# Protective Influence of Coffee Bean Crude Extract on Cadmium Chloride-Induced Alterations in Hematological Parameters of Swiss Albino Mice

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**Abstract -** Anemia is a condition primarily characterized by lower than normal red blood cell count, hemoglobin and hematocrit concentrations. Due to the danger brought about by this condition, several folkloric practices and herbal medicines have been used to prevent and correct it. One of these traditional practices is the use of coffee plant that is said to contain components that can act against anemia by protecting the cells from oxidative damage. Therefore, this study was undertaken to investigate the protective effects of coffee bean crude extract against cadmium chloride-induced anemia. The protective influence of the extract was tested by exposing the albino mice to cadmium chloride. Hematologic parameters were conducted in order to assess the activity of the extract against cadmium chloride-induced oxidative stress. Animals treated with only cadmium chloride revealed a marked decline in red blood cell count, hemoglobin and hematocrit levels. Normal red blood cell indices were observed and peripheral blood smear evaluation revealed the induction of normocytic, normochromic anemia. On the other hand, the experimental groups pretreated with coffee bean crude extract at a dose of 50 mg/kg body weight and 100 mg/kg body weight respectively showed protection against cadmium chloride-induced oxidative stress. Morphological evaluation of the red blood cells of these animals also revealed normocytic, normochromic cells. In conclusion, the plant extract has the ability to protect the cells against the damaging effects brought about by cadmium chloride administration.

**Keywords:** *anemia, coffee bean, Coffeacanephora, cadmium chloride, oxidative damage, red blood cell indices*


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**INTRODUCTION**

The red blood cells (RBC) or erythrocytes are the most common type of bloodcells having an average disk diameter of 6.0-8.0 μm and an average thickness of 1.7-2.2 μm. RBC's are disc-shaped when not subjected to external stress. The characteristic shape and corresponding deformability is an essential feature of the biological function of transporting oxygen from the lungs to the tissues and carrying carbon dioxide from the tissues to the lungs. Gas transport particularly oxygen delivery is accomplished by the attachment of RBC's major cytoplasmic content – hemoglobin (Silva, Dao, Han, Lim and Suresh, 2010).This role is essential for the normal functioning and survival of organisms. The most common disorder associated with these blood cells is termed anemia.

Anemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development (Al-Sayes, Gari, Qusti, Bagatian & Abuzenadah, 2011). According to the latest National Nutrition Survey of the Food and Nutrition Research Institute of the Department of Science and Technology, the prevalence of anemia in the Philippines has reached an alarming level having three out of ten Filipinos inflicted with anemia (Capanzana, 2009)

Anemia is described as a diminution from the normal value, in the total number of erythrocytes, amount of circulating hemoglobin and RBC mass of a particular patient. It can also be referred to as a reduction in red blood cell count, hemoglobin and hematocrit levels below the reference range for healthy individuals of the same age, sex and race, under similar environmental conditions (Mehta, 2012).

Since this condition leads todiminished oxygen carrying capacity of the blood and all human cells depend on oxygen for survival, a broad range of clinical consequences can be acquired from varying extents of anemia. Several kinds of anemia can be classified in a variety of ways. It is primarily based from three points of view: pathogenesis, red cell morphology and clinical presentation (Chulilla, Colas & Martin, 2009).

Cadmium which is an extremely toxic heavy metal is said to be a hemotoxin (Eteng, Onwuka, Akpanyung, Osuchukwu, Bassey & Nwankpa, 2012; Singh, Deora, Mogra, Patni and Sankhla, 2012; Vinodhini and Narayanan, 2009; Siddhu,Sirohi, Kashyap, and Ali Khan, 2008; Sinha, Manna & Sil, 2008; Ognjanovic, Markovic, Pavlovic, Zikic, Stajn & Saicic, 2008). It induces anemia by three mechanisms: hemolysis due to a deformity of peripheral red blood cells, iron deficiency through competing with duodenal iron absorption,


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and renal anemia derived from hypoproduction of erythropoietin (Horiguchi, Oguma, Kayama, 2011)

After exposure, cadmium entered the blood, bound with erythrocytic membrane, and stimulated formation of the reactive oxygen species and metallothioneins (Shekha, Sabir and Fatah, 2008) leading to alterations in the antioxidant system of erythrocytes and imposing oxidative damage upon the membrane (Sinha, Mannaand Sil, 2008). Anemia is one of the characteristic clinical manifestations of chronic cadmium intoxication as cadmium is known to reduce red blood cell count, hematocrit value and hemoglobin concentration (Sinha, Manna & Sil, 2008).

The plant, *Coffeacanephora,* particularly its extract, has been proven to have favorable biological effects because it serves as an antioxidant, inhibits sickling of red blood cells and ameliorates hemorheology thereby protecting blood cells from oxidative damage. It is cultivated in many countries primarily in equatorial Latin America, Southeast Asia and Africa. It appears as a small tree that grows 15 feet tall. The leaves are dark green and glossy and flowers irregularly, taking about 10 to 11 months for cherries to ripen producing oval-shaped beans. Of the two main species grown, *Coffeacanephora*more popularly known as coffee Robusta tends to produce a strong, full-bodied coffee with a distinctive earthy flavor, but usually with more bitterness than *Coffea arabica* due to its pyrazine content. This strain of coffee contains about 40%-50% more caffeine and is also less susceptible to pests and disease as compared to coffee Arabica. Further,it has been used traditionally in rendering a stimulating effect on humans because of its caffeine content and in reducing risk of Alzheimer's disease, dementia, heart disease, Diabetes Mellitus type 2, Parkinson's disease and gout(Li, Wu, Zhao, Xiong & Zhu, 2011).

*Figure 1. Coffeacanephora plant and green seeds*

The aim of the study, therefore, is to investigate theprotective role of coffee bean extract on cadmium chloride-induced anemia by assessment of hematological parameters such as red blood cell count, hemoglobin and hematocrit. Morphological assessment of red blood cells was accomplished on the basis of RBC indices such as Mean Cell Volume (MCV), Mean Cell Hemoglobin(MCH) and Mean Cell Hemoglobin Concentration(MCHC), and peripheral blood smear evaluation.The


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protective influence brought by the pretreatment of the extracts can be utilized to develop a therapy of low cost and is readily accessible to Filipinos debilitated by anemia.

## MATERIALS AND METHOD

### Plant Material

The green coffee beans were obtained from a coffee farm in Carmel School, Pallocan, Batangas City, Philippines. The vouchers of the plant specimen were submitted at Bureau of Plant Industry, San Andres Street, Malate, Manila for authentication.

### Chemicals

Cadmium chloride was used as the source of cadmium. It was purchased from Paramedic Supplies and Equipment in 1334 Rizal Avenue, Sta. Cruz, Manila, Philippines.

### Preparation of plant extract

The extraction process was carried out after the green coffee beans were dried at 63ºC for two days by using an oven in order to facilitate grinding. The dried sample was then ground before sieving at 600µm particle size. A water bath was maintained at 80ºC. Then, an Erlenmeyer flask was filled with 5g of powdered sample and 100ml of 80% methanol was added inside. The solvent was changed three times (every 30 minutes for a total of 90 minutes). Afterwards, it was filtered through Whatman filter paper no. 41. All extracts were placed in one Erlenmeyer flask and evaporated until approximately 10ml is left (Ismail, Anuar and Shamsudin, 2012).

### Experimental Animals

A total of 30 Swiss-albino mice weighing 20 to 25 grams were used. The mice were obtained fromUniversity of the Philippines-Manila. They were acclimatized under laboratory conditions two weeks prior the experimentation (Sinha, Manna and Sil, 2008). Ethical approval of all procedures requiring the use of laboratory animals was secured from the National Museum.

**Experimental Set-Up**(Sinha, Manna & Sil, 2008)

The animals were divided into five groups, consisting of six mice each and were treated as follows:

Group 1: Normal control (animals receiving only water as vehicle)

Group 2: Toxin control (animals receiving cadmium chloride orally at a dose of 2.5 mg/kg body weight for 3 days)



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Group 3: Animals treated with coffee bean extract at a dose of 50 mg/kg body weight for 5 days followed by cadmium chloride intoxication (2.5 mg/kg body weight for the next 3 days)
Group 4: Animals treated with coffee bean extract at a dose of 100 mg/kg body weight for 5 days followed by cadmium chloride intoxication (2.5 mg/kg body weight for the next 3 days)
Group 5: Vitamin C was administered orally at a dose of 100 mg/kg body weight for 5 days prior to cadmium chloride intoxication (2.5 mg/kg body weight for the next 3 days)

**Hematological Analysis**

Blood was collected by retroorbital puncture. Peripheral smear evaluation was done. Several hematologic parameters were assessed such as red blood cell count, hematocrit levels and hemoglobin concentrations (Singh, Sankhla, Deora, Mogra and Patni, 2012). Red blood cell indices including MCV, MCH and MCHC were also analyzed (Haziri, Mane, Haziri & Goga, 2012).

**Statistical Analysis**

All values were expressed as mean ± SD. Statistical differences among different groups were analyzed by OnewayANOVA to compare means of two or more samples and Post Hoc Tests for inter-group comparison. p-values of 0.05 or less were considered significant. All computations were done using PASW ver. 18.

**RESULTS AND DISCUSSION**

The present study was carried out to assess the activities of coffee bean methanolic crude extract on the hematological parameters such as red blood cell count, hemoglobin, hematocrit, red blood cell indices and peripheral blood smear evaluation of Swiss Albino mice.

The effects of coffee bean crude extract, vitamin C and cadmium chloride before and after administration among Swiss albino mice are summarized in Table 1. Results revealed that the experimental animals belonging to group 1 showed no change in the $5.8x10^{12}$/L red blood cell count and 42% hematocrit pre and post testing. Hemoglobin showed a nearly similar value of 13.5 g/dl to 13.6 g/dl. This group served as the normal control and received only water as vehicle accounting for the minimal variation in the obtained hematological values.

Meanwhile, group 2, which was administered with cadmium chloride only and served as the toxin control demonstrated marked variation in the red blood cell count, hemoglobin and hematocrit as well as the red blood cell indices as the results evidently declined when


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compared to the one's obtained during the pre-tests. Red blood cell count lowered from $5.5x10^{12}$/Lto $3.6x10^{12}$/L. Hemoglobin and hematocrit levels also declined from 14.5g/dl to 10.2g/dl and 42% to 27%, respectively. These results agree with the study of Sinha et al. in 2008 indicating the ability of cadmium to bind to the red blood cell membrane and stimulate the formation of reactive oxygen species thereby imposing oxidative damage upon the membrane; hence, induce anemia that resulted to decreased levels of the hematological parameters. Further, normal MCV, MCH and MCHC values were obtained indicating the induction of normocytic, normochromic anemia.

**Table 1**
**Comparison of the results of Hematological Parameters before and after administration of Cadmium, Vitamin C and Coffee Bean Extract**

| | TREATMENT | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hematological** | **Normal Control (Group 1)** | | **Toxin Control (Group 2)** | | **Experimental Group (Group 3)** | | **Experimental Group (Group 4)** | | **Positive Control (Group 5)** | |
| **Parameters** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** |
| **RBC Count ($x10^{12}$/L)** | 5.8 | 5.8 | 5.5 | 3.6 | 5.7 | 6.0 | 5.7 | 6.2 | 5.5 | 6.4 |
| **Hemoglobin (g/dl)** | 13.5 | 13.6 | 14.5 | 10.2 | 14.5 | 14.6 | 14.0 | 14.1 | 14.2 | 14.3 |
| **Hematocrit (%)** | 42 | 42 | 42 | 27 | 43 | 43.5 | 42 | 43.75 | 39 | 47.25 |
| **MCV (Fl)** | 42.1 | 45.42 | 46.37 | 45 | 45.44 | 42.5 | 43.68 | 40.56 | 40.91 | 43.83 |
| **MCH (pg)** | 13.28 | 14.92 | 16.37 | 18.33 | 15.44 | 14.33 | 14.56 | 12.58 | 15.82 | 12.19 |
| **MCHC (%)** | 22.14 | 23.03 | 24.52 | 27.78 | 23.72 | 23.56 | 23.33 | 22.0 | 26.41 | 20.05 |
| **Blood Smear** | NORMOCYTIC, NORMOCHROMIC | | NORMOCYTIC, NORMOCHROMIC | | NORMOCYTIC, NORMOCHROMIC | | NORMOCYTIC, NORMOCHROMIC | | NORMOCYTIC, NORMOCHROMIC | |

Hematological changes in all groups of albino mice pre-treated with coffee bean extract showed significant elevation of the red blood cell count, hemoglobin and hematocrit levels. Normocytic, normochromic cells were observed on peripheral blood smear evaluation and red blood cell indices. These results were detected in groups 3 and 4 administered with coffee bean extract at a dose of 50mg/kg body weight and 100mg/kg body weight, respectively. The red blood cell count obtained from group three increased from


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$5.7x10^{12}$/L to $6.0x10^{12}$/L. Hemoglobin and hematocrit values also elevated from 14.5 g/dl to 14.6 g/dl and 43% to 43.5% respectively.

Similarly, red blood cell count obtained in group four elevated from $5.7x10^{12}$/L to $6.2x10^{12}$/L. Hemoglobin and hematocrit levels also augmented from 14.0 g/dl to 14.1 g/dl and 42% to 43.75% respectively. Peripheral blood smear evaluation and the results obtained from the red blood cell indices suggest the appearance of normocytic, normochromic cells. According to the reported findings of Hassan, Hayat and Ahmad in 2012, this morphological classification of anemia has been a common finding in cadmium administrated mammals wherein the red blood cell membrane skeleton is initially altered by eposure to cadmium followed by deformation of the cell thus promoting hemolysis.

Also in agreement to the study accomplished by Flora, Mittal and Mehta in 2008, such results can be hypothesized to be attributed to the presence of 5-Hydroxymethyl-2-Furfural as a natural component of coffee bean that has favorable biological effects such as its antioxidant and antihypoxic activity (Liet al., 2010) thereby reversing the oxidative damage brought about by the formation of reactive oxygen species and free radicals that damages blood cells associated with heavy metal intoxication (Flora, Mittal & Mehta, 2008).

In agreement with the study conducted by Shekha et al. (2008), the group of animals administered with vitamin C prior to subsequent intoxication with cadmium chloride also revealed significant restoring of all hematological parameters. All the values obtained increased from $5.5x10^{12}$/L to $6.4x10^{12}$/L for red blood cell count, 14.2 g/dl to 14.3 g/dl for hemoglobin and 39% to 47.25% for hematocrit. Peripheral blood smear evaluation and the results obtained from the red blood cell indices suggest the appearance of normocytic, normochromic cells. This protective influence is attributed to its antioxidant activity and role in scavenging free oxygen radicals and in stabilizing the cell membrane, thus, maintaining its permeability (Shekha, Sabir and Fatah, 2008).

Meanwhile, the microscopic appearance of red blood cells of different groups of Swiss albino mice can be seen in Figure 2. Normocytic, normochromic cells having an average size and hemoglobin content that are within normal limits can be detected from all groups.

Table 2compares the hematological values obtained before and after the administration of cadmium chloride, vitamin C and coffee bean extract to the different groups of albino mice. The toxin control obtained a p-valuelower than 0.05 indicating the ability of cadmium chloride to significantly lower the red blood cellcount, hemoglobin,


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hematocrit and red blood cell indices. Meanwhile, the experimental groups obtained a p-value of 0.000 indicating a significant difference between the pre-test and post-test and the protective influence of coffee bean crude extract against cadmium chloride-induced oxidative stress on mice red blood cells.

**Table 2**
**Comparison on the effects of cadmium, vitamin C and coffee bean extract administration between the pre-test and post-test**

Paired Samples Statistics

| | Group | p-value | Interpretation |
|---|---|---|---|
| Group 1 | Pretest: Normal control<br>Posttest: Normal control | 0.000 | Significant |
| Group 2 | Pretest: Toxin control<br>Posttest: Toxin control | 0.005 | Significant |
| Group 3 | Pretest: Experimental A<br>Posttest: Experimental A | 0.000 | Significant |
| Group 4 | Pretest: Experimental B<br>Posttest: Experimental B | 0.000 | Significant |
| Group 5 | Pretest: Positive control<br>Posttest: Positive control | 0.001 | Significant |

*Significant at p-value < 0.05

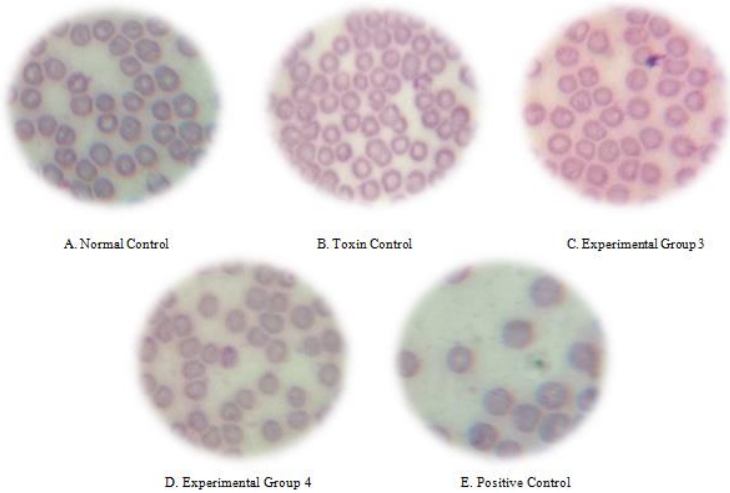


**Figure 2. Microscopic Examination of Red Blood Cells of Swiss Albino Mice**


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On the other hand, Table 3 presents the statistical difference when one group is compared with the other groups. All values showed a significant difference which indicates that while the administered toxin brought a marked diminution in the hematological parameters, the crude extract revealed a significant protective effect when given prior to cadmium chloride intoxication which is in accordance to the study carried out by Li et al. (2010).

**Table 3**
**Comparison of each group between and within groups**

| | p-value | Interpretation |
|---|---|---|
| Normal control | 0.000 | Significant |
| Toxin control | 0.001 | Significant |
| Group 3+ | 0.000 | Significant |
| Group 4 | 0.000 | Significant |
| Positive control | 0.000 | Significant |

**Significant at p-value <0.05*

## CONCLUSION

These findings suggest that administration of coffee bean crude extract prior to cadmium chloride intoxication can cause significant restoring of the hematological parameters of mice in terms of red blood cell count, hemoglobin and hematocrit levels. The crude extract was also able to preserve the normal morphology of the cells.

## RECOMMENDATION

Further pharmacological investigations through administration of different concentrations of the extract are highly recommended to assess the likely toxicological effects. The use of extracts from other parts of the plants can also be utilized to further explore its protective influence against toxicity on blood parameters brought about by cadmium administration. Lastly, quantitation of the specific 5-Hydroxymethylfurfural component is also suggested to know how much of the component will show therapeutic effect.

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