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Effect of extracted and commercial vitamin B₁₂ on hematological, physiological parameters in experimental animals

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Abstract--The study aimed to know the effect of commercial vitamin B12 and vitamin B12 extracted from *Lactobacillus rhamnosus* after testing its ability to produce the vitamin, purifying it in the laboratory and determining the optimal condition for its. The results showed effect vitamin B₁₂ on hematological, physiological parameters in experimental animals, it's have a negative effect on hemoglobin concentration, as it decreased to (11.9) µg/ml, with a significant difference from the control treatment (14.6) µg/ml. The results also indicated that the treatment of laboratory animals with B₁₂ extract at a concentration of (2, 4) µg/ml positive effect by a significant increase in hemoglobin concentration rate to (13.48,15.2) g/100ml, respectively, compared with the control treatment. The results of the current study of PLT showed a decrease in the rate of blood platelets to (325.3) 10³/µL infected with anemia compared to the control treatment, as it was (346) 10³/µL. When the animals were injected with vitamin B₁₂ extract, the PLT increased to (334.5) (387.5) 10³/µL for the two concentrations (2,4) µg/ml respectively. In addition, the platelet rate increased when treated with commercial B₁₂ for the two concentrations (4,2) respectively (331.2, 334.7) 10³/µL compared with the control and extracted B₁₂ treatments.

Keywords--Vitamin B₁₂ production, anemia, Vitamin B₁₂ Extract, Commercial Vitamin B₁₂.

Introduction

Anemia is a condition in which the concentration of hemoglobin (Hb) and the number of red blood cells (RBC) is lower than normal and insufficient to meet the physiological needs of the individual. It especially affects pre-school children and

women in pregnancy and childbirth. It is essential to ensure that anemia is properly recognized and its negative effects are prevented (3).

Vitamin B₁₂ deficiency is a common condition, and many are undiagnosed. Absolute deficiency occur up to 6% of those aged 60 years and older, whereas marginal deficiency occur in close to 20% of patients in later life(1) The manifestation of Vitamin B₁₂ deficiency ranges from subtle, non-specific clinical features to serious neurological and neuropsychiatric complication if left untreated. With an aging population, screening for vitamin B₁₂ level as part of anemia and cognitive impairment workup is more common. More cases are diagnosed, resulting in rising incidence of patients with vitamin B₁₂ deficiency. The common causes of vitamin B deficiency are food malabsorption and anemia. anemia is an autoimmune gastritis resulting from the destruction of gastric parietal cells and consequent impairment of intrinsic factors secretion to bind the ingested vitamin B₁₂, Other autoimmune disorders, especially thyroid disease, diabetes mellitus, and vitiligo, are also commonly associated with anemia. The cost and availability of auto-antibodies testing, such as intrinsic factor and anti-parietal cell antibodies(15).

For patients with pernicious anemia, lifelong vitamin B₁₂ therapy is indicated. Vitamin B₁₂ is absorbed in the terminal ileum. This absorption is almost entirely dependent on intrinsic factor binding to vitamin B₁₂. This bound complex in turn binds to the cubam receptor in the terminal ileum and is internalized. The complex is eventually released from lysosomes and transported across the cell membrane bound to transcobalamin in the blood circulation. Traditionally, vitamin B₁₂ replacement is administered intramuscularly. However, it is believed that oral vitamin B₁₂ can be absorbed passively independent of intrinsic factors(2).

Materials and Methods

Collection of Blood samples

Venous blood samples were drawn from all groups used in the experiment using sterile medical syringes with a capacity of 10 ml of blood. The samples were transferred to tubes containing an anticoagulant for the purpose of measuring physiological parameters, while the other part of the blood was placed in special tubes free of any anticoagulant and left at a temperature of Laboratory temperature for 10-15 minutes, then centrifuge at 3000 rpm for the purpose of separating the blood serum from the rest of its components. The serum was separated and placed in biochemical test tubes. The laboratory examination process included three axes

Estimation of Red Blood corpuscles

1- Total red blood cell count

He method of blood cell counter and Hymes Fluid solution was used as a dilution solution for total red blood cell count (8).

The examination was carried out according to the following steps:

2 ml of Heims solution was placed in a clean test tube, then 0.02 ml of the drawn blood was added to it and the mixture was shaken well. Then a drop of the mixture was transferred to the cell counter. After placing the slide cover, it was left for 2 minutes to stabilize the cells. Then the cell counter was transferred to the microscope and examined under great magnification.(40x) Then the number of red blood cells was calculated in five small squares in the middle large square. It calculates: $R.B.C_s \text{ } \mu\text{m}^3 = \text{Number R.B.C}_s \text{ counted} \times 10000$

2 - Hemoglobin Estimation

Hemoglobin meter and Drabkins solution were used as a dilution solution to estimate the hemoglobin concentration in the blood sample (14).

The examination was carried out according to the following steps:

5 ml of Drabkins solution was placed in a clean test tube, then 0.02 ml of the withdrawn blood was added to it. The tube was shaken well, then left for 10 minutes, then the hemoglobin meter was cleared with distilled water, then the tube was placed in the device, as the value (Hb) appeared on the screen The device is in units (g\dl).

3-Differential number of white blood cells

A blood smear was prepared and stained with Leishman Stain dye Annex 3 and examined with an oil lens to perform a differential count of white blood cells, as granulated and non-granulocytic white blood cells were counted in general (4).

The blood smear was prepared on a slide and left to dry in the laboratory atmosphere. Then the glass slide was dyed using Lechman dye. 8 drops of this dye were placed to cover the blood smear and left for two minutes, then 16 drops of phosphate buffer solution were added to the slide and mixed well with Lechman dye solution. It is left for 10 minutes and then the slide is washed with distilled water and left to dry using an oil lens. 100 white blood cells were counted from the slide and the different types of white blood cells were expressed in percentage formula.

$W.B.C \text{ } \mu\text{m}^3 = \text{Number cells counted} \times 50$

4- Total platelets count

The method of blood cell counter and ammonium oxalate solution was used as a dilution solution to calculate the total number of platelets (14).

The examination was carried out according to the following steps:

0.38 mL of ammonium oxalate solution was put into a clean point test tube, then 0.02 mL of the powdered alkaloid was added to the cell counter, after putting a lid on and leaving it for a quarter of an hour to stabilize the cells. Then the cell counter was transferred to the microscope and examined under the magnification of 40x. Platelet count in five small squares in the middle large.

It calculates: $\text{platelet } \mu\text{m}^3 = \text{Number of cells counted} \times 100$

Results and Discussion

Effect of extracted and commercial vitamin B₁₂ on blood parameters of laboratory animals:

1. Effect of number of red blood cells RBC:

The results in Figure (1) showed a significant increase ($P < 0.05$) in the number of RBC red blood cells for groups (G2,G3,G4,G5) compared to the control group G1 and the group of animals suffering from RAT1 anemia.

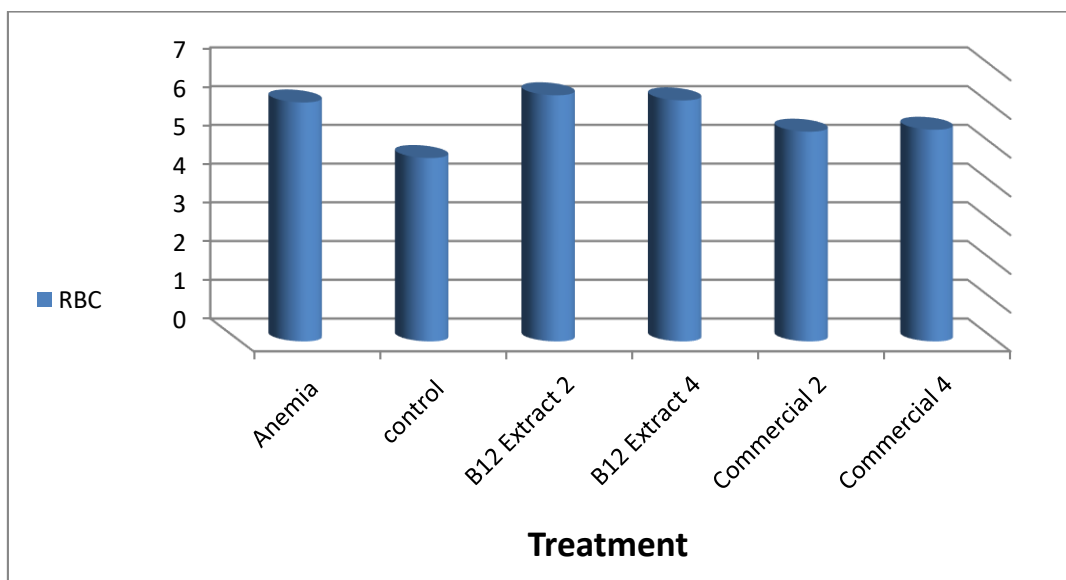


Figure (1) effect of number of red blood cells in animals treated with different concentrations of the vitamin.

2- The percentage of hemoglobin

The results indicate that laboratory animals infected with anemia have a negative effect on hemoglobin concentration, as it decreased to (11.9) $\mu\text{g/ml}$, with a significant difference from the control treatment (14.6) $\mu\text{g/ml}$. The results also indicated that the treatment of laboratory animals with B₁₂ extract at a concentration of (2) and (4) $\mu\text{g/ml}$ positive effect by a significant increase in hemoglobin concentration rate to (13.48) and (15.2) $\text{g}/100\text{ml}$, respectively, compared with the control treatment. Figure (2) .

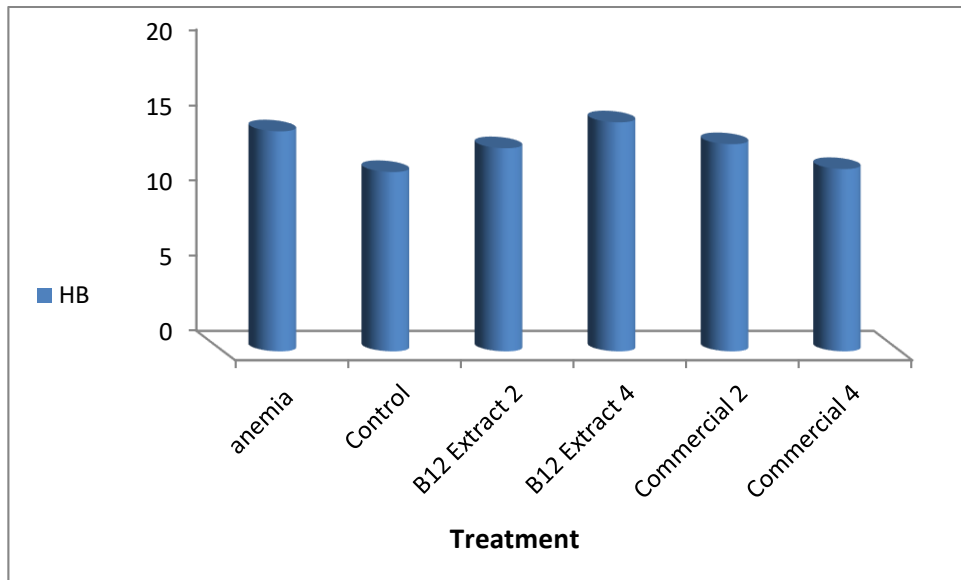


Figure (2) The percentage of hemoglobin in animals treated with different concentrations of the vitamin.

Sometimes the reason is due to the effect of B₁₂ and iron on membrane proteins, which affects the supply of blood cells with energy, as well as affects the transport of sugars inside the cell, which ultimately leads to a shortened lifespan of the globule and thus a rapid death, and thus leads to a decrease in the amount of hemoglobin in the blood (5).

3. Effect of number of white blood cells WBC:

The results also showed in figure (3) an increase in the numbers of WBC white blood cells compared to the control group, where a significant increase ($P < 0.05$) was observed in the groups G2, G3, G4, G5, but when comparing the groups with each other except for G1 with the RAT2 group, which represents animals affected by poverty. In the blood, a state of morale high ($P < 0.05$) was observed.

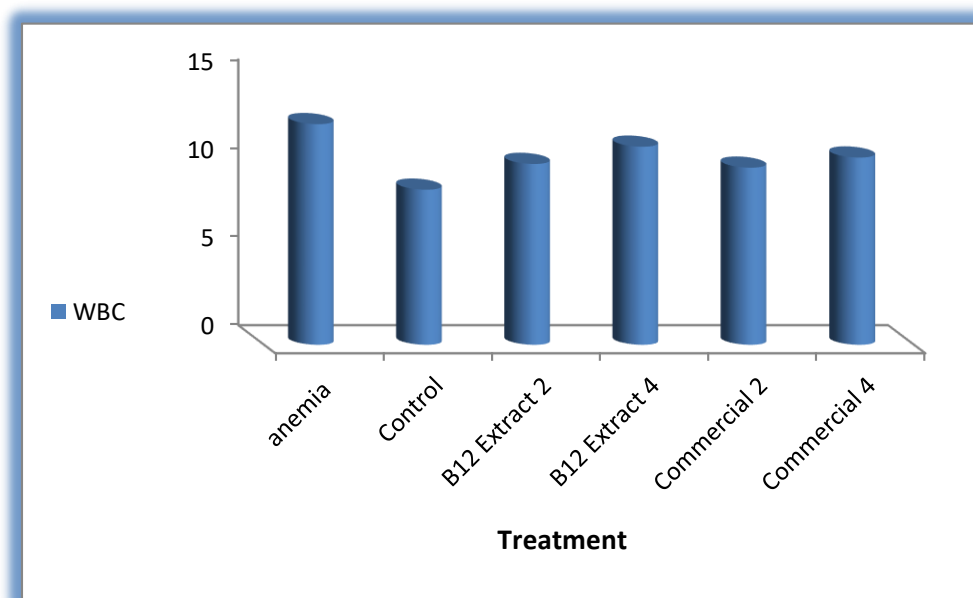


Figure (3) effect of number white blood cells in animals treated with different concentrations of the vitamin.

4- The rate of blood platelets PLT:

The results of the current study of PLT in Figure (4) showed a decrease in the rate of blood platelets to $(325.3) 10^3/\mu\text{L}$ infected with anemia compared to the control treatment, as it was $(346) 10^3/\mu\text{L}$. When the animals were injected with vitamin B₁₂ extract, the PLT increased to $(334.5) (387.5) 10^3/\mu\text{L}$ for the two concentrations (2,4) $\mu\text{g}/\text{ml}$ respectively. In addition, the platelet rate increased when treated with commercial B₁₂ for the two concentrations (4,2), respectively $(331.2, 334.7) 10^3/\mu\text{L}$ compared with the control and extracted B₁₂ treatments.

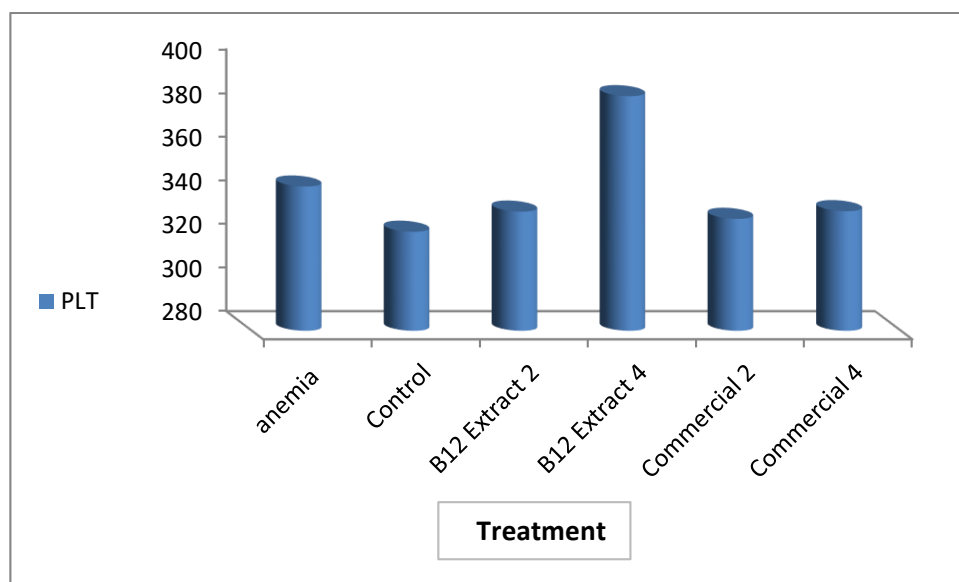


Figure (4) The rate of blood platelets in animals treated with different concentrations of the vitamin.

It is known that high blood platelets are an uncommon disorder that occurs when the body is exposed to the removal of the spleen, anemia, iron deficiency and abnormal activity of the bone marrow, which leads to an increase in the density and size of the blood platelet, sometimes the cause of the rise is not clear and this affects the density of blood and thus moves slowly, which leads to an increase in the risk and the occurrence of blood clots (6). Anemia is accompanied by low blood platelets, low Hb rate, small red blood cells, or sickle cell anemia.

The results of the current study showed that an increase in the number of red blood cells and the percentage of hemoglobin, as well as the increase in the number of white blood cells and platelets, may be attributed to the increase in the number of red blood cells and the percentage of hemoglobin, which is one of them Vitamin B₁₂ in addition to iron and folic acid or B₉, which are essential factors for normal blood formation and are important for the manufacture of DNA and in cell division, as the lack of vitamin B₁₂ and folic acid will cause an imbalance in the process of cell multiplication, especially in the red blood cell manufacturing system and Then it leads to anemia (15).

The treatments that included the administration of extracted and commercial vitamin B₁₂ to groups G2, G3, G4, G5 worked to modify the levels of the blood parameters under study through its important role in the formation of red blood cells (17) and in the synthesis of hemoglobin, which is It is important for red blood cells to transport oxygen and accordingly the hematocrit percentage increases as the percentage of volume (%) of red blood cells in the blood (7).

Some studies indicate that supplementation with vitamin B complex mixtures containing vitamin B₁₂ has enhanced the body's immunity as it significantly

improved pain and spasticity in diabetic neuropathy patients (16) as confirmed by (13) the role of Vitamin B₁₂ in the prevention of some diseases.

As for the platelet values of PLT, their values increased when they were dosed with vitamin B₁₂, and since vitamin B₁₂ works to maintain moderate levels of the amino acid, which helps reduce the risk of heart disease (10), where treatment with vitamin B₁₂ leads to Decreased homocysteine (12).

Elevated levels of the amino acid homocysteine have been identified as an independent risk factor for cardiovascular disease (9). This acid contains sulfur derived from methionine, which is normally present in the blood. Its high levels are believed to promote clotting, thus reducing its levels. Or balancing it by giving balanced doses of vitamins, it may work to lower homocysteine and then prevent cardiovascular disease by reducing platelet count. Also (11) mentioned the role of vitamin B₁₂ in maintaining the health and safety of the bone marrow that generates blood components.

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