Natural bioactive molecules melatonin and curcumin, and trace element selenium inhibit cadmium induced oxidative stress in mice

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Cadmium (Cd) is one of the most toxic and carcinogenic heavy metals in industrial discharges that cause considerable hazards to humans. Curcumin and melatonin are one of the most studied biometabolites known as scavenger of free radicals. Further, selenium is an essential dietary trace element having a protective role as an antioxidant. In this study, we explored the antioxidants effect of selenium, and bioactive molecules melatonin and curcumin over cadmium induced oxidative stress (OS) and some blood indices damages in adult Swiss strain male mice. The animals were treated with selenium, melatonin and curcumin and all pre-treated animals were administered with Cd (10 mg/kg) s.c. for three weeks. The nonenzymatic OS indices viz. lipid peroxides in the form of thiobarbituric acid reactive substance (TBARS) and reduced total glutathione (GSH), and enzymatic OS indices, such as catalase (CAT), glutathione S-transferase (GST) and superoxide dismutase (SOD) were estimated together with certain parameters of blood. Cd alone induced a significant increase in TBARS and a significant decrease in GSH, GST, CAT and SOD levels in all tissues. Furthermore, the blood parameters viz. counts of red and white blood cells, platelets, hemoglobin and packed cell volume were also depleted due to Cd exposure. The results indicated that though selenium, melatonin and curcumin act as natural antioxidants, the trace element selenium however, proved to be the best antioxidant comparatively.

Keywords: Heavy metal toxicity, Industrial pollution, reactive oxygen species (ROS)

Cadmium (Cd), one of the most toxic and carcinogenic heavy metals, causes considerable health hazards to humans. Exposure to Cd exposure is mainly through industrial discharges such as mining and smelting, alloys, phosphate fertilizers, plastics, batteries, pigments, metal plating and cigarette smoking. Even low concentration of Cd induces generation of reactive oxygen species (ROS) which leads to cellular dysfunction, apoptosis, DNA damage and carcinogenicity. Recently, Seth et al. have reported significant elevation of lipid peroxidation levels, and decrease in enzymatic and non-enzymatic antioxidants, such as catalase, superoxide dismutase, glutathione reductase, glutathione-s-transferase and reduced glutathione apart from marked structural alterations in neural tissue of BALB/c mice. Curcumin is most studied natural chemopreventive agent with potential against oxidative damage possessing antioxidant property and free radical scavenging effects. Melatonin the human pineal gland secretory product (n-acetyl-5-methoxy tryptamine) of the pineal gland, has been shown to be a scavenger of free radicals and has shown protective effects against Cd induced oxidative damages in vivo. Selenium is an essential dietary trace element known to play important metabolic roles in several species including human beings, particularly as antioxidant by reducing the oxidative stress in liver, kidneys and other organs. It is also known, that selenium has a certain protective role as an antioxidant from the toxic actions of Cd and other heavy metals.

Researchers have shown extensive interest in studying natural antioxidants for their bioactivity, such as chemopreventive effects in carcinogenesis and toxicity due to Cd exposure. The quest for exploring the role of various antioxidants in reversing the oxidative stress in various body tissues has been an interesting field of research for the basic scientists and clinicians. In the present study, we assessed the influence of melatonin, curcumin and selenium pretreatment on Cd induced oxidative damage in the liver, kidneys, red blood cells (erythrocytes) and on the other blood parameters as these organs are metabolically most active and involved in various vital biochemical processes of the body, such as synthesis, detoxification and, excretion.

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Materials and Methods
The study was approved by the Research and Ethics Committee of King Saud University, Riyadh, Saudi Arabia. Research work protocol and animal handling practice were according to a standard operating procedure approved by the Institute following international guidelines. Swiss strain adult male mice (25-30 g) were used under precise conditions with diurnal 12 h light-dark cycle, temperature 22±1°C, humidity 55-60% and free access to food and water. All assurances were taken to curtail animal stress and pain in the animals. All mice were randomly divided into five groups of 10 animals in each. Group I served as the control without any treatment; Group II received Cd only; Group III was treated with curcumin and Cd; Group IV received melatonin and Cd; and Group V was treated with selenium and Cd.

Treatment protocol
Curcumin (50 mg/kg), melatonin (10 mg/kg) and selenium (0.5 mg/kg) dissolved in 0.1% dimethyl sulfoxide (DMSO, purchased from Sigma Chemical Company, USA), were administered once a day orally by gastric gavage in a constant volume of 0.1 mL/10 g body wt., for three weeks. Groups I and II also received an equivalent volume of DMSO (0.1%). A control of 0.1% DMSO used as a solvent for the antioxidants was also used in this experiment but the results are not shown in the figures of the present study since it had no effect on the levels of measured parameters of OS as compared to the control group throughout and it is reportedly safe to be used as a drug solvent. One hour after the administration of selenium, melatonin and curcumin, Cd in the form of CdCl₂ (analytical grade, Riedel de Haen, Germany) was administered s.c. (10 mg/kg), to animals in Groups II to V. Thus, Cd treatment was given to all experimental groups (II to V) for 3 days. The selection of single doses of Cd, selenium, melatonin and curcumin used in the present study was the highest effective doses selected from the literature survey and from a range of three doses (low, medium and high) used for each in our pilot studies (data not shown). Our pilot studies further showed that selenium, melatonin and curcumin administered alone (without Cd) in the doses used herein did not show any alteration in the levels of any indices of OS measured in liver and kidney. Thus, the results of these antioxidants (selenium, melatonin and curcumin) alone, are also not included in the figures of the present study for easier expression of the targeted results only.

Biochemical analysis
The animals were sacrificed 24 h after the last dose of Cd administration, by execution and their liver and kidney were taken for further use by excised. Materials for use were washed with ice-cold normal saline, homogenized at ice-cold temperature, and were used for biochemical analysis. Lipid peroxidation was analyzed in the form of thiobarbituric acid reactive substance (TBARS) measuring malondialdehyde formation. Reduced glutathione (GSH) was assayed for estimation of total GSH where the gradient of the variation in absorbance was used. Glutathione S-transferase (GST) was estimated using 1-chloro-2,4-dinitrochlorobenzene (CDNB) as substrate at 340 nm. Catalase (CAT) activity was measured by measuring the decrease in the extinction of H₂O₂ at 240 nm by tracking the decomposition of hydrogen peroxide. Superoxide dismutase (SOD) activity was expressed as the quantity of enzyme that constrains the oxidation of epinephrine by 50% which is equal to U per gram tissue weight.

Hematological assays
After the treatment period the animal’s blood samples were collected according to published protocol and blood factors like red blood count, hemoglobin content, packed cell volume, total white blood count and blood platelets were measured in automated parameter hematology analyzer (T 450, USA). Erythrocyte lysates were made ready using another subset of collected blood samples, after clotting the blood samples were centrifuged at 2500 rpm in a refrigerated centrifuge at 10°C for 15 min and stored at 20°C for 15 min. Lysed erythrocytes were prepared by thawing frozen samples and three volumes of ice-cold distilled water were added. Cell membranes were removed by centrifugation at 1000 g for 20 min, and the supernatants were used for the estimation of oxidative stress indices as described above in the previous subsection ‘Biochemical analysis’

Statistical analysis
The statistical significance was calculated between the groups and was compared by ANOVA with post-hoc testing using Student-Newman-Keuls Multiple Comparison Tests.

Results
Administration of Cd to mice instigated a significant (P <0.001) increase in the TBARS in the
liver, kidneys, and erythrocytes. Pre-treatment of mice with melatonin, curcumin, and selenium ameliorated significantly \( (P < 0.001) \) the inductive effect of Cd on hepatic (Fig. 1), renal (Fig. 2), and red blood cells (Fig. 3) lipid peroxidation. Cd also caused a significant \( (P < 0.001) \) decrease in the GSH, GST, CAT and SOD levels in the liver (Fig. 1), kidneys (Fig. 2) and red blood cells (Fig. 3) and pre-treatment with selenium, melatonin and curcumin significantly \( (P < 0.001) \) ameliorated the decrease of hepatic and renal GSH, GST, CAT, and SOD content (Figs 1-3, respectively). More interestingly, we noted that all the three molecules used in the study were effective enough to ameliorate the Cd toxicity indicated by the measured OS indices. Cd exposure led to significant reduction in some of the perceived blood parameters in male mice like the red blood cell count, white blood cell count, platelets count, hemoglobin content, and packed cell volume when compared to the respective control groups (Fig. 4).

**Discussion**

The present results have clearly demonstrated that Cd is capable of inducing hepatic and renal oxidative stress by increased lipid peroxidation. However, the antioxidants comparatively ameliorated in the order selenium > curcumin > melatonin throughout in each parameter as compared to the Cd group. Cd is the most dangerous environmental and industrial pollutant toxic metal that induce oxidative damage prooxidant-antioxidant balance disturbance in cells and tissues. Although the mechanism of Cd toxicity is far from clear, some of the tissue damage is related to oxidative stress\(^{28}\). Cd is known to generate free radicals in an indirect manner by replacing redox active metals like iron and copper, which in turn involves in free radical generation\(^{29}\). Other authors have also reported for Cd induced oxidative stress by increased lipid peroxidation and altered antioxidant status in liver and kidneys\(^{28}\). Furthermore, the present results also show a significant decrease in the level of GSH, GST, CAT, and SOD both in the liver and kidneys due to Cd exposure. Earlier, other authors have also reported for such effects\(^{3,30-32}\). Blood parameters results are also in agreement with other studies\(^{33,34}\). Cd exposure has been related with the toxic effects on a variety of tissues including neural\(^{7}\), but the first to be affected are blood since Cd has been reported to bind

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**Fig. 1** — Effect of selenium, melatonin and curcumin pre-treatment on hepatic (A) Lipid peroxidation expressed as TBARS; (B) Total glutathione (GSH); (C) Glutathione-S-transferase (GST); (D) Catalase (CAT); and (E) Superoxide dismutase (SOD) content in the mice exposed to cadmium (Cd). \( **P < 0.01 \) and \( * * * P < 0.001 \) significantly different from control at \( P < 0.001 \), and \( * \) significantly different from Cd treated group at \( P < 0.001 \) by Newman Keul’s student’s t test after one-way ANOVA. The three antioxidants were effective in the order selenium > curcumin > melatonin

**Fig. 2** — Effect of selenium, melatonin and curcumin pre-treatment on renal (A) Lipid peroxidation expressed as TBARS; (B) Total glutathione (GSH); (C) Glutathione-S-transferase (GST); (D) Catalase (CAT); and (E) Superoxide dismutase (SOD) content in the mice exposed to cadmium (Cd). \( * * * P < 0.001 \) significantly different from control at \( P < 0.001 \), and \( * * * P < 0.001 \) significantly different from Cd treated group at \( P < 0.001 \) by Newman Keul’s student’s t test after one-way ANOVA. The three antioxidants were effective in the order selenium > curcumin > melatonin

**Fig. 3** — Effect of selenium, melatonin and curcumin pre-treatment on erythrocyte. (A) Lipid peroxidation expressed as TBARS; (B) Total glutathione (GSH); (C) Glutathione-S-transferase (GST); (D) Catalase (CAT); and (E) Superoxide dismutase (SOD) content in the mice exposed to cadmium (Cd). \( * * * P < 0.001 \) significantly different from control at \( P < 0.001 \), and \( * * * P < 0.001 \) significantly different from Cd treated group at \( P < 0.001 \) by Newman Keul’s student’s t test after one-way ANOVA. The three antioxidants were effective in the order selenium > curcumin > melatonin
to the membrane of erythrocytes and plasma albumin and is then transported to the liver. Disturbance in the oxidative stress indices of the red blood cells and liver in the present study could be a possible reason for the depletion in the blood parameters. In the recent past, researchers are globally involved in evaluating and screening natural antioxidants in search of ideal ones that can prevent from oxidative stress related disease and can counteract the heavy metal toxicities. In the present study, the three natural antioxidants selenium, curcumin and melatonin were successfully demonstrated to ameliorate Cd induced oxidative damage in the liver, kidneys and red blood cells of mice in the effective order of selenium > curcumin > melatonin. Thus, all the three natural antioxidants (selenium, curcumin and melatonin) proved to be useful in the therapy of Cd poisoning. Our results clearly demonstrated that the natural element selenium was the best antioxidant in annuling the deleterious effects of Cd oxidative stress in liver, kidneys and erythrocytes.

Liver, kidneys, erythrocytes and other blood parameters were chosen as the target organs in the present study for exploring the effect of acute Cd intoxication and were found to be sensitive and convenient markers for screening purposes and may be useful in screening other toxicants and antioxidants using the parameters of oxidative damage as used herein. The present study shows that the antioxidants selenium, melatonin and curcumin exert antioxidative effects both directly as chemical antioxidants due to their ability to scavenge free radicals and by modulating cellular defenses which exert antioxidant effects.

Thus, clinically also the present findings suggest for the inclusion of selenium, melatonin and curcumin in regular diets in one way or the other for obvious reasons as natural protective agents. These three antioxidants are clinically proven for use in human beings although within limitations. The present comparative effects of the antioxidants are in the order selenium > curcumin > melatonin for OS and clearly demonstrate for an ameliorating effect on AOS, especially for people in the areas that have chances of exposure to Cd occupationally or environmentally. Furthermore, the present study shows comparative potential for further research on these three antioxidants, especially the trace element selenium in the blood cells and tissues of liver and kidneys.

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Conflict of interest
The authors declare that they have no conflict of interest.

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