC, HGB, WBC, MCV, HCT, MCH, MCHC, and the body weight of rats administered with potassium dichromate remains unchanged when compared to those in the control group.

Incidentally, the available body of knowledge suggests potassium dichromate-induced toxicity and induction of oxidative stress through the generation of ROS. In this study, the researcher reported that $K_2Cr_2O_7$ injection in rats induced oxidant stress and that this could be confirmed by the increased levels of ROS, products of lipid peroxidation, and oxidized proteins. The enzymatic and non-enzymatic antioxidants are considered to be the pivotal defense by scavenging the ROS and/or preventing biological macromolecules from oxidative injury. A detailed evaluation of the activity of antioxidant enzymes revealed that $K_2Cr_2O_7$ treatment induced a decline in the activity of CAT, GR, GPx, GST, and SOD, thereby confirming the findings made in previous empirical and research evidence. However, it was observed in the present study that LM significantly reversed these abnormal changes. The preventive effect of LM against $K_2Cr_2O_7$-induced oxidant stress observed in this research is consistent with previous studies that showed the ability of LM to interfere with oxidant processes (Lu et al., 2014). The nephroprotective effect of LM may be explained, at least partially, by its ability to chelate Cr(VI) [thereby reducing the amount of Cr(VI) that reaches the kidneys].

Moreover, NF-kB is considered to be an intracellular sensor of oxidative stress (Liu et al., 2001). Reactive oxygen generation following chromium exposure activates a transcription factor, NF-kB, which is a critical activator of genes involved in inflammation and apoptosis prevention (Ye, Zhang, Young, Mao, & Shi, 1995; Shi et al., 1999). It has been reported that hexavalent chromium exposure could increase ROS formation, activate AKT, NF-kB, and MAPK pathways as well as increase the production of cytokines, including TNF-? (Wang et al., 2010).

In the present study, Cr(VI)-induced ROS generation may account for the activation of NF-kB. It was established that potassium dichromate per se administered rats showed increased kidney levels of TNF-?, IL-6, and IL-10 compared to control rats. Pretreatment with LM decreased the levels of TNF-? compared to potassium dichromate per se administered rats. Therefore, we believe

that antioxidant property of LM scavenges ROS and thereby inhibits NF-kB activation and TNF-?, IL-1?, and IL-10 release. These findings are consistent with other studies in which the anti-inflammatory property of LM is well established (Song, Yang, Ye, Chen, Shi & Shaikh, 2016). Mitochondria are targets of metal toxicity that is in many cases related to oxidative stress and mitochondrial dysfunction (García-Niño et al., 2013). The mitochondrial respiratory chain is one of the major sources of ROS in cells. However, they possess a very effective antioxidant system which maintains redox balance (Molina-Jijón, 2011). The experimental results in renal mitochondria isolated from rats administered with potassium dichromate demonstrated a decrease in the activities of mitochondrial respiratory enzymes (such as NADH dehydrogenase, succinate dehydrogenase, and cytochrome-c-oxidase), a decrease in mitochondrial antioxidant, GSH, and an increase in mitochondrial MDA levels, as an index of mitochondrial lipid peroxidation when compared to control rats.

In the present study, the decrease in mitochondrial respiratory enzyme activities, following potassium dichromate administration, altered the normal electron transport chain and inhibited mitochondrial dysfunction. The findings of the present study are also corroborated with results in previous studies. MTT reduction assay is a useful measure of mitochondrial activity as well as cell viability (Sahu et al., 2014). Mitochondrial redox activity, as evidenced by MTT reduction, was significantly decreased in mitochondria of kidney tissues of the rats administered with potassium dichromate, suggesting decreased mitochondrial function and cell viability. Pretreatment with LM preserved the respiratory enzyme redox activities, restored the mitochondrial antioxidant activities, and reduced the oxidative stress as evidenced from the test results of LM treated rats when compared with those of potassium dichromate per se treated rats.