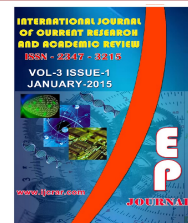




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### Protective Role of Aqueous Leaf Extract of *Moringa oleifera* on Blood Parameters in Cadmium Exposed Adult Wistar Albino Rats

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#### KEYWORDS

Cadmium,  
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anaemia

#### A B S T R A C T

Cadmium is one of the toxic, hazardous metals widely dispersed in the environment in high levels. Studies have shown that excessive exposure of this biological hazard can even cause death. It is well known that the most important tissue in our body in which metabolic changes are reproduced is the blood. The present study was conducted to evaluate the role of *Moringa oleifera* leaf extract on haematological parameters in cadmium exposed rats. Twenty-four adult male *Wistar Albino* rats, weighing between (180-200) g were broadly divided into four groups, each had six animals, with group I being the control. Pre-treatment with *Moringa* leaf extract, 100 mg/kg/bw, prior to the administration of cadmium showed a significant increase ( $p < 0.0001$ ) in the haematological parameters, like red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular haemoglobin (MCH) & a decrease in mean corpuscular volume (MCV) as compared to the cadmium treated group. Therefore, our results suggest that aqueous leaf extract of *Moringa oleifera* has a positive effect on cadmium induced anaemia & other manifestations induced by this biological toxicant.

### Introduction

Cadmium, an environmental metallic toxicant with varying degrees of solubility, absorption and toxicity (Falks et al 1990), exists in different oxidation or transitional states (Donald 1996). The compound has a high acute toxicity on developmental effects (Calabrese & Kenyon 1991). It has been observed to have a high affinity for some

organs like, the liver & kidney (Cai et al., 2001). *Moringa oleifera* (*Moringaceae*), a small, fast-growing, ornamental plant species originally from India has been scientifically evaluated for its possible medicinal applications (M. H. S. Mughal et al., 1999). For centuries, different parts like,

root, bark, pods, flowers, seeds, gum leaves, etc., of this miraculous tree have been used in traditional medicine for the various pharmacological & nutritional purposes (M. H. S. Mughal et al., 1999). Cadmium has been recognized as a biological toxicant (Hounkpatin A. S. Y et al., 2013). It is associated with several clinical complications like, bone abnormalities, haematological alterations, renal & hepatic dysfunctions, etc., (Jarupet al., 2000). A study conducted on rats has also reported that there is a significant decrease in the red blood cell (RBC) count & haemoglobin (Hb) concentrations, packed cell volume (PCV), mean corpuscular volume (MCV) & mean corpuscular haemoglobin (MCH), leading to anemia, on cadmium exposure (Hounkpatin A. S. Y et al., 2013). This might be due to the fact that cadmium on entering into the blood stream, binds to the erythrocyte membranes causing an increased haemolysis & destruction, leading to alterations in the haematological parameters (Branka I et al., 2001). But, the production of reactive oxygen species (ROS) can be attributed to one of the most common causes for the reduction in the haemoglobin concentration which also results in the destruction of the red blood cell membrane and its functions (Hounkpatin A. S. Y et al., 2013). *Moringa oleifera*, also called as the miracle tree, is the best known species of *Moringaceae* family, having a wide range of nutritional, medicinal & nutraceutical uses throughout the world (M. H. S. Mughal et al., 1999). Leaves of this tree are a good source of iron, vitamins (A, B, C), minerals including calcium, copper, sulphur, potassium, carotenoids - leutin, alpha & beta-carotene, xanthine, chlorophyll, phytochemicals with known powerful antioxidant properties (Sabale et al., 2008, Ho, 1994, Siddhuraju et al., 2003). Hence extracts from different parts of this plant have been used in the treatment of various conditions like fever,

bronchitis, eye/ear infections, inflammation of mucous membrane, diarrhea, gastric ulcer, & also as an anti-cancer, anti-inflammatory and hepato-protective agents (Aslam et al., 2008; Caceres et al., 1991; Ghasi et al., 2000; Kumar et al., 2003). Studies have shown that, the leaves of this plant contain vitamins and Fe in significant amounts & hence help to improve iron and blood status in rats (Vermaet al., 1976, Dhar and Gupta, 1982). It is well documented that *Moringa oleifera* extract facilitates iron absorption, as adequate amount of this element is essential for haemoglobin (Hb) synthesis and also for the animal tissues like, the kidneys and bones to take part in manufacture of the red blood cells/RBCs' (Hisham M. Osman et al., 2012). Although, many studies have shown that extracts from different parts of *Moringa oleifera* could be used to combat various metal intoxications like cadmium, arsenic, lead etc., (Gupta et al., 2005, Sirimongkolvorakulet al., 2012), literature lacks information about the effect of *Moringa oleifera* leaf extract on red blood cell count, haemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV) & mean corpuscular haemoglobin (MCH), in cadmium exposed rats.

Therefore, the study was conducted to evaluate the effect of aqueous leaf extract of *Moringa oleifera* on haematological parameters in cadmium exposed adult Wistar Albino rats.

### **Materials and Methods**

The chemicals used for the biochemical assay were obtained from Durga Laboratories, Mangalore, Karnataka, India. The study was conducted in the year: 2011-2012. In this experiment, guidelines of Institutional Ethics Committee and to that of

Federal laws for the use of animals were followed strictly for all the experimental procedures and animal maintenance.

### Plant materials

Samples of *Moringa oleifera* leaves were collected from various plantations in & around Mangalore, Karnataka, India. These were then identified and authenticated by a plant taxonomist.

### Preparation of extract

The leaves obtained from this plant were first cleaned thoroughly, dried in room temperature & then crushed into coarse powder. From this, about 20 g of the powder was taken and soaked separately in 100 ml of water and chloroform. All these were done by keeping it in a Shaker for 3 days. It was then filtered through cheese cloth and reduced to around 10% of its original volume (organic solvent). A rotary evaporator was used to concentrate the filtrate in vacuum. The aqueous extract was dried using water bath. The whole process of extract preparation was carried out in Yenepoya Medical College, Yenepoya University, Mangalore, Karnataka, India (Mukerjee, 2002).

### Experimental animals

Adult male *Wistar Albino* rats, weighing between (180-200) g were obtained from the animal house of Kasturba Medical College (KMC), Manipal University (MU), Mangalore, Karnataka, India. The Institutional Animal Ethical Committee (IAEC) approval was also obtained. The animals were housed in the Institutional Experimental Animal Laboratory. They were having free access to food (standard diet) & water. The study was conducted for a duration of ten days.

### Experimental design

Aqueous leaf extract of *Moringa oleifera* was used. A total of twenty four (24) adult male *Wistar Albino* rats, weighing between (180-200) g were taken into the study. They were then broadly divided into four (4) groups. Each group had six (6) animals, as follows:

Group i - control group, received normal saline only.

Group ii - experimental control group, pre-treated (oral) with *M. oleifera* leaf extract, 100 mg/kg/bw) for 10 days.

Group iii - received a single oral dose of cadmium chloride (10 mg/kg/bw).

Group iv - pre - treated with *M. oleifera* leaf extract (100 mg/kg/bw) for 10 days, followed by cadmium chloride (10 mg/kg/bw) given orally for one day.

### Haematological measurements

Using 23 G needles, about 4 ml of blood was collected directly via cardiac puncture. The sample was then placed in EDTA tube following which it was used immediately for measurement of blood parameters using automated apparatus. The blood parameters estimated were red blood cells (RBCs) count, hemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume/MCV & mean corpuscular haemoglobin /MCH (Hisham M. Osman et al., 2012). Mean corpuscular volume (MCV) & mean corpuscular haemoglobin concentration (MCH) was calculated by using standard formula (C.L.Ghai, 2013).

### Statistical analysis

The data were expressed as means  $\pm$ SD, with a total of 6 animals per group. The differences between the groups were

compared for statistical significance by using the student t test with the level of significance taken as  $P < 0.05$ .

### **Result and Discussion**

The results depicted in Table 1 show a significant increase ( $p < 0.05$ ) in the RBC count, Hb concentration & packed cell volume in group II as compared to group I. The group which received pre-treatment with aqueous leaf extract of *Moringa oleifera* before cadmium exposure showed a significant increase in the above mentioned haematological parameters as compared with the cadmium alone treated group.

Cadmium, a toxic environmental pollutant has no known biological functions & when accumulated in the body from various sources, leads to various disease conditions (Seymore, 1994, Othumpangat et al., 2005). Study has reported that the most important tissue in our body in which metabolic changes are reproduced is the blood (Hounkpatin A. S. Y et al., 2013). The alterations in the blood parameters are therefore considered to be one of the most reliable indicators of toxic effects of drugs, chemicals, heavy metals, etc., (Lodia and Kansala, 2012). *Moringa oleifera*, a multipurpose tree, has been used for medicinal purposes in the treatment of various disorders (Goyal B R. et al., 2007).

Our findings revealed that cadmium exposure decreased the red blood cell (RBC) count, haemoglobin (Hb) concentration & packed cell volume (PCV) significantly along with an increase in the mean corpuscular volume (MCV) & a reduction in the mean corpuscular haemoglobin (MCH)

respectively, indicating the toxic effects of cadmium on the haematological parameters, which is in accordance with the previous studies (Hounkpatin A. S. Y et al., 2013, Ognjanovic et al., 2003). It is well known that the increased destruction of erythrocytes as evidenced from decreased red blood cell count & packed cell volume, is due to oxidative damage to the erythrocytes, induced by cadmium, causing destruction of cell membrane by increasing lipid peroxidation (Branka I et al., 2001). The drop in haemoglobin (Hb) concentration can be attributed to the production of reactive oxygen species/ROS (Hounkpatin A. S. Y et al., 2013). All these can lead to cadmium induced anaemia as reported in other studies (Branka I et al., 2001, Kostic M.M. et al., 1993).

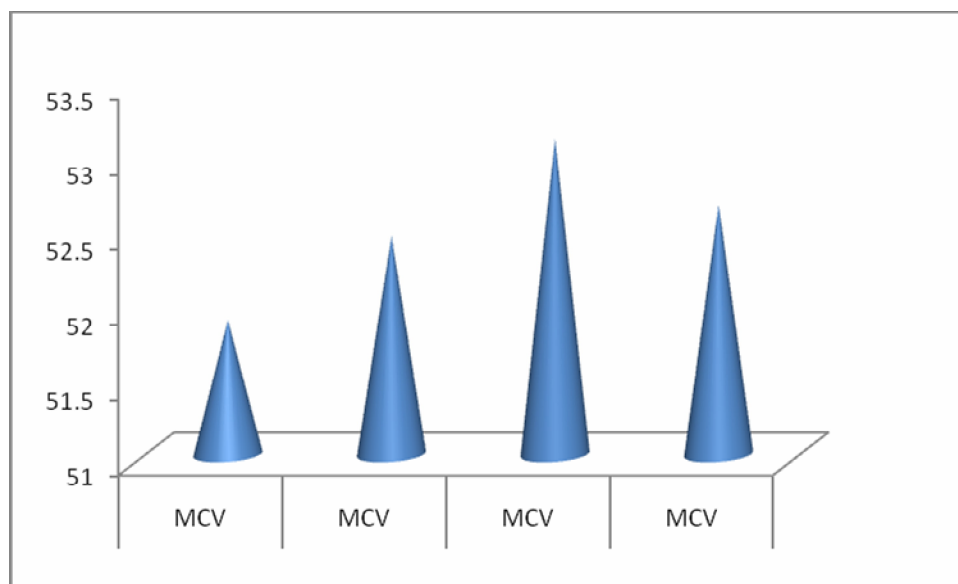
Pre-treatment with *Moringa* leaf extract, prior to cadmium exposure increased the haematological parameters, like red blood cell (RBC) count, haemoglobin (Hb) concentration & packed cell volume (PCV) but the mean corpuscular volume (MCV) decreased & mean corpuscular haemoglobin (MCH) increased, as compared to the cadmium treated group. The value of MCH in group IV is almost same as that in the normal control group. This might be due to the fact that the *Moringa oleifera* leaf extract contains some phytoconstituents which should have boosted haematopoietic activities (Ujah O. F et al., 2013). This is supported by the fact that the leaves of this miraculous plant are an outstanding source of vitamins (A, B, C), minerals (iron) & proteins. Therefore it has been prescribed for the treatment of various conditions & appears to be aid full in the management of anaemia also (Ujah O. F et al., 2013).

**Table.1** Effect of pre-treatment with *Moringaoleifera* leaf extract on haematological parameters in cadmium exposed rats (values are expressed as mean  $\pm$  SD)

| Groups   | Number of animals (n) | RBC count (millions/cu.mm) | Hb (g%)           | PCV (%)           |
|--|-----------------------|----------------------------|-------------------|-------------------|
| Normal control (Gr.I)                                      | 6                     | 6.92 $\pm$ 0.09            | 12.16 $\pm$ 0.06  | 35.91 $\pm$ 0.28  |
| Pre-treated with control with Moringa leaf extract (Gr.II) | 6                     | 7.14 $\pm$ 0.05*           | 13.20 $\pm$ 0.05* | 37.45 $\pm$ 0.33* |
| Cadmium treated (Gr.III)                                   | 6                     | 6.47 $\pm$ 0.22*           | 11.24 $\pm$ 0.08* | 34.36 $\pm$ 0.07* |
| Pre-treatment with moringa + cadmium (Gr.IV)               | 6                     | 6.75 $\pm$ 0.03*           | 11.86 $\pm$ 0.07* | 35.55 $\pm$ 0.15* |

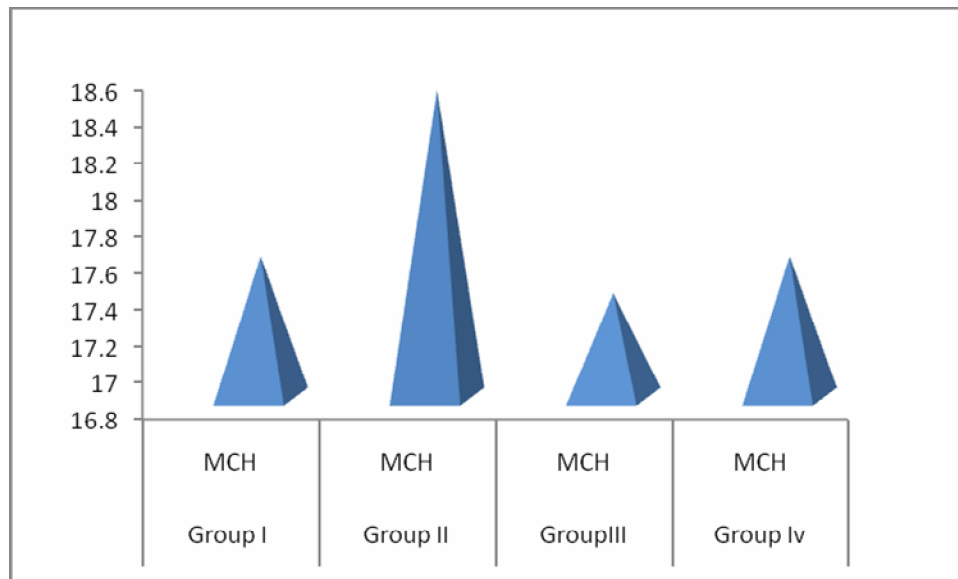
Gr.I vs Gr.II, Gr.III&Gr.IV - \*p < 0.05

Figure I & II shows the changes in the blood indices in cadmium exposed rats. A significant increase (p < 0.05) in mean corpuscular volume (MCV) & a decrease in the mean corpuscular haemoglobin (MCH) in group III as compared to group I & II whereas, group IV showed a decrease in MCV & increase in MCH when compared to group III. The value of MCH observed in group IV is almost similar to the normal control group.



**Figure 1:** Effect of pre-treatment with *Moringaoleifera* leaf extract on mean corpuscular volume (MCV) in cadmium exposed rats (values are expressed as mean  $\pm$  SD)

Gr.I vs Gr.II, Gr.III & Gr.IV - \*p < 0.05



**Figure.2** Effect of pre-treatment with *Moringaoleifera* leaf extract on mean corpuscular volume (MCV) in cadmium exposed rats (values are expressed as mean ± SD)

Gr.I vs Gr.II, Gr.III&Gr.IV - \*p < 0.05

Therefore it has been prescribed for the treatment of various conditions & appears to be aid full in the management of anaemia also (Ujah O. F et al., 2013).

### Conclusion

The *Moringa oleifera* aqueous leaf extract, due to its erythropoietic potentials, has a positive effect on the haematological toxicity induced by cadmium. Hence, the leaf extract of *Moringa oleifera* appears to be supportive in the management of anaemia.

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