Cymbopogon schoenanthus (Ethkher) ameliorates cadmium induced toxicity in swiss albino mice

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Abstract  Cadmium is among the toxic and hazardous metal widely dispersed in the environment in high levels. Current studies have provided new insights into antioxidant properties of bioflavonoid which have emerged as probable therapeutic and nutraceutical agents. The present study is geared to investigate the possible role of Cymbopogon schoenanthus (L.) Spreng. (or Ethkher) on heavy metal cadmium (Cd) induced oxidative stress in mice. Mice were randomly divided into four groups and treated for 15 days as follows: group 1: normal control-treated (saline); group 2: Ethkher leaves extract-treated (100 mg/kg); group 3: cadmium chloride (CdCl2) treated; group 4: CdCl2 plus Ethkher leaves extract. The results showed a significant reduction in hemoglobin, RBC and hematocrit in cadmium-treated mice as compared to control. Exposure to Cd caused a significant increase in the number of white blood cells (P < 0.05) indicating the occurrence of systemic inflammation. The results of this study also revealed that the mice intoxicated with Cd showed a significant increase in bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGTP) activities. Cd intoxication leads to suppression in humoral immunity. However, pretreatment with Ethkher extract reversed almost all the abnormalities in the blood parameters showing noteworthy protection against cadmium induced toxicity in mice. The outcome of the present study revealed that the Ethkher possessed significant immunomodulatory activity and had a preventive effect on the hematological alterations in Cd intoxicated mice.

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1. Introduction

Cadmium is a hazardous and toxic heavy metal that produces severe noxious health issues in humans (Lee and White, 1980; Jarup et al., 1998; ATSDR, 1999; Singh et al., 2007). It is released into the atmosphere through mining and smelting operations, fuel combustion (Zhang et al., 2012; Chen et al., 2014), burning of wastes from municipal, sludge from sewage (Edwards et al., 2013), and the application of phosphate fertilizer (Gill et al., 2013). Humans can obtain Cd from crops, leaves of vegetables and tobacco (Fan et al., 2009; García-Esquinas et al., 2014), soil (Guo and Zhou, 2006), and fruits and oily seeds (Schwarz et al., 2014). It is known for the cytotoxicity, carcinogenicity, mutagenicity and known to spur free radical production, causing oxidative degeneration of lipids, proteins, DNA, and initiating various disease conditions in humans and animals (Gunnarsson et al., 2003; Manca et al., 1991; Shaikh et al., 1999; World Health Organization, 1992).

The function of a typical cell depends on the equilibrium between the reactive oxygen species (ROS) produced and the mechanisms that propel antioxidant. This balance is obstructed by an increase in the ROS instigating an oxidative stress (Fidan and Dundar, 2008). ROS are part of life and when there are stressful situations there is an increased outlay of energy resulting in more ROS generation and when the levels of these ROS surpass the ability of the body to counteract, they start to damage cells, tissues and organs. Antioxidants possess radical-scavenging properties and prevent lipid peroxidation and other free radical mediated processes. Therefore, they can defend the human body from numerous diseases associated with the reactions of radicals (Takao et al., 1994). Recently, polyphenols have been found to possess radical-scavenging properties and prevent lipid peroxidation and other free radical mediated processes. Therefore, they can defend the human body from numerous diseases associated with the reactions of radicals (Takao et al., 1994).

Cymbopogon schoenanthus (L.) Spreng., (common name lemon grass) (CS) is an aromatic herb and is widespread in North Africa, Arabian Peninsula, India & Pakistan. In Saudi Arabia, there are two species of the Cymbopogon genus, Cymbopogon commutatus (Sakhbar) and the Cymbopogon schoenanthus (L.) Spreng. (or Ethkher) (Atyat, 1995; Hilo, 1996; Chaudhary, 1999). The Saudi folk medicine uses the Cymbopogon schoenanthus (L.) Spreng. for treating people with a kidney stone (Hilo, 1996). The traditional treatment has used as Antihelminthic, antidiarrhea, antirheumatic, carminative, diaphoretic stomachic, diuretic, emmenagogue, for treating fever, for treating jaundice and as tonic. Its aqueous extract is known to heal intestinal troubles, food poisoning and aids in digestion (Atyat, 1995; Khadri et al., 2010).

However, the impact of Ethkher, as a potent antioxidant on cadmium chloride induced oxidative stress on hematology and humoral immunity parameters, on mice has not been studied. Therefore, the primary goal of this evaluation is to explore the potential effect of Ethkher against cadmium chloride intoxication in mice.

2. Materials and methods

2.1. Plant and extract preparation

The dried leaves of Cymbopogon schoenanthus (L.) Spreng. (called Ethkher in Saudi folk medicine) were obtained from the local market and the process of extraction for the hydro-alcoholic extract done as highlighted by Saggu et al. (2014).

2.2. Total flavonoids content

The amounts of flavonoid contents were measured by a colorimetric assay as was emphasized by Rohman et al. (2010). For the calibration curve, rutin was used as a standard and was expressed as mg rutin equivalents (RE) per gram of sample (mg/g) (Fig. 1).

2.3. Determination of total phenolic content

The accumulated amounts of phenolic content were assessed quantitatively as reported by Jindal and Singh (1975). Gallic acid (0.1 mg/ml) was prepared and different concentrations were used for the standard curve and values were expressed in mg/g gallic acid equivalent.

2.4. Assessment of total antioxidant capacity

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) method was utilized for the estimation of total antioxidant capacity as recorded by Brand-Williams et al. (1995). The presence of antioxidants lowers the concentration of DPPH at 515 nm and the engrossing nature disappears as the process continues. Analysis of samples was done in threes, and the outcomes were calculated as averages. A negative control was taken after adding DPPH solution to 0.1 ml of the methanol.

2.5. Animals

The study was conducted in Swiss albino male mice (30–32 g) in Department of Home Economics, Faculty of Specific.
Education, Kafr ElShaikh University, Egypt. The animals were bred and maintained under standard conditions: temperature (25 ± 2 °C) and a photoperiod of 12 h. Commercial pelleted diet and water were given ad libitum.

2.6. Experimental design

Cadmium chloride salt was dissolved in double distilled water and according to the body weight of the animals (5 mg/kg) was injected into mice intraperitoneally (i.p.), daily for 15 days.

The mice were randomly assigned into four experimental groups; each group contained 6 mice, including saline group as control (1 ml/kg for 15 days), CdCl2 group (5 mg/kg body weight, i.p for 15 days), Ethkherr extract (100 mg/kg for 15 days) and CdCl2 plus Ethkherr extract. For co-administration of CdCl2 and Ethkherr extract, Ethkherr extract was gavaged at a dose of 100 mg/kg immediately after i.p. Injection of CdCl2 for 15 days. A low effective dose of 100 mg/kg of the Ethkherr extract was chosen and no signs of mortality and toxicity were observed up to 3000 mg/kg (data not shown). Autopsies were done on the 15th day of post treatment.

2.7. Sample Preparation and Biochemical analysis

Overnight fasted animal blood specimens were taken from the orbital sinus (Riley, 1960) using capillary tubes under mild ether anesthesia. A blood specimen for hematological studies was collected into tubes containing ethylene-diamine-tetraacetic acid (EDTA) as an anticoagulant. ALT, AST (Reitman and Frankel, 1957), ALP (Bessey et al., 1946), bilirubin (Pearlman and Lee, 1974), GGTP (Rosalki, 1975) were estimated.

2.8. Hematological parameters determination

The parameters such as hemoglobin (Hb), red blood cells (RBC), hematocrit (HCT), white blood cells (WBC), neutrophils, lymphocytes, monocytes, were analyzed using automated analyzer.

2.9. Humoral immunity parameters determination

Important immunological tests that include the plaque forming cell (PFC) assay and the quantitative hemolysis of sheep red blood cell, (QHS) test were carried out in the spleen as recorded by Bin-Hafeez et al. (2003) and Simpson and Gozzo (1978).

2.10. Statistical analysis

All data are presented as mean ± SE for at least six replications for each prepared sample. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test to assess significant differences among different groups. The results are considered to be significant when \( P < 0.05 \). All statistical analyses were performed using SigmaPlot, Systat Software program version 11.

3. Results

3.1. Analysis of phytochemical and total antioxidant potential

In this study partial characterization of the Ethkherr extract was done in relation to the total phenolic contents and its total antioxidant capacity (IC50). The extract contains 75 mg/g of the gallic acid equivalent of total phenols which may be responsible for its observed antioxidant activity (Table 1).

The DPPH test was performed to evaluate the free radical scavenging effect of the Ethkherr extract using the IC50 parameter. In this case, IC50 is the concentration of antioxidant required for 50% scavenging of DPPH radicals during a particular time. Ethkherr extract showed the dose-dependent activity and the IC50 value of the extract were found to be 110 µg/ml. It was also found to contain high flavonoid contents (10.42 mg/g of RE) (Table 1).

3.2. Effect of Cd intoxication on the hematological parameters in swiss albino mice

Cadmium when bioaccumulated from different sources, leads to various pathological conditions. Houkpatin et al. (2013) highlighted that the most important tissues in the human body are blood where metabolic changes are reproduced. Therefore, any variations in the parameters of blood are regarded as the most reliable indicators of toxic effects of drugs, chemicals, heavy metals, etc.

In the present study, a significant decrease was observed in hemoglobin (Hb) (\( F_{12,3,;} P < 0.001 \)), hematocrit and red blood cells (RBC) in the cadmium-treated group when compared to control (Fig. 2A, 2B and 2C). The levels of the hematocrit and total RBCs count indicated an adverse effect of Cd as evident in Fig. 2B and C. Cd intoxication indicated a significant decrease from the control treated group in hematocrit and total RBCs count indicated an adverse effect of Cd as evident in Fig. 2B and C. Cd intoxication indicated a significant decrease from the control treated group in hemoglobin and total number of RBCs (\( F_{13,2,;} P < 0.001 \) and \( F_{33,62,;}; P < 0.001 \) respectively). Similarly, pretreatment with Ethkherr extract showed an increase in all of the above said parameters as compared to the cadmium-treated group (\( P < 0.001 \)).

3.3. Alteration in differential counts of peripheral blood leukocytes in Cd intoxicated mice

In the case of differential WBCs count, significant decreases in the value of lymphocytes (\( F_{120,3,;}; P < 0.001 \)) were found as compared with the control group. However, the pretreatment with the Ethkherr extract helps in restoring the count of lymphocytes. A similar trend was observed in monocytes when compared with the Cd-treated group but in the case of neu-

| Table 1 | Total flavonoids; total phenolics contents and percentage inhibition of antioxidant activity (IC50) in Ethkherr leaves extract. Value represents are mean ± S.D of four determinations. |
|-----------------|-----------------|-----------------|-----------------|
| Parameter       | Ethkherr extracts |
| Total phenolic compound (mg/g gallic acid) | 2.8 ± 75 |
| IC50 (µg/ml)    | 110.04 ± 0.35   |
| Total Flavonoids (TF) (mg/g dry weight)  | 10.42 ± 0.08   |
trophil the value increased significantly after treatment (Table 2) $F_{28,3,3}$; $P < 0.001$).

3.4. In vivo hepatoprotective activity of Ethkher extract

The data shown in Table 3 indicate that, AST, ALT, ALP and GGTP activities were markedly increased ($P < 0.001$) in mice intoxicated with Cd (group 3) and in combination with Ethkher extract (group 4) when compared with the control group (group 1). The leakage of above enzymes in the blood reflects the malfunction of liver indirectly due to Cd-induced hepatotoxicity. When compared to the control group administration of Cd to the overnight fasted animals resulted in a 3.0-fold increase in Serum ALT level and a 4.0-fold rise in AST levels (Table 2). The upsurge in the levels of ALP, total bilirubin and GGTP were also clearly evident in Cd-treated animals, indicating liver damage ($P < 0.001$). Pretreatment with the Ethkher extract (100 mg/kg) prevented Cd-induced elevations compared to the control group (Table 3).

3.5. Effect of cadmium on humoral immunity parameters

Cd intoxication leads to suppression in humoral immunity showing a reduction in all the parameters viz., PFC response (Fig. 3A), and QHS response (Fig. 3B). The decrease in PFC response and QHS was significant ($P < 0.01$–$P < 0.001$) when compared to control treated animals (group 1).
Table 2  Effect of Ethkher extract on differential counts of peripheral blood leucocytes in Cd intoxicated mice. Value are mean ± S. E. N = 6 animals per group. Statistical significances: (aP < 0.01, compared with normal control group; bp < 0.05, compared with Ethkher extract group and cP < 0.05, compared with Cd group, where P < 0.001 in all compared groups).

| Parameters               | Control          | Groups                      |
|--------------------------|------------------|-----------------------------|
|                          | WBC (thousands/mm³) | Etkher                              | Cd (5 mg/kg) | Ethkher + Cd |
|                          | 10.76 ± 0.75     | 10.68 ± 0.15               | 9.15 ± 0.07a,b | 10.07 ± 0.04  |
| Lymphocytes (%)          | 71.39 ± 0.16     | 70.21 ± 0.12               | 66.7 ± 0.05b  | 69.70 ± 0.3a,c |
| Neutrophils (%)          | 31.60 ± 1.1      | 30.76 ± 0.25               | 38.80 ± 1.1a,b | 28.20 ± 0.68a,b,c |
| Monocytes (%)            | 1.86 ± 0.02      | 1.76 ± 0.05                | 0.38 ± 0.02a,b | 0.83 ± 0.05a,b,c |

4. Discussion

Tabuk is one of the important regions of Saudi Arabia, characterized by the presence of an abundant flora of medicinal value and Ethkher is one of the important therapeutic plants being used today for the extraction of different phytochemical components for the treatment of fatal diseases like, cancer, diabetes hypertension etc. The present work investigated the impact of Ethkher leaves extract against cadmium chloride-induced perturbations on hematological and humoral immunity parameters.

In the current study, the hydroalcoholic extract of Ethkher was tested for their total antioxidant activity, total phenolic and total flavonoid content (Table 1). Phenolics are non-enzymatic antioxidants and the antioxidant property owes to the redox properties, which permit their action as reducing agents, hydrogen donors and singlet oxygen quenchers (Thirugnanasampandan and Jayakumar, 2011; Buck et al., 1976).

After 48h of Oral administration of Ethkher extract showed no signs of morbidity or mortality after 48h in doses up to 3000 mg/kg, warranting a possibility that the extracts were safe to be used and are nontoxic. Plants with LD values past than 5000 mg/kg are regarded as nontoxic.

In our study some of the blood parameters like Hb decreased along with RBC and WBC count. This decrease might be because of the oxidative damage to the erythrocytes induced by cadmium, resulting to the damaging of the cell membrane by increasing lipid peroxidation or oxidative stress (Hounkpatin et al., 2013). The differential leukocyte count showed low lymphocyte and high neutrophil values in Cd intoxicated group. Although the peripheral cell counting is not adequate to indicate a change in quality and quantity of precursor cells, it is still the way to determine the toxicity on hematological indices.

Liver function tests revealed that the Cd intoxication in mice produced a significant (P < 0.001) rise in enzyme levels such as AST, ALT, ALP, GGTP, as well as total bilirubin, when compared to the control treated group. There was a significant (P < 0.001) retrieval of these enzyme levels on the administration of the Ethkher extract (Table 2). For the assessment of liver injury these enzymes are principally used and can be found in the cytosol and any damage liberates the enzyme into the blood circulation and hence it is determinable. Increased amounts of these enzymes are indices of cellular spillage and loss of functional integrity of cell membranes in the liver (Drotman and Lawhan, 1978). Administration of the Ethkher extract resulted in the restoration of increased serum enzymes which may be because of the membrane stabilizing action to prevent leakage of intracellular enzymes. This coincides with the generally accepted opinion that the serum levels of transaminases return to their normal state with the curing of hepatic parenchyma and the reformation of hepatocytes (Thabrew and Joice, 1987).

In this study using plaque-forming cell assay the number of antibodies secreting cells from spleen was determined. Pretreatment with the Ethkher extract secured them from Cd-induced inhibition of humoral immunity. However, there was no inhibitory effect on the identified parameters of humoral immunity by the plant extract. As a matter of fact all the parameters of humoral immunity that were inhibited as a result of Cd intoxication showed recovery in the animals treated with Ethkher extract (Fig. 3). The humoral mediated immunity involves the interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody secreting plasma cells. Antibodies perform the task as the effectors of the humoral response by binding to the antigen and neutralizing its effect by ensuring that it is eliminated through cross-linking to form clusters that can be ingested easily by phagocytic cells. The effect was significant.

Table 3  Effect of Ethkher extract on liver function markers and total bilirubin in Cd intoxicated mice. Values are mean ± S. E. N = 6 animals per group. Statistical significances: (aP < 0.01, compared with normal control group; bP < 0.05, compared with Ethkher extract group and cP < 0.05, compared with Cd group, where P < 0.001 in all compared groups).

| Group                | ALT (U/L) | AST (U/L) | ALP (U/L) | GGTP (U/L) | Total Bilirubin (mg/dl) |
|----------------------|-----------|-----------|-----------|------------|------------------------|
| Control              | 35.1 ± 1.4| 42.3 ± 5.7| 107.3 ± 4.0| 6.5 ± 2.0  | 0.18 ± 0.02             |
| Ethkher extract      | 34.0 ± 2.4| 40.2 ± 2.2| 102.2 ± 4.3| 5.8 ± 1.5  | 0.19 ± 0.03             |
| CdCl₂                | 105.4 ± 2.1a,b | 169.3 ± 10.2a,b | 192.7 ± 7.8a,b | 15.3 ± 1.8a,b | 1.42 ± 0.05a,b          |
| CdCl₂ + Ethkher extract | 82.4 ± 4.7a,b,c | 95.4 ± 2.1a,b,c | 124.4 ± 3.4a,b,c | 10.2 ± 1.4a,b,c | 0.32 ± 0.03a,b,c        |
P < 0.01) when compared to the control. The pretreatment with Ethkher extract indicates that the immunostimulation has achieved through humoral immunity.

Cd-induced immunosuppression mechanism is not entirely known. Any alterations in the immunological parameters may reflect an intense stress effect of Cd (Sovenyi and Szakolczai, 1993). Previous reports showed that the heavy metals such as Cd prevent immune cell activity through direct toxicity to cellular molecules and structures. Nonetheless, the metals can also alter the behavior of cells through affecting specific genes by transmitting or influencing signals controlling gene expression (Koropatnick and Zalups, 1997).

5. Conclusions

Ethkher extract suffices as a possible source of natural antioxidants, phenolics, alkaloids that contain potent immunomodulatory action. The extracts may be protecting the liver through free radical scavenging activity and this may be due to the ubiquity of antioxidants and flavonoids in the extract. The further precise and full inquest may shed light on this aspect to be used as in polyherbal formulations.

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