Compare between of extracted and commercial vitamin B_{12} on biochemical parameters in experimental animals

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ABSTRACT

The objective of this study is to provide an on the effectiveness of vitamin B_{12} for the treatment of anemia extracted from bacteria *Lactobacillus rhamnosus* after testing its ability to produce the vitamin, purifying it in the laboratory and determining the optimal condition for its and compared with commercial vitamin B_{12} that treatment of anemia .

The results shown The results shown in show a significant decrease ($P<0.05$) in cholesterol in groups G2,G3,G4,G5 than the control group G1 was (70.35, 91, 99.55,82.7) mg/dl consecutively compared with the control its 117.3 200mg/dl and the triglycerides decreased significantly ($P<0.05$) in groups G2, G3, G4, and G5 than in the control group G1.

On the other hands ,The results show that the percentage of liver enzymes in groups G2, G3, G4, and G5 did not increase compared to the G1 B the increase in it was significant ($P<0.05$) and refer As for AST enzyme, it that the percentage of liver aspartate transporter enzyme AST was significantly increased ($P<0.05$) and it was higher in the group treated with extracted vitamin B12 compared to the control group G1 .

Keywords: Vitamin B_{12} production, anemia, Vitamin B_{12} Extract, Commercial Vitamin B_{12}.

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 (5).

Vitamin B_{12} 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(2) The manifestation of Vitamin B_{12} deficiency ranges from subtle, non-specific clinical features to serious neurological and neuropsychiatric complication if left untreated. With an aging population, screening for vitamin B_{12} level as part of anemia and cognitive impairment workup is more common. More

cases are diagnosed, resulting in rising incidence of patients with vitamin B_{12} 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 B_{12}, 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(3).

For patients with pernicious anemia, lifelong vitamin B_{12} therapy is indicated. Vitamin B_{12} is absorbed in the terminal ileum. This absorption is almost entirely dependent on intrinsic factor binding to vitamin B_{12}. 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 B_{12} replacement is administered intramuscularly. However, it is believed that oral vitamin B_{12} can be absorbed passively independent of intrinsic factors(4).

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:

How blood tests work

It included biochemical parameters.

Biochemical parameters Measurement

1. Estimation of Total serum cholesterol

The enzymatic method described (9) was used to estimate the total cholesterol in the serum. The optical absorbance was read at a wavelength of 500 nm.

Extracting the value of the cholesterol concentration in the serum of the sample according to the following equation:

$$\text{Total S. Cholesterol(mg/dL)} = \frac{\text{A sample}}{\text{A Standard}} \times 200$$

2. Estimation of Serum Triglycerides

The enzymatic method described (7) was used and the optical absorbance was read at the wavelength of 505 nm.

Extracting the exchange value of triglycerides in honey serum according to the following equation:

$$\text{Triglycerides Conce.(mg/dL)} = \frac{\text{A sample}}{\text{A Standard}} \times 100$$

3. Estimation of high density lipoproteins (HDL) in Serum

The method of precipitation of lipoproteins present in high-density lipoprotein (HDL) in the blood serum, which includes (LDL.Chylomicrons) using (Phosphotungistic Acid) in the presence of magnesium ions (6).

Extracting the value of the HDL concentration in the serum of the sample according to the following equation:

$$HDL.Conce.(mg/dL) = \frac{A\ sample}{A\ Standard} \times 100$$

4. Calculation of low density lipoproteins

The equation described (12) was used to calculate low-density lipoproteins, and this equation is:

LDL . Cholesterol (mg/dl) = Total cholesterol – (VLDL +HDL)

5. Determination of the activity of the two enzymes that transport the amino group alanine and aspartate transaminases (ALT, AST) in serum.

This method is based on the determination of the amount of pyruvate and oxaloacetate liberated by reacting it with dinitrophenyl hydrazine (8).

efficient tube(Blank)	sample tube	solutions
-	0.1	sample (with serum)
0.5	0.5	ALT or AST buffer solution
The tubes were mixed well and incubated at 37°C for 30 minutes		
0.5	0.5	Diphenyl hydrazine solution
0.1	-	sample (with serum)
The tubes were mixed well and incubated at 20-25°C for 20 minutes		
0.5	0.5	sodium hydroxide solution

The contents of the tubes were mixed well, then the absorbance was measured for it at the wavelength 540nm. The standard curve was obtained for the determination of pyruvate using different concentrations of them, and as indicated in the instructions for using the estimation kit. One micromole of pyruvate during one minute under reaction conditions.

Results and Discussion

1. Effect of vitamin B_{12} on the values of cholesterol(Ch)

The results shown in Figure (1) show a significant decrease ($P<0.05$) in cholesterol in groups G2,G3,G4,G5 than the control group G1 was (70.35, 91, 99.55,82.7) mg/dl consecutively compared with the control its 117.3 200mg/dl .

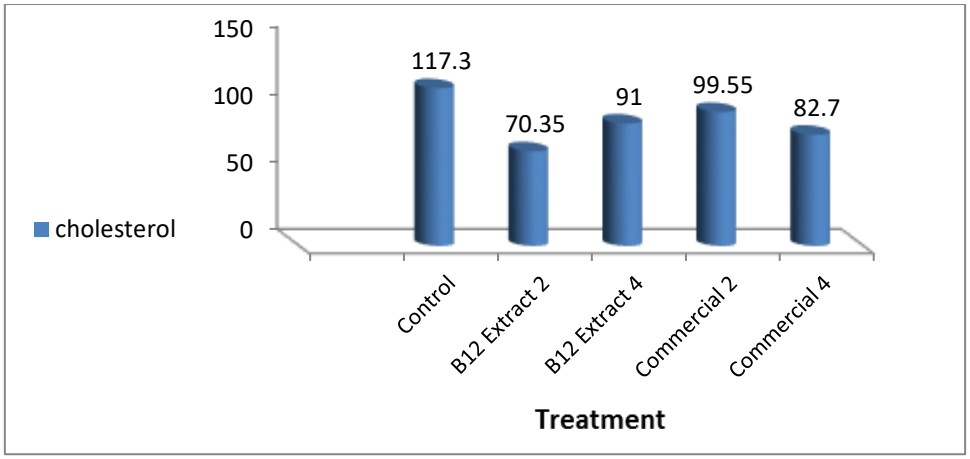

Figure (1) Effect of vitamin B_{12} on the values of cholesterol(Ch)

2. Effect of vitamin B_{12} on the values of Triglycerides (TG)

Figure (2) show a that the percentage of triglycerides TG decreased significantly ($P<0.05$) in groups G2, G3, G4, and G5 than in the control group G1.its was (90.5 , 84, 81.5) mg/dl consecutively compared with the control its 128.5 mg/dl.

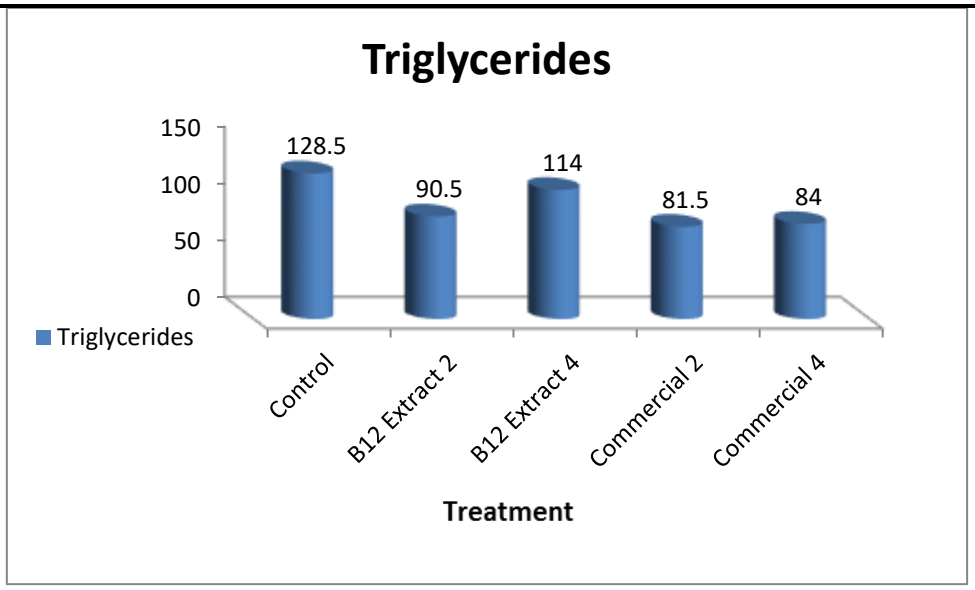

Figure (2) Effect of vitamin B_{12} on the values of Triglycerides (TG)

3. Effect of vitamin B_{12} on the values of high-density lipoproteins (HDL), and low-density lipoproteins (LDL):

It was also observed that the percentage of high high-density lipoproteins (HDL) was significantly decreased ($P<0.05$) in the groups treated with vitamin B_{12} extracted from the control group. Its refer in Figure (3) was (39.15, 54.45, 42.6, 40.65) mg/dl for groups G2, G3, G4, and G5 consecutively compared with the control G1 its 31.85 mg/dl

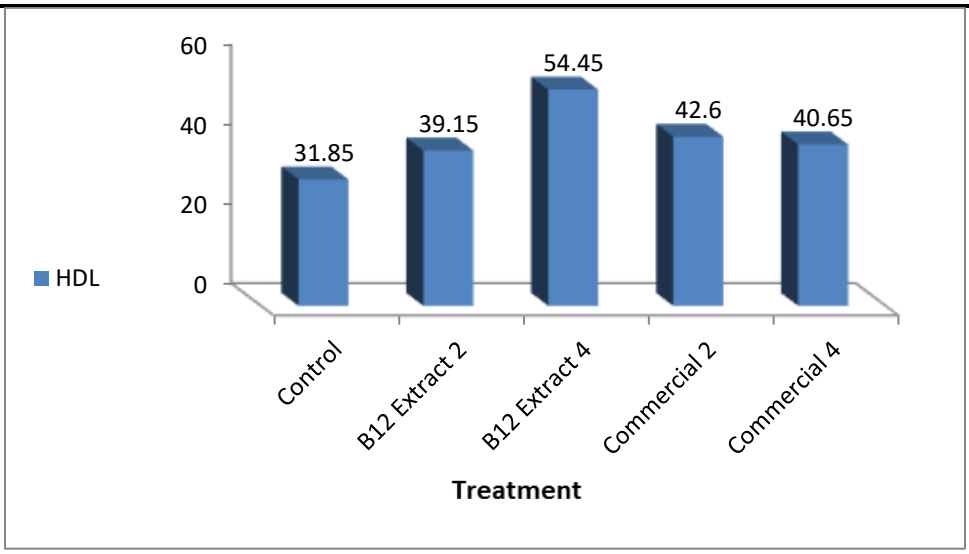

Figure (3) Effect of vitamin B_{12} on the values of high-density lipoproteins (HDL)

On the other hand , Table (1) A significant decrease ($P<0.05$) in the results of harmful LDL lipoproteins was also observed in the group treated with vitamin B_{12} extracted from the G1 control group. It was (45.33 , 37.67 , 41, 45) mg/dl for groups G2, G3, G4, and G5 consecutively compared with the control G1 its 60.5 mg/dl

The results of the study indicated that the normal levels of cholesterol, triglycerides, LDL and HDL concentrations were maintained in female albino rats treated with extracted and commercial vitamin B_{12} at a concentration of (2 and 4) µg/ml for both species in comparison with the control group G1.

4.The effect of vitamin B_{12} on the activity of liver enzymes AST and ALT.

The results shown in Table (1) that percentage of liver enzymes in groups G2, G3, G4, and G5 did not increase compared to the G1 control group, but when comparing the G1 control group with the vitamin B12-treated group, the increase in it was significant ($P<0.05$) and refer As for AST enzyme, it was observed in Table (1) that the percentage of liver aspartate transporter enzyme AST was significantly increased ($P<0.05$) and it was higher in the group treated with extracted vitamin B12 (39.75 , 40.75 , 42.2 , 39.95) IU/L compared to the control group G1 its 55.4 IU/L.

Table (1) shows the results of the after dosing with Vitamin B_{12} Extract and Commercial B_{12} for low-density lipoproteins , AST and ALT

LDL ≤100mg/ dl	AST Upto 48IU/L	ALT Upto 55IU/L	sample name
60.5 a	55.4 a	55.5 a	RAT1
45.33 b	39.75 b	47.33 b	B_{12} Extract 2
37.67 c	40.75 b	39.85 d	B_{12} Extract 4

41 bc	**42.2 b**	**44.75 bc**	**Commercial2**
45 b	**39.95 b**	**41.35 cd**	**Commercial 4**

a-b: Different letters in the same column indicate significant differences at the 0.05 . probability level

The treatment of groups G2, G3, G4, G5 with extracted and commercial vitamin B12 separately led to an improvement in cholesterol and triglyceride values. The role of vitamin B_{12} as an antioxidant and in repairing liver tissue cells and working to rid the body of excess cholesterol, also has a role in Repair of hepatocytes leads to the return of the liver to its normal state and its secretion and regulation of the levels of important lipoproteins needed in the blood plasma (11)(1).

If the levels of LDL are high, it will be deposited on the walls of the blood vessels, which leads to a decrease in their diameter and reduces their elasticity and thus blockage, and as a result there is a problem with blood flow, and this is called atherosclerosis when it occurs in the arteries that feed the heart, it increases the risks It is worth mentioning here that HDL helps prevent the deposition of harmful LDL cholesterol on the walls of blood vessels and remove it from the circulatory system, as vitamin B_{12} helps reduce cardiovascular disease, low-density lipoprotein levels, and high blood pressure. and low levels of high-density lipoprotein, obesity and diabetes (11) .

The high activity of enzymes (ALT, AST) in the blood is the best evidence of liver damage, so high levels of them in the blood can be used as a measure of the pathological changes that occur in the liver(12).

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