Research Article

Moringa oleifera aqueous leaf extract: role on total leucocyte count and its differentials in cadmium toxicity in adult Wistar Albino rat model

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Abstract

Background: Cadmium (Cd), an environmental metallic toxicant with varying degrees of toxicity, exists in different oxidational or transitional states and causes various blood disorders. Cadmium (Cd) is known to affect various organs on both acute and chronic exposures. Studies have revealed that the most important tissue in body in which metabolic alterations are mainly reproduced is the blood. *Moringa oleifera,* originally from India, has been scientifically assessed for various medicinal applications. **Aims and Objective:** To examine the effect of *M. oleifera* aqueous extract taken from the leaf on total leukocyte count including its differentials (neutrophils and lymphocytes) in cadmium-treated rats. **Materials and Methods:** Twenty-four adult Wistar Albino rats, weighing between 180 and 200 g were broadly divided into four groups, six animals in each group, with group I being the control. Group IV was pretreated with the extract and then cadmium chloride orally for 1 day. Data were presented as mean ± SD and $p \leqslant 0.05$ considered significant. **Result:** The results portrayed that pretreatment with *Moringa* leaf extract, 100 mg/kg/bw, earlier to the cadmium administration exhibited a significant increase ($p \leqslant 0.001$) in the total leukocyte count including its differentials in comparison with the cadmium-exposed group, whereas the total WBC count was significantly decreased in group III in comparison with groups I and II. This study depicts a marked increase in the total leukocyte (T-WBC) count and its differentials in the animals pretreated with *Moringa oleifera* leaf extract, before the infusion of cadmium could be an indication that *M. oleifera* offers protection against damage to hematological parameters induced by cadmium. **Conclusion:** Therefore, our study suggests that aqueous leaf extract of *M. oleifera* enhances the immune mechanisms to fight back foreign substances when exposed to cadmium toxicity.

KEY WORDS: Cadmium; Blood Disorders; ; Leukocyte Count; Immune Mechanisms

Introduction

Cadmium is present in the environment and has a wide variety of industrial applications.[1] Cadmium is a type of carcinogen as recommended by International Agency for Research on Cancer.[2] It is mainly found in ambient air and in paints.[3] Environmental exposure to cadmium can occur through diet, drinking water, or fume inhalation.[4] Cadmium leads to alterations in the antioxidant defense system in the body.[5] Research has revealed that high doses of environmental contaminants, mainly cadmium, are present in fish, shrimp, various vegetables, seafood, giant snails, etc. Moreover, it is also known that contamination of food by cadmium and other toxic metals is directly related to their presence in the environment. Excessive levels of cadmium is toxic to humans as, once absorbed, it is carried via blood to soft tissues and can also affect the blood parameters such as red blood cell (RBC)

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count and white blood cell (WBC) count.[6] *Moringa oleifera* is from *Moringaceae* family. This plant family is known to possess hypotensive, anticancer, and antibacterial activity and is also rich in a number of vitamins, minerals, and other more common phytochemicals such as carotenoids, including carotene or provitamin. It is also known to alter the blood parameters.[7] Although levels of these phytochemicals (bioactive compounds) were higher in the leaves than the seeds, mineral content of *M. oleifera* showed variations in composition with changes in location. Hematology, which has been defined as the study of blood, forms an important part of various diagnostic processes as it includes the examination of its cellular and fluid portions including the tissues that form, store, and circulate blood cells. Studies have documented that the white blood cells contribute to the defense mechanisms of the body. It is known that the major function of phagocytes is to ingest and destroy defend the invading microorganisms and defend the body against them.[8] Although literature shows that *M. oleifera* extracts have been used to combat metal intoxications with cadmium, research lacks information about the effect of *M. oleifera* leaf extract on the total leukocyte count and its differentials (neutrophils and lymphocytes) in cadmium-exposed rats. So, the current experiment was done to evaluate the end result of aqueous leaf extract of *M. oleifera* on the total leukocyte count including its differentials in cadmium-induced toxicity in adult Wistar Albino rats.

Materials and Methods

The chemicals required for the study were procured from Durga Laboratories, Karnataka, South India. The experiment was conducted in the year 2011–2012. All the experimental procedures and animal maintenance were followed according to the rules and regulations laid down by Ethical Committee of the Institute.

Plant Materials

We collected *M. oleifera* leaf samples from cultivated areas in the coastal region of Karnataka, India, which were identified and validated by a plant taxonomist.

Extract Preparation

The leaves were washed meticulously and, after drying, were grinded into coarse powder; 20 g of the powder was soaked in 100 ml water and chloroform, which was kept in a shaker for 3 days. After filtering, original volume was reduced to its 10%. The filtrate was further concentrated in a rotary evaporator and dried in a water bath. The procedure was done at Yenepoya Medical College, Yenepoya University in Mangalore, South India.[9]

Experimental Animals

In-bred adult male Wistar Albino rats were provided by the Institute. The rats were of 180–200 g weight. Food and water was provided *ad libitum.*

Experiment Protocol

Twenty-four male rats of weight 180–200 g were divided into four groups, with six animals in each:

Group I: control (infusion of normal saline).

Group II: pretreated with *M. oleifera* leaf extract, 100 mg/kg/bw orally for 10 days.

Group III: one dose of cadmium chloride (10 mg/kg/bw) orally.

Group IV: pretreated with the extract (100 mg/kg/bw) for 10 days and then cadmium chloride (10 mg/kg/bw) orally for 1 day.

Hematological Measurements

We collected 4 mL of blood by cardiac puncture; 23 G needles were used. The sample was then placed in EDTA tube. It was immediately used for determining[10] total white blood cell (T-WBC)/leukocyte count[11] along with differential count of neutrophils and lymphocytes only.[12]

Statistical Analysis

Data were presented as mean ± SD. Student *t* test was used for comparison; $p \leqslant 0.05$ was considered as significant.

Result

Figure 1 presents *M. oleifera* leaf extract's effect on the T-WBC count in cadmium-induced Wistar Albino rats when compared with the other groups. The total leukocyte count in the control group was 4.58 ± 0.15.

taThe total WBC count decreased significantly (***$p < 0.0001$) in group III (3.41 ± 0.18) when compared with groups I and II rats (4.89 ± 0.09), whereas group IV showed a significant (***$p < 0.0001$) increase in the T-WBC (4.50 ± 0.14) when compared

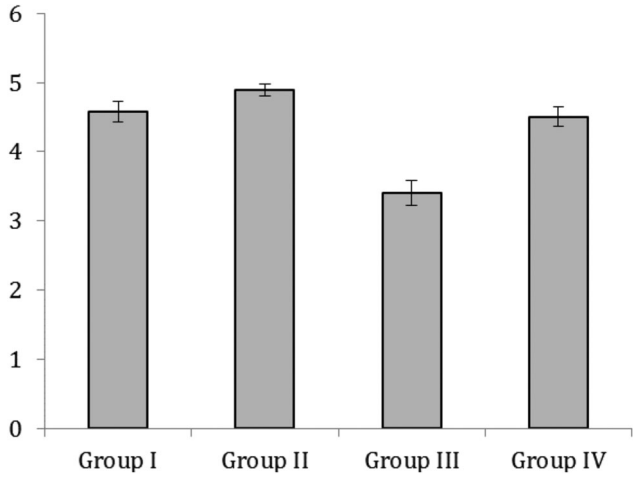

Figure 1: Effect of aqueous leaf extract of *Moringa oleifera* on T-WBC Count in cadmium-induced Wistar Albino rats (group III vs. groups I and IV; ***$p \leqslant 0.0001$).

Table 1: Effect of *Moringa oleifera* extract on neutrophil count in cadmium-exposed Wistar Albino rats

Groups (n = 6)	Neutrophil count (%)
I	50.91 ± 0.91
II	52.58 ± 1.2
III	48.91 ± 0.73***
IV	50.50 ± 1.04 **

Group III vs. group I, ***$p \leqslant 0.001$; group III vs. group IV, **$p \leqslant 0.01$.

with cadmium-alone–exposed group. But, there was no significant difference in T-WBC count between groups IV and I.

The changes in neutrophil count (differential of WBC) are depicted in Table 1. Neutrophil count decreased significantly (***$p \leqslant 0.001$) in cadmium-exposed rats (48.91 ± 0.73) when compared with the control rats (50.91 ± 0.91). A significant increase in the neutrophil count was also observed between group IV (50.50 ± 1.04) and group III (**$p \leqslant 0.01$).

The variations in lymphocyte count (differential of WBC), as observed in Table 2, showed significant differences between group III (46.76 ± 0.22) vs. group I (47.83 ± 0.25; ***$p \leqslant 0.0001$) and between group III vs. group IV (47.58 ± 0.24; **$p \leqslant 0.002$).

Discussion

Cadmium, a toxic environmental pollutant, when accumulated in the body at high levels from different sources, leads to various disease conditions.[13] Literature has revealed that blood being the most important tissue in our body in which metabolic changes are reproduced,[8] any alterations in blood parameters have always been considered to be the most reliable indicator of toxic effects of any substance, such as drugs and heavy metals.[14] In this study, on cadmium exposure, a substantial reduction in total leukocyte count revealed the toxic effects of cadmium on the hematological parameters, which is in accordance with previous studies.[15,16] Decrease in the neutrophil and Lymphocyte counts with cadmium also indicates the effects of cadmium toxicity.[6] The fall in T-WBC and its differentials (neutrophils and lymphocytes) suggests decrease in immune in fighting foreign substances.[17] The toxic properties of cadmium is generally because of its binding with reactive agents. It causes inhibition of enzymatic reactions, affecting general growth, development, and reproduction.[3] *M. oleifera*, being a multipurpose tree, with various pharmacological properties has been used in the treatment of innumerable disorders.[18] Leukocytes are formed naturally against any foreign substances in the body. Literature has shown that the principal function of WBCs as phagocytes is to defend against invading microorganisms or is xenobiotic.[19] This study depicts a marked increase in the T-WBC count and its differentials in the animals pretreated with *M. oleifera* leaf extract, before the infusion of cadmium, which could be an indication that *M. oleifera* offers protection against damage to hematological parameters induced by cadmium.[20] The principal function of phagocytes is to protect our body against invading microorganisms by ingesting and destroying them and, hence, contributing to cellular mechanism. However, our study depicted an increase in the T-WBC count along with its differentials in the *M. oleifera* alone-treated group, which shows that cellular inflammatory processes is enhanced, and this may account for its antibacterial activity also.[21–23]

Table 2: Effect of aqueous leaf extract of *Moringa oleifera* on lymphocyte count (differential of WBC) in cadmium-exposed Wistar Albino rats

Groups (n = 6)	Lymphocyte count (%)
I	47.83 ± 0.25
II	48.35 ± 0.3
III	46.76 ± 0.22 ***
IV	47.58 ± 0.24**

Group I vs. group III, ***$p \leqslant 0.0001$; group III vs. group IV, **$p \leqslant 0.0002$.

Conclusion

Current research findings reveal that the aqueous leaf extract of *M. oleifera* protects against cadmium-induced hematological toxicity in rats. Future studies in this aspect will help to explore the multisided mechanisms leading to these beneficial outcomes.

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