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وحماية المستهلك

THE EFFECT OF YOGHURT FORTIFIED WITH NANO-ZINC ON SOME NUTRITIONAL INDICATORS FOR EXPERIMENTAL MICE

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ABSTRACT

The research aimed to develop therapeutic products by producing yogurt fortified with nano-zinc and studying the effect of feeding this product on some vital indicators of laboratory mice . One of the most important results reached is that nano-zinc has a clear role in reducing daily and final weight gain in a way that is directly proportional to the amount of nano-zinc added compared to the C+ positive control group, it also significantly reduced (P>0.05) the levels of total cholesterol (TC), triglycerides (TG), and bad fats low density lipoprotein (LDL) and very low density lipoprotein (VLDL), and a significant increase (P≤0.05) in the values of good fats high density lipoprotein (HDL). The results also showed a significant decrease (P>0.05) in the blood sugar content. Glucose and the effectiveness of the liver enzymes glutamic oxaloacetic ransaminase (GOT), glutamic pyruvi transaminase (GPT) and alkaline phosphotase (AIP) compared to their levels in the C+ positive control treatment fed a high-fat diet. Nano-zinc also improved the efficiency of the immune system by increasing the number of white blood cells (WBC) and maintaining the concentration of hemoglobin (Hb) within normal limits.

Key words: Yogurt, Nano-zinc, Liver enzyme levels, Blood lipids, Blood glucose levels, White blood cells.

تأثير اليوغرت المدعم بالزنك النانوي على بعض المؤشرات التغذوية لفئران التجارب

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الخلاصة

هدف البحث الى تطوير منتجات علاجية وذلك من خلال انتاج لبن رائب مدعم بالزنك النانوي ودراسة تأثير التغذية بهذا المنتج على بعض المؤشرات الحيوية لفئران التجارب. ومن أهم النتائج التي تم التوصل لها هو للزنك النانوي دور واضح للحد من الزيادة الوزنية اليومية والنهائية للفئران بشكل يتناسب طردياً مع كمية الزنك النانوي المضافة مقارنة مع مجموعة السيطرة الموجبة, كما خفض وبشكل معنوي (P>0.05) من مستويات الكوليسترول الكلي والدهون الثلاثية والدهون السيئة وزيادة معنوية (P≤0.05) في قيم الدهون الجيدة وكما أظهرت النتائج وجود أنخفاض معنوي (P>0.05) في محتوى الدم من سكر الكلوكوز ومن فعالية أنزيمات الكبد مقارنة بمستوياتها في معاملة السيطرة الموجبة المغذاة على عليقة عالية الدهن كما وحسن الزنك النانوي من كفاءة الجهاز المناعي بزيادة أعداد خلايا الدم البيضاء والمحافظة على تركيز الهيموغلوبين ضمن الحدود الطبيعية.

الكلمات المفتاحية : لبن رائب، زنك نانوي، مستويات إنزيمات الكبد، دهون الدم، مستوى كلوكوز الدم، خلايا الدم البيضاء.

[*] **The research is extracted from the master's thesis of the first researcher.**

INTRODUCTION

Functional foods are defined as those foods that have many benefits to human health, and it was agreed in 2012 at the Tenth International Conference held in California that functional foods are those foods that contain specific quantities of substances that are biologically beneficial to human health and are used to treat some diseases **(Sadiq, 2019)**, and in the seventeenth session organized by the US Department of Agriculture and the Agricultural Research Service (ARS) They modified the definition of functional foods as those foods that contain specific amounts of substances with health benefits within limits that are not considered toxic to the body **(Khalil & Lafta, 2023).** Notably, with persistent nutrient deficiencies prevailing in various human societies, particularly throughout various life stages, there is an increasing adoption, importation, and consumption of fortified food **(Victor *et al*., 2013)**. Milk is considered one of the basic sources of human food, which prompted countries to take intense interest in providing milk and its products and strive to develop them in order to achieve self-sufficiency (**Mohammed, 2015**). One of the oldest methods by human practiced is fermentation beings for the transformation of milk into products with an extended storage **(Khalil & Lafta, 2023)**. Milk has positive effects on many body structures as the nervous, immune, and cardiovascular systems **(Alkhalidy & Doosh,2022)**. The milk base to enhance or maintain the appropriate yogurt properties stabilizers, such as, pectin or gelatin, are often added to including texture, mouthfeel, appearance, viscosity/consistency and to the prevention of whey separation (wheying- off) **(Lafta, 2014).** In the past decades 44% of Americans have changed their eating habits to lower their cholesterol level the International Dairy Statistics reported that world per capita slowly decreased consumption of butter from 1999 to 2002 in most European countries, Asia and the United States, this shift in eating habits has caused a dramatic increase in no-cholesterol, low-cholesterol, reduced-cholesterol and low fat products available in the marketplace **(Doosh *et al* ., 2013)**.A powerful trace element that is essential for immune system health is zinc. A lack of zinc in the body is strongly linked to immune system changes and increased vulnerability to viral infections, such as COVID-19-causing SARS-CoV-2. Additionally, Zinc assists in the structural composition of the zinc finger protein GFi-1B and acts as a catalyst in the metabolism of heme. Its relevance extends to controlling angiogenesis and erythroid cell development by regulating erythroid chain-specific gene expression **(Angelova *et al*., 2014).** Zinc is ubiquitous in all biological systems and plays exceptionally versatile roles. Zinc participates in various vital functions at the cellular and subcellular levels, classified into catalytic, structural, and regulatory roles **(King, 2011).**

Objectives of the Study:

1. Preparation of cholesterol-free milk using beta cyclodextran.
2. Preparation of coated and uncoated zinc nanoparticles after selecting the best type of coating.
3. Manufacture of cholesterol-free yoghurt and fortified with different percentages of zinc nanoparticles, encapsulated and unencapsulated, in proportions that fill a quarter and a half and all the daily need from it.
4. The investigation focuses on analysing the physicochemical, rheological, and sensory characteristics of the produced item.
5. Conducting a feeding experiment on laboratory rats to find out the effect of fortifying the diet with the zinc-fortified product on some health indicators.

MATERIALS AND METHODS

In this study, we employed 40 mice males of the Albino strain, procured is Biotechnology Research Center at Al-Nahrain University. The experiment used mice that were nearly 6 weeks old, our investigation focused on evaluating the impact of zinc nanoparticles on glucose and lipid levels. Specifically, we assessed the all profil lipid TC, TG, HDL, LDL and VLDL, as well as total white Hb concentration, liver enzyme activity GOT, GPT, and ALP.The animals were housed in controlled environments, ensuring proper ventilation and maintaining temperatures within the range of 2 ± 25 °C. Lighting conditions consisted of 12 hr without lighting followed by 12 hours of lighting. mice was split into a four group based on their assigned diets. They were accommodated in specialized plastic cages, receiving a continuous supply of both food and distilled water, accessible to them ad libitum. To facilitate adaptation to the experimental conditions, the mice were provided with food in their cages for 3 days prior to commencing the study, a process known as adaptation, repeated twice a week throughout the experiment.

1. First Group (C-): Throughout the investigation, the negative control group just had standard diet.
2. Second Group (C+): received a high-fat diet (Long-chain fats found in large animal products and sheep fat) duration the experiment.
3. Third Group (T1): The T1 group was subjected to a high fats diets (Long-chain fats found in large animal products and sheep fat) with oral administrations of 0.1 ml of yogurt fortified with zinc nanoparticles, encapsulated at a concentration of 7.5 mg / 100 ml, throughout the experimental period.
4. Fourth Group (T2): The T2 group was exposed to a high-fat diet (Long-chain fats found in large animal products and sheep fat) and received by oraldose of 0.1ml of yogurt fortifieds with nano-encapsulated zinc with concentration 15mg/100 ml throughout duration of the experiment.

Table (1): Nutritional components of the diet of laboratory mice (g/100g).

Ingredients	**g \ 100**
Caseins	19
corn oils	7
Fibero Cellulos	6
Vitamins mix	1
Minerals mix	3.5
Colins	0.2
Milk Fat	0
Cholesterol	0
Starch	46.8

Note: the sucrose used to complete 100g of the diet.

- **Preparation of Mouse Diet:** The mice diet prepared according to **(American Institute of Nutrition, 1993)**
- **Collect Samples:** in conclusion test, the mice was subjected to an 8-hour fasting period before being anesthetized using a ketamine and xylazine intramuscular injection. The abdominal cavity was opened from the bottom, and 1 ml of bloods withdrawn taken out is hearts and place in a tube of test until the necessary tests were performed:

1. **Plasma:** To prevent coagulation, ethylenediaminetetraacetic acid (EDTA) anticoagulant was added to this portion of the blood, and it was used for conducting standard blood tests.
2. **Serum:** After centrifuging the clot-filled blood at 3000 rpm for 15 minutes, the blood was chilled for 30 minutes. Using an Eppendorff tube and a Pasteur pipette, the serum was added. To avoid infection, it can either be used right away or kept in a deep freezer at -100°F (-20°C).

Biochemical Analysis:

- Glucose Concentration Measurement: The modified method of the German company Humanan **(Bablok *et al*., 1988)** was followed. The glucose concentration was measured according to the following equation: glucose concentration (mg/100 ml) = sample absorbance reading / standard solution absorbance reading × 100, where the standard solution concentration is known.
- **Assessment of the efficiency of glutamate oxidase transaminase GOT and glutamic pyruvate transcription factors GPT :**

  Both the manufacturer's instructions and the modified colorimetric procedure from **(Thefeld *et al*., 1994)** that was altered by the Swiss business AGAPPE were followed. The following formula can be used to determine these two enzymes'activity in international units (L/U):

  -Activity of GPT enzyme (unit/L) = absorbance rate / time (minutes) × 1745.

  -Activity of GOT enzyme (unit/L) = absorbance rate / time (minutes) × 1745.
- **Alkaline phosphatase enzyme determination in the blood serum of mice:**

Following the guidelines provided by the treatment firm, the colorimetric approach **(Schlebusch *et al*., 1974)** created by the Swiss business AGAPPE was used.

- **Determination of total Serum cholesterol Level:**

  According to method **(Young, 1997)** created using the German firm Human and per the directions wich treatments company, the enzymatic hydrolysis procedure for cholesterol was applied. According to the link between:

  Cholesterol concentration (mg/100ml) is the difference between the sample's absorbance reading and the standard solution's absorbance reading multiplied by the standard solution's 200 concentration.
- **Estimation of Serum Triglycrides Level**: The modified method of the German company Human mentioned in **(Young , 1997)** was followed to measure the enzymatic hydrolysis of cholesterol according to the followin equation: Triglycerides concentration (mg/100 ml) = Absorbance reading of the sample / Absorbance reading of the standard solution x 200 Concentration of the standard solution.
- **Estimation of Serum High-Density Lipoprotein HDL Level:**

  The enzymatic analysis method for high-density lipoprotein cholesterol (HDL-C) was followed according to Method No. **(Burstein *et al*., 1970)** developed by the German company Human, following the instructions provided by the manufacturer. HDL concentration (mg/200 ml) was calculated using the following formula: HDL concentration (mg/200 ml) = Absorbance reading of sample/ Absorbance reading of standard solution x 50 Concentration of standard solution x 2.

- **Estimation of Serum Low-Density Lipoprotein LDL and Very Low-Density Lipoprotein VLDL Levels:** The Friedewald equation **(Al-Shorfani,2006)** was used to calculate the levels of LDL and VLDL as follows: (LDL Cholesterol) = (Total Cholesterol)

- (HDL Cholesterol) + (Triglyceride/5), where (Triglyceride/5) represents (VLDL) Cholesterol.

blood test:

- **Total Leukocyte Count:** The method **(Haen, 1995)** was used to calculate the total white blood cell count.
- The total number of white blood cells (cells/ml) has calculat like follow: totals whites bloods cells count (cells/ml) = counted cells number / (4 × 20 × 10).
- **Estimation of Hemoglobin Concentration:** Hemoglobin concentration was determined using method **(Makarem, 1974)**, where hemoglobin oxidizes to methemoglobin in the presence of basic iron potassium cyanide. It then combines with methemoglobin using potassium cyanide to form cyanomethemoglobin, which absorbs light at a wavelength of 540 nm.
- **Statistical Analysis:** In order to analyze the data, a full random design was implemented. (CRD), and the statisticals programs SAS (Statistical Analysis System - 2018) was used to determine how different factors affected the attributes that were being studied. To evaluate significant differences between means, the least significants differenc (LSD) tests as employed. The study was conducted using currently existing statistical analysis tools **(SAS, 2018).**

RESULTS AND DISCUSSION

- **Effect of Zinc-Fortified Nano-Encapsulated Yogurt on mice Weight:**

Table (2) presents the results of the impact of yogurt fortified with nano-coated zinc on the daily and final weight gain rates of mice. These observations were made 28 days after the experiment started, comparing groups of mice which fed Standard diet (control group C) refers to a highest fat diet (C+ control group), therapeutic yogurt fortified with 7.5 mg of zinc nanoparticles per day (T1 group), and therapeutic yogurt fortified with 15 mg of zinc nanoparticles per day (T2 group). The standard diet resulted in a daily weight gain of 0.2439 grams/day, with a final weight gain of 6.83 grams after 28 days.From the table, it is evident that the highest daily and final weight gains were recorded in the C+ positive control group, amounting to 0.3150 grams/day and 8.82 grams, respectively. The notable differences in results between the C- and C+ groups can be attributed to the dietary variations. The C+ group was feeding a high diet of fat, leading to a more significant weights gain. These findings align with previous research **(Sadiq, 2019)**.

Table (2): Average weight gain of different groups of mice after 28 days.

Treatment	BW (g)		BW gain after 28 days (g)	Average daily increase in body weight (g)
	Average starting weight (g)	Average final weight(g)		
C-	26.12	32.95	6.83	0.2439
C+	26.77	35.59	8.82	0.3150
T1	27.12	34.95	7.83	0.2796
T2	26.00	32.89	6.89	0.2460
L.S.D value	3.028 NS	3.189NS	2.094NS	0.137NS
NS :P>0.05 (non-material).				

It is noteworthy from the results that dosing with the milk product containing nano-zinc, despite highest fats diets, led to a reduction in weights gain that was directly proportional to the concentration of nano-zinc consumed. Daily is average and final weights

gain for the groups of mice were (T1 treatment) 0.2796 grams/ day and 7.83 grams, continuously. Meanwhile, the weight gain rate for the T2 treatment group of mice was (0.2460) g/day and (6.89) g, respectively. compared to C+ group, which was fed by a diet with high fatty livel, a significant decrease in weight observ in the groups which treated. This indicates that zinc contributed to reducing weight gain while maintaining normal growth. Notably, there was a significant increase by these group compared to the group of negative control C-. These results align with findings by **(Al-Shaikh & Doosh, 2017).** There was statistically significant difference in our study results statistical analysis ($P > 0.05$) in the means weights of the treated mices during study periods. Moreover, significant differences in daily weight gain and average daily weight gain were observed between C+, C-, and all treatment.

Effect of Nano-Zinc-Fortified Yogurt on Blood Glucose Levels and Liver Enzymes GOT, GPT, and ALP:

(Table, 3) presents the results of the impact of nano-zinc-fortified yogurt on blood glucose levels in groups of mice treated with C-, C+, T1, and T2 after a 28 day experiment. The results show that the highest concentration of blood glucose is found in the serum of mice in the C+ treatment group, reaching 210.00 mg/100 ml. This finding aligns with the discovery by **(Lafta & Doosh, 2017)**, who noted that feeding mice a high-fat diet led to increased blood plasma glucose levels, reaching 196 mg/100 ml compared to the group fed a standard diet.

(Table, 3) reveals a significants decreas by glucose in blood level in the T1, T2 treatment groups, where levels reached 81.00 and 76.00 mg/100 ml, respectively. This decrease was statistically significant in $P \leq 0.05$ levels when compared to C+ group. Notably, supplementing mice with nano-zinc-fortified yogurt at concentratin 7.5and15 mg/100 ml contributed to bringing blood glucose levels back to normal. This effect may be attributed to increased intestinal contents viscosity and thickness, particularly in the jejunum, which impedes or inhibits sugar absorption. These results agree with the results of **(Kielczykowska *et al*., 2014)**, who found that seleniums, in concentration of 0.5 mg/kg of B.W, restored normal blood gluco-levels in mice subjected to lithium poisoning.

Through statistical analysis, it was determined that the C+, T1, T2 group, also as C- and C+ treatments, had significantly different blood glucose readings ($P \leq 0.05$).

(Table, 3) indicated the results of the effect of yogurt fortified with nano-zinc on the concentration of liver enzymes GOT and GPT in the above-mentioned groups of C-, C+, T1, and T2 mice after 28 days. The highest serum GOT level was recorded in mice in the C+ treatment group, which were fed a high-fat diet, at 69.11 U/L. In contrast, treatment group C, which was fed a standard diet, had a level of 56.00 U/L. The lowest concentration was observed in the T2 treatment group with a value of 35.28 U/L, followed by the T1 treatment group with a value of 38.00 U/L. Statisticals analys shown a significants differenc ($P<0.05$) withe groups C+, T1, and T2, while there was no significant difference between group C and groups T1 and T2.

Table (3): Level of glucose, GOT and GPT from livers, and zinc nanoparticles of groups of experimental mice after 28 days.

Treatment	Glucose mg/100ml	GOT unit/liter	GPT Unit / liter	AIP Unit/ litre	Zinc Mg / l
C-	132.00	56.00	27.00	84.33	11.7
C+	210.00	69.11	66.32	128.00	89.2
T1	81.00	38.00	43.71	39.00	67.0
T2	76.00	35.28	61.04	65.21	69.3
L.S.D value	32.821 *	18.944 *	15.307 *	31.805 *	17.923 *
*(P≤0.05)					

Regarding the GPT Enzyme: The GPT enzyme exhibited its highest level in the serum the C+ group experimental animal, which was fed a highest fats diets, reaching 66.32 units/liter. Following closely, the T2-treated mice showed a GPT level of 61.04 units/liter. In contrast, the lowest level was observed in the C- group, registering 27.00 units/liter, followed by the T1 treatment group with a level of 43.71 units/liter. This indicates a notable decrease in GPT levels when compared to the C+ treatment group. Statistical analysis results show a significants differenc (P≤0.05) in GPT concentration between C+ and the other treatment, including C-, T1, and T2.

Concerning the AIP Enzyme: The AIP enzyme's concentration in the control group (C-) reached 84.33 units/liter. In contrast, the control C+ group recorded the highest level in serum, which amounted to 128 units/liter, like fined of the high percentage of fat of diet. The results come with a decreases in AIP concentration in groups fed a high-fat diet but supplemented with nano-zinc. In the T1 group, AIP concentration was 39.00 units/liter, while the T2 treatment group recorded 65.21 units/liter. Statistical analysis reveals a significant decrease (P≤0.05) in these levels compared to the C+ control group, indicating a return to normal values. Elevated AIP levels in circulation typically signify hepatic membrane malfunction **(Plaa, 2010)**.

Regarding Zinc Concentration: The more high levels of zinc is found in the blood of the C+ group of experimental animal, which were fed a highest fats diets, reaching 89.2 mg/L. Following this, the T2 treatment group exhibited a zinc level of 69.3 mg/L. Conversely, the most lower level was recorded by the C- group of experimental animal reaching 11.7 mg/L, followed by the T1 treatment group, with a concentration of 67.0 mg/L. This indicates a decrease in zinc levels compared to the C+ treatment group. Statistically analysis shows that there is a significants differenc (P<0.05) in zinc concentration between the treatments including C-, T1, T2, and the C+ treatment.

The Effect of Nano-Zinc-Fortified Yogurt on Triglycerides, TC, HDL, LDL, and VLDL Levels:

(Table, 4) It shown effect by yogurts fortified nano-zinc in the levels of LDL, TG, VLDL, and HDL cholesterol in groups of experimental mice.

subjected to treatments after 28 days C-, C+, T1, and T2. The groups of mice treated with the positive control C+ exhibited the highest cholesterol level at 138.0 mg/100 ml. This may be doing attributed to the high-fat content of their diet compared with C- negative control groups, which had a cholesterol level of 99.0 mg/100 ml. These results are consistent with the results of **(Al-kafaji & Ajeena).**in which an increase in total cholesterol levels was reported In

groups of experimental mice that were fed a highest fats diets compared with other groups of mice that were feeding with a standard diet with values of 186.0 and 94.0, respectively.

Table (4): Percentages of total cholesterol, triglycerides, HLDL and VLDL in the serum of lap mice after (28) days.

Treatment	Total cholesterol mg / 100 ml	Triglycerides TG mg / 100 ml	High-density lipoproteins (HDL) Mg / 100 ml	Low-density lipoproteins, (LDL) Mg / 100 ml	Very low-density lipoproteins (VLDL) Mg / 100 ml
C-	99.0	133.5	40.70	88.00	17.6
C+	138.0	182.0	86.17	159.00	48.8
T1	90.0	93.0	69.60	15.20	15.6
T2	93.0	120.0	70.90	15.30	15.3
L.S.D value	28.516 *	41.681 *	17.549 *	32.487 *	7.533 *
* (P≤0.05)					

Regarding a (TG) in T1 treatment groups of mice, reached 90.0mg/100ml. Notably, this level exhibited a significants decreas (P≤0.05) when compared to the C+. In the case of the T2 treatments groups, the TC level was recorded at 93.0 mg/100 ml, and this decrease was also statistically significants (P≤0.05) compared to the group (C+). These fended align with the results of **(Hamid & Doosh, 2021)** It indicated a significant decrease in cholesterol levels when mice were treated with zinc alone or zinc with bread. The results of the statistical analysis indicate **Table (4)** It was found that there was a significants differences (P<0.05) between (C+) and the other groups treatments. Conversely, there was no significant difference observed between the T1 group and control C- treatment group. This suggests that zinc nanoparticles at a concentrations of 7.5 mg/100ml effectively reduced cholesterol levels to within the normal range.

From the results presented in **Table (4)** it is evident that yogurt fortified with nano-zinc has an impact on triglyceride (TG) levels. The levels of the T.G in the blood was highest in C+treated mice in the control group, also identified a three-fold increase in TG when mice was fed a cholesterol-rich diet for a month. The TG level in the C-control rat group, however, was 133.5 mg/100 ml. For the T1 and T2 treatment groups of mice, TG levels reached 93.0 mg/100 ml and 120.0 mg/100 ml, respectively. Notably, the lowest TG level was observed in the T1-treated group, even surpassing negative controls groups. The results of statistical analysis in TG levels between groups T1 and T2 compared to both control groups for both C- and C+ indicater significants difference (P<0.05). This parametrs is consistent with those of **(El-Demerdash & Nasr, 2014)** who found that mice given selenium at a dose of 200 micrograms per day had lower TG levels than mice in the control group (109 mg/100 ml vs. 97 mg/100 ml).Regarding (HDL) values, the high concentration is recording in the serum of mice in the positive control group, C+, reaching 86.17 mg/100 ml.

Following that, the T2 treatment showed of 70.90 mg/100ml, whereas the T1 treatment exhibited a level of 69.60 mg/100 ml. was low than other recorded level was in the C- control treatment, measuring 40.70 mg/100 ml. This decrease in HDL levels can be attributed to the increased body weights of the group C+ mice. This observation is consistent with the findings of **(Mahdi *et al.*, 2023**), who noted a correlation between weight gain in experimental animals

and a reduction in HDL levels. Therefore, we can observe an increase in the average final weights of the group C+ mice due to the lower HDL percentages compared to the control group, C.

A substantial differences (P≤ 0.05) betweens C+ and the T1, T2 treatments is shown by the statistical analysis results, which are shown in Table 3. The action of zinc nanoparticles shows a favorable effect in raising the levels of high-density serum lipoproteins, which are sometimes referred to as "benign." This result is regarded as one of the good health signs.

These findings align with the results reported by **(El-Demerdash & Nasr, 2014)**, which demonstrated a significants increas in beneficial HDL levels of the TC in mice dose with selenium at a 200 micrograms. This suggests that selenium has positive health effects. Regarding serum low-density lipoproteins LDL levels, the highest concentration was observed in the C+ treatment group, reaching 159.00 mg/100 ml. In contrast, the C- treatment group had LDL levels of 88.00 mg/100 ml, While the results indicated a significant decrease (P<0.05) for the T1 and T2 treatment groups, levels of 15.20 and 15.30 mg/100 ml, respectively, compared to the C+ treatment group. Interestingly, despite the high-fat diet consumed by the T1 and T2 groups, this decrease can be attributed to the influence of zinc nanoparticles added to their diet.

Regarding the VLDL levels in the previously mentioned groups of mice (C-, C+, T1, and T2), a most high level has observed in the C+ treatment group, which was fed a high-fat diet, amounting to 48.8 mg/100 ml. The results indicate a significants decreased in VLDL value for the groups of mice fed a high-fat diet fortified with nano-zinc. Specifically, the values for the T1 and T2 treatments reached 15.6 and 15.3 mg/100 ml, respectively. In contrast, the C- treatment group had levels of 17.6 mg/100 ml. Notably, the lowest value was recorded in the T1 treatment group, surpassing even the C- treatment. Statistical analysis reveals a significants differenc (P≤0.05) in levels of lipid VLD betweenness the C- and C+ treatment, with no significants difference betwen the C-T1, and T2 treatment.

From the comprehensive results presented in (**Tables 2, 4)**. it is evident that the high fats diets fed to mice in (C+) group that led as to elevated the levels T.C, such as all lipid profile parameters. These factors are directly associated with obesity and weight gain in mice, along with other fat-related disorders. However, the fortification of the diet with zinc nanoparticles, introduced in the diets of the T1 and T2 treatment groups, played most significants role with prevent excessived weights gaining. Additionally, it reduced TG, TC levels, they bringing it doing to normaling range compared to the positive control group (C+), despite the increased fat content. This observation aligns with the findings of **(El-Demerdash & Nasr, 2014)**, who reported significants decreas by totally cholesterol level, reduced LDL levels, and an increase in HDL percentages when fortifying rat diets with selenium.

Regarding the Effect on White Blood Cells (W.B.C) and Hemoglobin (Hb): Table (5) presents the impact zinc nanoparticle-fortified yogurt on (W.B.C) and hemoglobin (Hb) for the groups of C-, C+, T1, and T2 mice involved in the experiment after 28 days. The group of T1-treated mice exhibited the highest white blood cell count, reaching 15.9×10^3 cells/mm³. This significant increase was observed at a level of (P≤0.05) compared to both the negative control group C- and the positive control group C+, which recorded counts of 8.6×10^3 and 12.6×10^3 cells/mm³, respectively. Statistical analysis indicates significants differenc (P>0.05) with treatments T1andT2 compared like C+ control group, was no significants differenc with the C- group. Notably, white blood cell counts increased in T1 and T2 treatments fortified with zinc nanoparticles, suggesting that zinc nanoparticles enhance white blood cell activity, thereby

aiding the body in resisting infections, viruses such as influenza and the common cold, and even more dangerous cancer cells. This observation aligns with previous findings **(Yazdi *et al.*, 2013)** that reported an increase in white blood cell counts in mice 30 days After taking 100 micrograms/day of selenium supplements, compared to their levels before supplementation, which were within normal limits. Furthermore, Selenium supplements have been shown to enhance cellular immunity against various bacterial and fungal infections and cancerous tumors.

Table (5): The number of total white-bloods cells and level of hemoglobin (Hb) and Packed cell Volume (P.C.V) in the experimental mice blood after (28) days.

Treatment	The total number of white blood cells cells/mm³ (W.B.C)	Hemoglobin grams/liter (Hb)	Packed cell volume% (P.C.V)
C-	8.6×10^3	12.5	30.22
C+	12.6×10^3	15.9	28.00
T1	15.9×10^3	10.9	34.79
T2	14.1×10^3	11.2	34.00
L.S.D value	* 3.093	* 2.108	
.(P≤0.05) *			

Regarding Hemoglobin (Hb) Levels:

by side of hemoglobin (Hb) levels, the most higher recorded level was in the C+ control group, at 15.9 g/dL. This increase was statistically significant (P>0.05) compared to the C- control group, which had a hemoglobin level of 12.5 g/dL. Following closely was the T1 treatment group, with a level of 10.9 g/dL, while the T2 treatment group had a level of 11.2 g/dL. The results of the statistically analys indicates a significants differenc (P≤0.05) between the various treatments. This suggests that zinc nanoparticles contributed to an increase in hemoglobin levels, restoring them to normal levels compared to the C+ positive control group, which was fed a high-fat diet. Zinc nanoparticle deficiency may pose a risk of anemia in humans, especially those with HIV. The current study's results demonstrate that fortification with nanoparticle zinc at concentrations of 7.5 and 15 mg/100 ml increased hemoglobin levels compared to the C+ treatment group, while maintaining normal hemoglobin levels compared to the C-. This underscores the vital role of nanoparticle zinc, which exhibited a positive impact, serving as an indicator of good health. The results in **(Table, 5).** indicate the percentages of compressed cell volume (P.C.V.). The highest percentage was recorded by the T1-treated group of mice, reaching 34.79%, Followed by the group of mice treated with T2, which amounted to 34.00%, and the control treatment C-, which amounted to 30.22%, and the lowest percentage recorded by the group of mice in the control treatment C+, which amounted to 28.00%. The results of the statistical analysis indicated that there was a significant difference (P<0.05) between the control treatment C+ and the rest of the treatments represented by C-, T1 and T2, and there was no significant difference between the control treatment C- and the treatments T1 and T2 supported with nano-zinc, and this result is consistent with what was found**(Eghianruwa & Anika, 2011)**.

CONCLUSION

Through the study, it was concluded that adding nano-zinc at concentrations of 7.5 and 15 mg/100 ml contributed to prolonging the shelf life in addition to improving the physicochemical properties of yogurt. It was also clear from the nutritional experiment that nano-zinc contributed to adjusting the level of glucose in the blood and also reduced the levels of total cholesterol and fats. The trilogy of high-density lipoproteins, low-density lipoproteins, and very low-density lipoproteins, and the effectiveness of liver enzymes. Nano-zinc also improved the efficiency of the immune system by increasing the number of white blood cells and maintaining hemoglobin concentration within normal limits. In group T2, which was fed yogurt fortified with 15 milligrams of zinc, the cholesterol level increased compared to group T1.

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