

Protective role of Moringa Leaf Extract against Cyclophosphamide Induced Anemia in rats

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Abstract: The effect of Moringa leaf extract on the albino rats induced anemia by cyclophosphamide was investigated. Thirty five Albino rats weighing 180-200g were used. Groups B, C, D and E were injected with cyclophosphamide to induce anemia in the mice, while group A served as a normal control without anemia and treated with 0.5ml of normal saline, Group B served as the positive control rat with anemia and was treated with 0.5ml of normal saline, Group C rat with anemia and was treated with 50 mg/kg body weight of moringa leaf extract, Group D mice with anemia and was treated with 100 mg/kg body weight of moringa leaf extract. Group E mice with anemia and was treated with 200 mg/kg body weight of moringa leaf extract. The feeding lasted for three weeks. The results of this research revealed an increase in the levels of White Blood Cell (WBC), Bilirubin and a lower in Hemoglobin (Hb) and Packed Cell Volume (PCV) caused by cyclophosphamide compared to control samples. The increase in the White Blood Cell is attributed to stimulation of the resistant system response brought about by using the induced by the cyclophosphamide; there was also an indicator of anemia and hemolysis in the blood of the experimental mice. After 21 days of oral intake of 50, 100 and 200mg /kg body weight moringa leaf extract there was clearly reduction in white blood cells and bilirubin levels, with a corresponding increase on packed cell volume and hemoglobin.

[Maha A. Hijazi and Hanan A. Jambi. **Protective role of Moringa Leaf Extract against Cyclophosphamide Induced Anemia in rats.** *Nat Sci* 2018;16(5):95-99]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). <http://www.sciencepub.net/nature>. 13. doi: [10.7537/marsnsj160518.13](https://doi.org/10.7537/marsnsj160518.13).

Keywords: Moringa leaf extract, anemia, cyclophosphamide, hematology.

1. Introduction

Anemia is a widespread public health problem associated with an increased risk in morbidity and mortality, especially in pregnant women and young children. It is a condition caused by both nutritional (vitamin and mineral deficiencies) and non-nutritional factors. One of the most contributing factors is the iron deficiency which is considered the number one contributor to the global burden of diseases (**Brandy, 2007**). Anemia can result in impaired cognitive development, reduced physical work capacity and in severe cases, increased risk of mortality, particularly during the perinatal period. Anemia may also result in reduced growth and increased morbidity (**WHO/UNU/UNICEF, 2001**).

Moringa oleifera belonging to the family of Moringaceae is an effective remedy for malnutrition. Moringa is rich in bioactive components in its leaves, pods, and seeds. In fact, moringa is said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yogurt, 15 times more potassium than bananas and 25 times more iron than spinach (**Rockwood et al., 2013**). Moringa oleifera. The most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh, and Afghanistan. It is a perennial which for centuries has been advocated for traditional medicinal and industrial

uses. All parts of the Moringa tree are edible and have long been consumed by humans. It is known that the Moringa has many benefits based on its nutrition. The ratio of grams per gram, Moringa leaves dry powder contains 25 times more iron than spinach, in which iron is one of the therapeutic agents for anemia. (**Kasoloet, al., 2012**). Leaves of Moringa oleifera are also known as a great source of vitamins and minerals including calcium, copper, sulfur, vitamin A and B-vitamins (Lowell, 2002). In addition, M. oleifera is very rich in iron and it was estimated that the dried 100 g leaf powder to contain about 28.29 mg (**Odoro et al., 2008 and Osman et al., 2012**).

Recently, attention has been drawn to several benefits of Moringa Oleifera, including its ability to enhance blood formation and boost the immune system. Other medicinal properties have also been ascribed to various parts of Moringa Oleifera. Almost every part of the plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil has been used for various ailments in medicine [**Odebiyi and Sofowora 1999**], including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepato-renal disorders (**Morimitsu et al., 2000**) and (**Siddhuraju and Becker 2003**). The aim of this study was to evaluate the effect of moringa leaf extract on hematological profile in anemic albino rats induced by cyclophosphamide-

2. Material and Methods:

Thirty five albino rats of Wistar strain weighed 180-200g, were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia.

The animals were kept under Room temperature and were acclimatized in the new environment for a period of 7 days with free access to food and water before the commencement of the experiment.

Cyclophosphamide and Kits for biochemical analyses were purchased from The Gamma Trade Company for Pharmaceutical and Chemicals, in Riyadh.

Methods:

Preparation Moringa leaf extract:

The leaf of Moringa were collected, dried in oven at 45°C for 72 hours and milled to the powder using the grinding machine. 500 g of moringa leaf powder were macerated in 1200 ml of ethanol with thorough shaking at regular interval for 72hours at room temperature (25±1°C). The extract was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using the rotary evaporator to obtain the slurry of the extract the semi-pastry extract was dried and stored in the refrigerator and used for the study.

Experimental design

Thirty five albino rats were used in this study. The experimental rats were randomly distributed into five (5) groups of 7 rats each. Anemia was induced in the rats and this was performed by intraperitoneally injection of cyclophosphamide (10 mg/kg b/w). The rats were feed graded doses of moringa leaf extract *through* oral intubation method. The groups and doses administered are summarized below:-

Group A:

(Negative control rats without cyclophosphamide induced anemia): The rat were treated with (0.5ml of normal saline). For three weeks.

Group B:

(Positive control rats with cyclophosphamide induced anemia): Anemia rats without treatment. The rats were treated with (0.5ml of normal saline). For three weeks.

Group C:

Anemia rats treated with 50 mg/ moringa leaf extract kg body weight of moringa leaf extract). For three weeks.

Group D:

Anemia rats treated with 100 mg/ moringa leaf extract kg body weight of moringa leaf extract). For three weeks.

Group E:

Anemia rats treated with 200 mg moringa leaf extract /kg body weight of moringa leaf extract). For three weeks.

At the end of each week of treatment, blood samples were collected from one rat in each group into tubes containing EDTA and were immediately used for determination of hematological parameters, for hematological study (Jain, 1986).

Analytical Methods:-

Determination of Total Red Blood Cell Count (RBC), total white blood cell count (WBC), Packed Cell Volume (PCV) and Hemoglobin (Hb) Concentration was carried out according to the method of (Dacie and Lewis 2000). Total bilirubin (TB) was estimated by acid diazo method as described by (Doumas *et al.* 1973), using assay kits (Randox Laboratories Ltd).

Statistical analyses:-

Results are presented as mean ± standard error of mean (SEM). Within and between groups, comparisons were performed by the analysis of variance (ANOVA) (using SPSS17.0 for windows Computer Software Package). Significant differences were compared by Duncan's new Multiple Range test; a probability level of less than 5% ($P < 0.05$) was considered significant (Duncan, 1955).

3. Results and Discussion:

The effects of moringa leaf extract on the hemoglobin concentration (Hb g/dl) in anemia rats induced by cyclophosphamide during the experimental periods for three weeks are presented in Fig 1. Results were showed that, a decreasing of hemoglobin concentration in rats injected with cyclophosphamide Group B (positive control group). This is an indication of hemolysis and the decreasing in hemoglobin has a corresponding increasing in methemoglobin content which affects the oxygen carrying capacity of the blood, caused by the toxicant (Tilak *et al.* 2007). Treatment with moringa leaf extracts were increased the hemoglobin level after one week for groups C, D and E compared to Group B (positive control group). These results are in agreement with reports of (Adeyemo 2007) and (Vinodhini and Narayanan 2009). Treated with 50, 100 and 200 mg/kg (body weight) moringa leaf extract for groups C, D and E were improved the hemoglobin concentration during the experimental periods compared to Group B (positive control group).

The effects of moringa leaf extract on red blood cells count (RBC) of anemia rats during the experimental periods for 21 days are presented in Fig. 2. The results revealed that the red blood cells count (RBC) were decreased in anemia rats Group B (positive control group) compared to group A normal control group. treated with 50,100 and 200 mg/kg body weight of moringa leaf extract for group C, D and E were increased in red blood cells count

compared to anemia rats Group B (positive control group) after one, two and three weeks respectively.

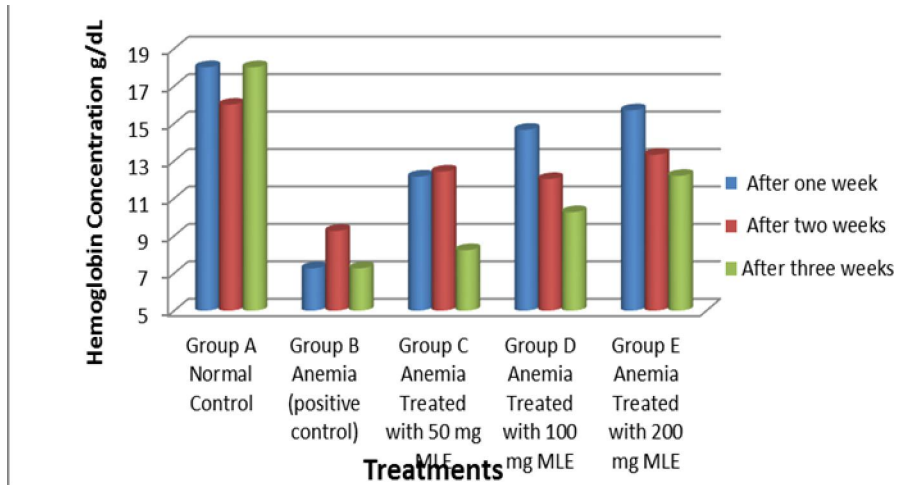


Fig. 1 Effect of moringa leaf extract on Hemoglobin Concentration (Hb g/dl) anemia rats induced by cyclophosphamide during the experimental periods for three weeks.

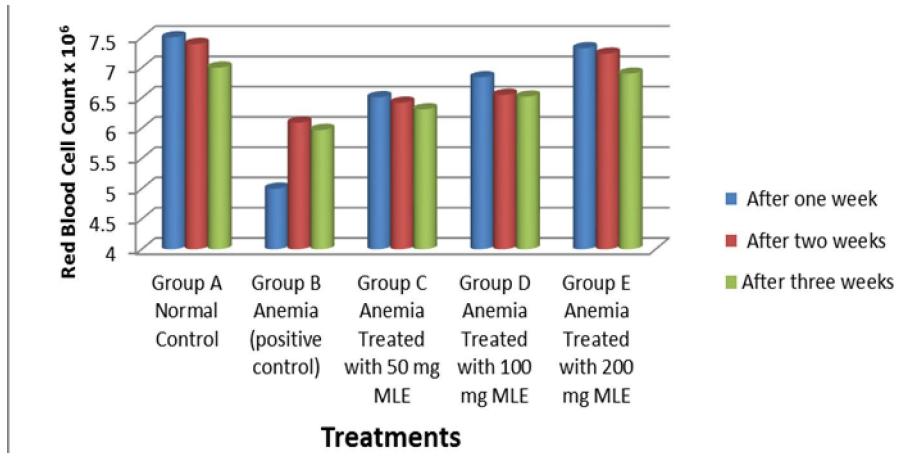


Fig. 2. Effect of moringa leaf extracts on red blood cells count (RBC) anemia rats induced by cyclophosphamide during the experimental periods for three weeks.

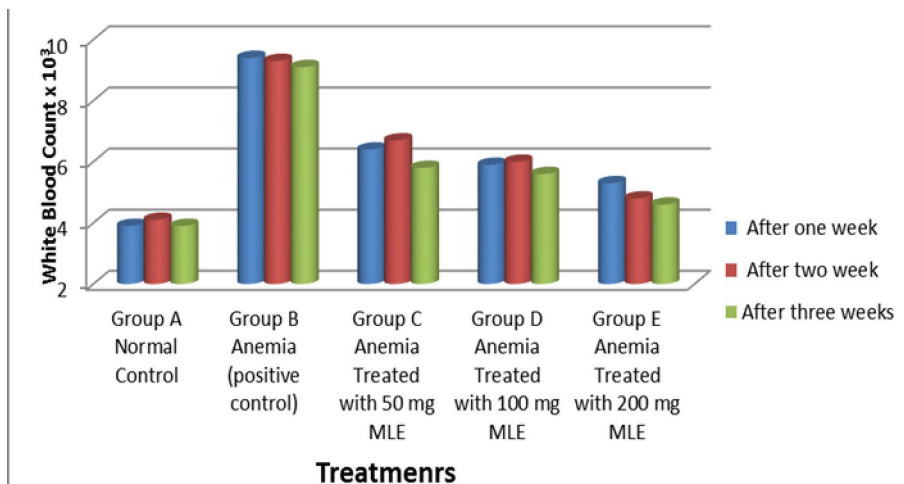


Fig. 3. Effect of moringa leaf extract on White Blood Count (WBC 10³) anemia rats induced by cyclophosphamide during the experimental periods for three weeks.

Effect of moringa leaf extract on white blood count (WBC mm³/L) of anemia rats induced by cyclophosphamide during the experimental periods for three weeks is shown in Fig.3. Anemia rats induced by cyclophosphamide Group B (positive control group) had increased the white blood count values compared to the control group A, C, D and Group E after one, two and three weeks respectively. The elevated White Blood Count can be due to the stimulation of immune defense system (Kashinath, 1990). Similarly,

literature has shown that increased concentration of antigen in the body results in high values of White Blood Count (Schalm et al., 1975) and (Hoeney, 1985). In this study, it was observed that treated with moringa leaf extract at dosage 50, 100 and 200mg/kg (body weight) were decreased of White Blood Count by the third week. This trend agrees with earlier results obtained by (Adisa et al 1999) and (Ezekiel and Onyeyili (2007).

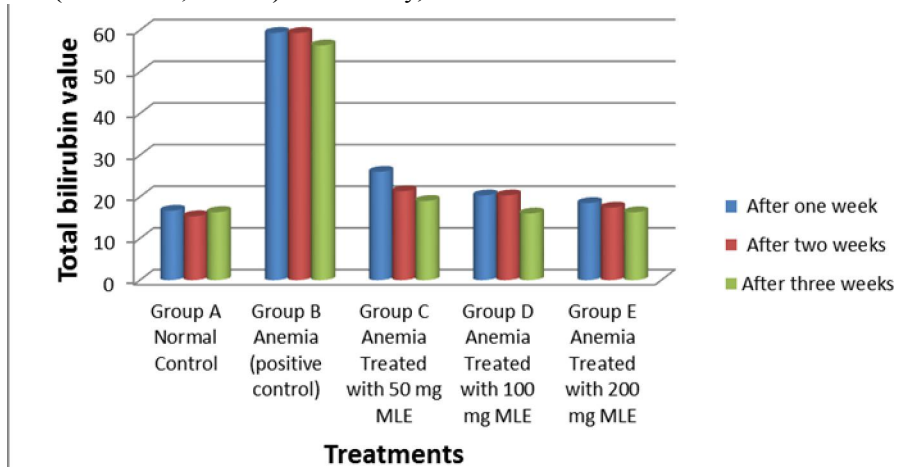


Fig. 4. Effect of moringa leaf extract on total bilirubin value anemia rats induced by cyclophosphamide during the experimental periods for three weeks.

Effects of moringa leaf extract on total bilirubin value anemia rats induced by cyclophosphamide during the experimental periods for three weeks are illustrated in Fig. 4. Anemia rats induced by cyclophosphamide Group B (positive control group) were increased the total bilirubin value compared to the group A normal control and other treatments during the experimental periods. This increasing of total bilirubin value can be due to damage to the liver

cells and obstruction of the bile duct (Arthur et al, 1986, Khaleifat et al, 2002). The treated with 100 mg moringa leaf extracts were decreased the serum total bilirubin level close to normal by the third week (group D). Results from this study conform to the report of previous researchers (Nwamba et al, 2006) but inconsistent with the report by (Kori-Siakpere Ovie 2008) and (Klyszejko and Lyezywek, 1999).

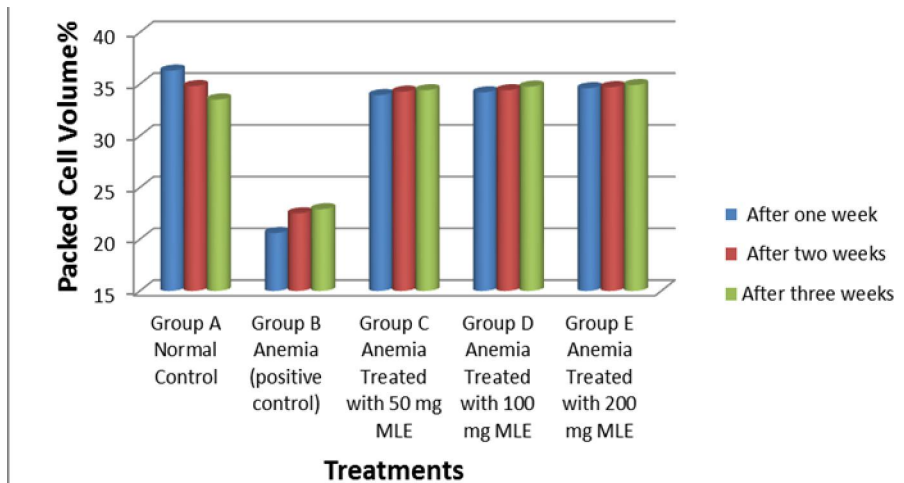


Fig. 5. Effect of moringa leaf extract on Packed Cell Volume (PCV%) anemia rats induced by cyclophosphamide during the experimental periods for three weeks.

Effect of moringa leaf extract on Packed Cell Volume (PCV %) anemia rats induced by cyclophosphamide during the experimental periods for three weeks are presented in Fig. 5. The results revealed that, Packed Cell Volume (PCV %) were decreasing in Group B (positive control group) compared to the normal control (group A). The treated with of 50, 100 and 200 mg/kg (body weight) moringa leaf extract for groups C, D, and E were showed the increased in the serum level of packed cell volume compared to Group B (positive control group) after one, two and three weeks.

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