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Effect of Grape Berries Juice Treated with Zinc Oxide Nanoparticles on the Performance and Health of Rabbits

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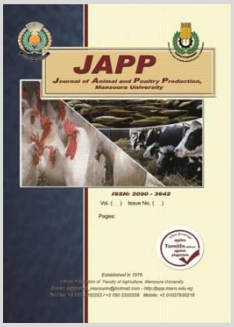

ABSTRACT

This study was conducted to evaluate the effect of grape juice treated with zinc in both forms [chelated zinc (CZn) and zinc oxide nanoparticles (ZnONPs)], on rabbits performance. Forty-two male rabbits were divided into seven groups (6 each). The 1st group was administered 20ml/kg BW water daily (negative control).The 2nd group was taken 20ml/kg BW untreated grape juice (without zinc) daily (positive control).The 3rd group was treated with 20ml/kg BW grape juice that has been treated with CZn at 1.5 gL^{-1} daily, While rabbits of the 4th, 5th,6th, &7th groups were taken 20ml/kg BW of grape juice that has been treated with ZnONPs at 60, 120, 240 & 480mgL^{-1}, respectively, daily. Results showed that growth performance, feed efficiency, serum proteins, and hematological parameters of rabbits treated with either ZnONPs or CZn were significant ($P< 0.001$) improved until they reached their maximum in G6 and then began to decline after that in G7. Blood glucose levels followed the same trend. Liver and kidney function continued to gradually increase with increasing ZnONPs rate. ZnONPs treatments had significantly decreased in total cholesterol and triglycerides, but high &low-density lipoprotein were not affected compared to control. ZnONPs grape juice administration had significantly raised the serum GSH content and SOD activity compared to the control group. It can be concluded that using grape juice treated with ZnONPs up to 240mgL^{-1} has no adverse effects on rabbits health. On the other hand, using the grape juice treated with ZnONPs at 480mgL^{-1} has adverse effects on the rabbits health.

Keywords: Grape juice, zinc oxide nanoparticles, health, rabbits

INTRODUCTION

Zinc is a necessary trace element for plants, animals, and humans; it is required for the enzymes activity and takes part in enzymatic functions in animals bodies (Ahmadi *et al*., 2013). Also, they showed that zinc is necessary for many enzymes activity and takes part in enzymatic functions and several metabolic in the body of animals. In recent years, nanotechnology has a tremendous potential to revolutionize almost all veterinary and animal sciences (Raguvaran *et al*., 2015). Partha *et al.* (2015) reported that nanomaterials have more significant growth promoting and antibacterial effects than conventional materials.

Additionally, Hekmet *et al.* (2018) stated that ZnONPs is widely used in the pharmaceutical, cosmetic, and photocatalyst pigments industries. Therefore, ZnONPs was marketed as a feed supplement or additives with unique features and activities increasing the surface area of particles, deeply penetrating tissues through fine capillaries, and efficient uptake by cells. So, the ordinary mineral can pass through the stomach wall and into body cells slowly than nanoparticles, as described by Bunglavan *et al*. (2014). Besides, Abdel-Wareth *et al*. (2020) mentioned that ZnONPs treatments significantly improved the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and urea concentrations in male Californian rabbits.

Importantly, it is necessary to understand the various adverse effects of ZnONPs on cellular and organ functions to provide better approaches for them. Meanwhile, Chong *et al.* (2021) reported that ZnONPs need to collect and reliable; disseminate accurate and unbiased information on the risks and benefits of ZnONPs, to evaluate if the potential advantages outweigh the risk associated.

Grape berries (*Vitis vinifera L.*) contain sugars, tannins, succinic acid, tartaric acid, malic acid, and mineral substances, which have been used for medicinal and therapeutic purposes (Tomaz *et al*., 2017). Thus, the fresh grape juice stimulates the gut and kidneys function, aids in the release of all toxic substances, and prevents the formation of gallstones, kidney stones, and bladder stones (Ozturk *et al*., 2020).

An early study has been carried out on Flame Seedless grapevines using ZnONPs (Mekawy, 2021). Meanwhile, this investigation was performed to study the possible toxic effects of oral administration of Flame Seedless grape juice treated with different doses of ZnONPs on rabbits' health. This may give us more information on the hazards of nanomaterials on human health.

MATERIALS AND METHODS

This study was conducted to evaluate the effect of grape juice obtained from Flame Seedless grapevines vineyard located at grown at the Experimental Farm of Sids Agricultural Research Station, Beni-Suef Governorate that treated with foliar application of zinc in both forms (CZn12.5% at a dose of 1.5gL^{-1} and ZnONPs at dose of 60, 120, 240 & 480mgL^{-1}) preharvest during their growing seasons, grape clusters were collected, each treatment separately at harvest time and berries crushed in a blender, then filtered with cheese cloth as described by Mekawy (2021), and then fed to rabbits to test toxicity of the residual zinc content in grape juice that treated with both forms (CZn or ZnONPs) and their effects on the performance of rabbits.

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Experimental design

Forty-two V-line male rabbits, aged three months, average body weight (BW) was 1.465±0.12 kg were used as experimental animals in this investigation for three months. Rabbits were divided individually into seven equal groups (6 males/each). The 1st group (G1) was orally administered of 15mL/kg BW distilled water and served as a negative control, the 2nd group (G2) was orally administered 15mL/kg BW grape juice obtained from Flame Seedless grapevines without treated with chelated zinc and served as a positive control, the 3rd group (G3) was orally administration of 15mL/kg BW grape juice obtained from Flame Seedless grapevines that have been treated with CZn at a dose of 1.5gL^{-1} daily, While rabbits of the 4th (G4), 5th (G5) 6th (G6), &7th (G7) groups were taken 15mL/Kg BW orally administration of grape juice obtained from Flame Seedless grapevines that have been treated with ZnONPs at a dose of 60, 120, 240 & 480 mgL^{-1}, respectively, daily.

Rabbits were held in galvanized metal bunny battery with separate feeders. All rabbits were housed under the same conditions. *Ad libitum* pelleted diets were given during the trial period, and fresh water was available from automatic nipple drinkers. Chemical analysis of the basal rations is shown in Table (1). Both feed intake and body weight were recorded weekly. Body weight gain and feed conversion ratio were calculated.

Table 1. Chemical analysis of the experimental ration for growing rabbits.

Ingredients	% DM	Calculated analysis: [1]	% DM
Clover hay (12%CP)	30.00	Crude protein %	17.02
Barely	29.00	Digestible energy (Kcal/Kg)	2500
Yellow corn	10.00	C/P ratio	147
Soybean meal (44%CP)	18.00	Ether extract %	2.72
Wheat bran	8.00	Crude fiber %	13.25
Molasses	3.00	NDF%	37.63
DL-Methionine	0.10	ADF%	21.52
Vit..& Min. mix.*	0.40	Hemicellulose %	16.11
Salt	0.50	Calcium %	1.10
Limestone	1.00	Total Phosphorus %	0.80
		Methionine %	0.36
		TSAA	0.61
		Lysine %	0.75
Total	100		

*** Each 1.5Kg. of Vita. mix contained : 50,000,000 IU Vit. A; 1,000,000 IU D3; 10,000 mg Vit. E; 1170 mg Vit. K3;735 mg Vit.B1; 15000 mg Vit B6;15 mg Vit.B12 ; 500 mg Vit.B5 Panathonic acid; 30,000 g Nicotinic acid; 84 mg Biotin; 500 g Folic acid; 300g choline cholride. Each 1.5 Kg Min. mix contained 25g Zn (oxide); 33.4g Mn; 26.7g Fe; 2.67g Cu; 67mg cobalt;1mg Se and.0.334 gI;**

[1] According to Feed Composition Tables for animal and poultry feed stuffs used in Egypt.

Blood samples

After 12 hours of fasting, rabbits were slaughtered, and blood samples were taken between 07:00-08:00 a.m., placed in 5mL, a sterile vacutainer tube. 1mL of the blood was put into a bottle containing ethylene diamine tetraacetic acid (EDTA) as an anticoagulant for haematological assay. The remaining 4 ml of the blood sample was put into a sterile vacutainer tube without an anticoagulant for serum biochemical assay. Serum preparation by centrifugation at 1.370*g* for 15 min. and then transferred into sterilized tubes and stored at - 20 °C.

Hematological studies

The hematological parameters were determined using an automatic Vet hematology analyzer (Abacus Junior, Radim, Italy) after putting the electric mixer samples. Each sample had been estimated in a duplicate manner (the mean of each duplicate was introduced to the statistical analysis).

Biochemical Analyses

Serum total protein (TP), albumin (AL), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), creatinine, urea, uric acid, and glucose were assayed according to Young (2000) method using biosystems automated reagent kits obtained from Costa Brava 30, Chemical Company, Barcelona (Spain). Globulin (GL) is calculated by the differences between TP and AL. Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDLc) were determined using an enzymatic colorimetric method using commercial kits (Vitro Scient, Germany) according to the manufacturer's instructions. The amount of low-density lipoprotein cholesterol (LDLc) level was calculated by using equation according to Fridewald *et al.* (1972):

$$\mathbf{LDLc = TC - HDLc - (TG/5),}$$

where (TG/5) = very-LDLc (VLDLc).

Antioxidant status

Serum glutathione (GSH), superoxide dismutase (SOD), and lipid peroxidation (LPX) were determined according to the manufacturer's instructions of assay kits (Biodiognostic Company, Dokki, Giza, Egypt).

Statistical analysis:

The statistical analysis was determined by the SPSS program for Windows software. ANOVA was used to test the effect of treatment, and the differences among means were detected by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of experimental groups on growth performance and feed efficiency

Growth performance and feed efficiency for the experimental groups are presented in Table (2). The body weight gain and growth rate of male rabbits treated with ZnONPs grape juice (G4, G5, G6, & G7) showed a significant increase in growth rate (P <0.001) (percentage change = 54.11, 67.01, 94.83&63.09, respectively) relative to the control group (G1).The growth rate reached the highest value in G6 and began to decline thereafter in G7. While the food conversion ratio took the opposite trend, this indicates improved feed efficiency through various treatments.

These findings are in agreement with those obtained by Mohamed *et al*. (2015 & 2017) in sheep and Tag-El Din (2019) in rabbits, who reported that ZnONPs supplemented diets improved digestion, nutritive values, growth rate, and feed efficiency.

These results showed that ZnONPs enhanced the digestibility coefficients and nutritive values of nutrients more than Zn in typical form, thus improving feed efficiency and growth rate. Perhaps it is due to a greater activity of biological processes and more particular surface area, high surface effectiveness, and powerful absorption capacity of elements in the nanoscale (Wang *et al*., 2007; Zhang *et al*., 2008).

Bunglavan *et al.* (2014) illustrated that the size of nanoparticles metal as feed additives is supposed to be smaller than 100 nm. As a result, they can pass via the gastrointestinal tract and into the body's tissues faster than ordinary particles with larger sizes. Nano-supplements may also be used in protein micelles or capsules or some other natural feed component. Since these molecules bioavailability is limited, developing suitable vectors remains a challenge by the intestinal epithelial barriers and their sensitivity to gastrointestinal deterioration by digestive enzymes. Manipulation of the nanoscale material also paves the way for bettering food/feed molecules' functionality, which improves the product quality.

Table 2. Growth performance and feed efficiency of male rabbits as affected by treatments.

Traits	Experimental groups							±SE	Sig.
	G1	G2	G3	G4	G5	G6	G7		
IW (kg)	1.463	1.458	1.460	1.463	1.458	1.453	1.450	0.018	NS
FW (kg)	2.193^{d}	2.393^{c}	2.603^{b}	2.588^{b}	2.673^{b}	2.860^{a}	2.630^{b}	0.031	***
WG (kg)	0.730^{e}	0.935^{d}	1.143^{bc}	1.125^{c}	1.215^{b}	1.407^{a}	1.180^{bc}	0.026	***
GR (%)	49.897^{d}	64.129^{c}	78.288^{b}	76.897^{b}	83.333^{b}	96.834^{a}	81.379^{b}	2.454	***
% Change	----	28.521	56.897	54.110	67.009	94.066	63.093		
FI (kg)	5.503^{b}	5.490^{b}	5.248^{c}	5.270^{c}	5.773^{a}	5.793^{a}	5.175^{d}	0.0186	***
FCR	7.538^{a}	5.872^{b}	4.591^{cd}	4.684^{c}	4.751^{c}	4.117^{d}	4.386^{cd}	0.1697	***
% Change	----	-22.11	-39.09	-37.86	-36.97	-45.38	-41.82		

$^{a, b \text{ and } c}$: Means within each row with different super scripts are significantly differ ($P< 0.05$). Sig= Significant; NS= Not significant; *** = $P< 0.001$; IW= Initial weight; FW= Final weight; WG= Weight gain during 90 days; GR= Growth rate; FI= Feed intake during 90 days; FCR= Feed conversion ratio.

Effect of experimental groups on serum protein profile and liver function enzymes

Data in Table (3) illustrated that the administration of grape juice without or with Zn or ZnONPs (G2, G3, G4, G5, G6 & G7) showed significant increase ($P< 0.001$& 0.05) in TP, AL and GL concentrations (percentage change = 18.61, 43.05, 38.42, 53.91, 57.09 &10.48in TP; 18.24, 37.74, 48.02, 47.70, 55.57 & 15.09in AL and 19.18, 51.21, 23.67, 63.43, 59.42 & 3.38in GL, respectively). While Table (5) showed that ZnONPs grape juice significantly increased ($P< 0.001$), most estimated liver enzymes (ALT, AST, ALP & GGT) except total bilirubin was significant ($P< 0.05$) decreased, but all values within the normal range. However, there were no significant ($P> 0.05$) differences between G4, G5, and G6 in all liver function parameters. It was evident from Tables (3 & 4) that liver efficiency continued to gradually improve with increasing the rate of ZnONPs in grape juice until it reached its maximum in G6 and then began to decline thereafter in G7 for serum proteins, while liver enzymes continued to increase with increasing the nanoparticles rate until it reached its maximum in G7.

It is known that the liver is a vital organ due to its various functions, such as synthesizing plasma protein (Tacke *et al*., 2009), processing injury erythrocyte cells, generation of hormones, detoxification (Yu *et al*., 2011), glucose and lipid metabolism (Liu *et al*., 2012). Results showed that ZnONPs treated juice administration showed a significant increase in TP, AL, and most liver function enzymes concentrations, while total bilirubin was significantly decreased. These results are incompatible with the results obtained by Mohamed *et al*. (2015 & 2017) in sheep, Fatma *et al*. (2016), and Tag-El Din (2019) in rabbits. Serum TP, AL, and GL levels in G4, G5 & G6 groups had the highest levels, followed by G2, G3 & G7 groups, while G1 recorded the lowest ones. This superiority in ZnONPs groups compared to the control group may be attributed to the rising feed intake and feed efficiency (Table 1), metabolic rate, and T3 & T4 hormones, which were expressed in the metabolites in the blood (Mohamed *et al*., 2017). Serum TP and its fractions are used as a biological indicator of an animal's health and productivity (Gabbedy, 1971; Mohamed *et al*., 2015).The data showed that the liver was in good health as the liver is the organ responsible for albumin synthesis. The elevated GL level and decreased A/G ratio in the treated rabbits enhanced immune response and resistance of the rabbits to disease, according to Bovera *et al*. (2015).

Table 3. Serum protein profile of rabbits as affected by treatments.

Traits	Experimental groups							±SE	Sig.
	G1	G2	G3	G4	G5	G6	G7		
TP (g/dL)	5.250^{d}	6.227^{c}	7.510^{ab}	7.267^{b}	8.080^{a}	8.247^{a}	5.800^{cd}	0.236	***
% change	----	18.610	43.048	38.419	53.905	57.086	10.476		
AL (g/dL)	3.180^{d}	3.760^{c}	4.380^{b}	4.707^{ab}	4.697^{ab}	4.947^{a}	3.660^{c}	0.143	***
% change	----	18.239	37.736	48.019	47.704	55.566	15.094		
GL (g/dL)	2.070^{b}	2.467^{ab}	3.130^{a}	2.560^{ab}	3.383^{a}	3.300^{a}	2.140^{b}	0.293	*
% change	----	19.179	51.208	23.671	63.430	59.420	3.382		
A/G ratio	1.651	1.656	1.715	1.544	1.720	1.667	1.585	0.197	NS
% change	----	0.313	3.856	-6.486	4.198	0.977	-4.012		

$^{a, b \text{ and } c}$: Means within each row with different super scripts are significantly differ ($P< 0.05$). Sig= Significant; NS= Not significant; * = $P< 0.05$; *** = $P< 0.001$; TP= Total protein; AL= Albumin; GL= Globulin; A/G= Albumin/ Globulin

Table 4. Liver function enzymes of rabbits as affected by treatments

Traits	Experimental groups							±SE	Sig.
	G1	G2	G3	G4	G5	G6	G7		
AST (IU/L)	60.000^{c}	59.000^{c}	72.787^{b}	71.287^{b}	72.830^{b}	73.000^{b}	91.500^{a}	2.790	***
% change	----	-1.667	21.312	18.812	21.383	21.667	52.500		
ALT (IU/L)	25.500^{d}	26.500^{cd}	34.240^{b}	30.740^{bc}	33.600^{b}	35.500^{b}	36.500^{a}	1.618	***
% change	----	3.922	34.275	20.549	31.765	39.216	43.137		
ALP (IU/L)	203.00^{b}	217.00^{b}	232.00^{b}	225.50^{b}	225.50^{b}	221.50^{b}	277.50^{a}	9.597	***
% change	----	6.897	14.286	11.084	11.084	9.113	36.700		
GGT (U/L)	4.760^{a}	5.500^{ab}	8.577^{a}	5.550^{ab}	5.110^{b}	7.227^{ab}	7.170^{ab}	0.951	*
% change	----	15.546	80.189	16.597	7.353	51.828	50.630		
TB (mg/dL)	0.950^{a}	0.947^{a}	0.640^{b}	0.420^{ab}	0.397^{b}	0.227^{b}	0.520^{ab}	0.151	*
% change	----	-0.316	-32.632	-55.789	-58.211	-76.105	-45.263		

$^{a, b \text{ and } c}$: Means within each row with different super scripts are significantly differ ($P< 0.05$). Sig= Significant; * = $P< 0.05$; *** = $P< 0.001$; ALT= alanine aminotransferase; AST= aspartate aminotransferase; ALP= alkaline phosphatase; GGT= gamma-glutamyl transferase (γ-GT); TB= Total Bilirubin.

ALT, AST, ALP, and GGT enzymes are the most significant markers of hepatocyte activity. The effect of treatments on these enzymes' concentrations did not have clear trends among the experimental treatments. Still, there was not a negative effect in general due to the ZnONPs treatment relative to control. Previous studies showed that ZnONPs had no significant adverse impact on broilers' liver enzymes activity (Ahmadi *et al*., 2014) and sheep (Mohamed *et al*., 2015 & 2017).Wang *et al*. (2006) reported that hepatocellular damage caused by Zn-micro-particles is more severe than that caused by nanoparticles. One explanation for these variations may be due to the doses and length of time the animal was exposed to ZnONPs. Levels greater than 50 mg/kg of ZnONPs have been reported to cause oxidative stress and raise ALT and AST levels in the plasma (Sharma *et al*., 2009).

The blood CRT and BUN are good indicators for renal function. The current study reported that creatinine, urea, and uric acid concentrations showed significant differences among experimental groups (Table 4). Besides, an oral administration of ZnONPs grape juice recorded a significant effect on increasing serum creatinine ($P< 0.01$) and urea ($P< 0.05$) concentration, while uric acid was not ($P> 0.05$) affected yet, while all values within the normal range. Kidney markers continued to increase with increasing the nanoparticles rate until it reached its maximum in G7.Thus, the slightly increased serum CRT and BUN levels of the treated groups could be suggested that mild renal impairment was likely due to the administration of ZnONPs. This result was also obtained from other investigations (Wang *et al*., 2006; Najafzadeh *et al*., 2013; Ismail and El-Araby, 2017).Also, on exposure to high zinc salts, Llobet *et al*. (1988) illustrated that the plasma CRT and BUN were elevated significantly after exposure to high doses of Zn-acetate dihydrate in drinking water. However, in the present study, treated rabbits showed no such over elevation.

Table 5. Kidney function markers of rabbits as affected by treatments

Traits	Experimental groups							±SE	Sig.
	G1	G2	G3	G4	G5	G6	G7		
CRT (mg/dL)	0.893^{b}	0.920^{b}	1.090^{a}	0.937^{b}	1.080^{a}	1.140^{a}	1.210^{a}	0.039	**
% change	----	3.024	22.060	4.927	20.941	27.660	35.498		
BUN (mg/dL)	42.667^{c}	50.000^{bc}	49.617^{bc}	48.500^{bc}	52.670^{ab}	58.650^{a}	56.107^{ab}	2.539	*
% change	----	17.187	16.289	13.671	23.444	37.460	31.500		
UA (mg/dL)	1.287^{ab}	1.307^{ab}	1.217^{b}	1.207^{b}	1.217^{b}	1.300^{ab}	1.457^{a}	0.065	NS
% change	----	-6.216	-5.439	1.554	-5.439	1.010	13.209		

$^{a, b \text{ and } c}$: Means within each row with different super scripts are significantly differ ($P< 0.05$). Sig= Significant; NS= Not significant; * = $P< 0.05$; ** = $P< 0.01$; CRT= Creatinine; BUN= blood urea nitrogen; UA= Uric acid

Serum Glucose (GLU) and cholesterol values are shown in Table (6). It could be shown that the present data of GLU among experimental groups followed the same trend as that of TP, AL, and GL in ZnONPs compared to control. These results may be attributed to increased voluntary feed intake (Table 2), rumen fermentation, enzyme activities, and high thyroid gland secretion (Mohamed *et al*., 2017).The anti-hypoglycemic effect of ZnONPs is due to their antioxidant efficacy, which protects against the cytotoxicity of free radicals generated by diabetes(Wadood *et al*., 2007).The present results concerning lipid profile illustrated that ZnONPs treatment has significantly ($P< 0.05$) decreased TC, TG, VLDLc but HDLc and LDLc were not ($P> 0.05$) affected compared to control. The lowering cellular cholesterol biosynthesis is related to increased LDLc receptor activity (Ness *et al*., 1996) and reduced total lipids (Bhandari *et al*., 2005).The significant decrease in lipid profile in the rabbit treated with ZnONPs grape juice may be attributed to Zn direct impacts on lipid metabolism and its role in lipoprotein lipase activity changes (Ismail and El-Araby, 2017). The existence of the Zn-NPs form provides it a stronger hypolipidemic influence than the normal form that has no significant impact (Samman and Roberts, 1988).

Tag-El Din (2019) reported that plasma TG was reduced by 10.20mg/dl for rabbits treated with 30 mg Nano-Zn than control, while plasma TC level was insignificantly reduced by 9.50 and 3.64% for rabbits treated with Nano-Zn by 30 and 60 mg/kg, respectively than control, whereas, HDL cholesterol was increased by 9.34% for those fed 60 mg Nano-Zn. Changes in serum TG and TC concentrations may be attributed to the Zn role in enzyme activity. It is a component of many enzymes (metallo enzymes) involved in lipid digestion and absorption (Al-Daraji*et al*., 2011). The present results are disagreement with El-Katcha *et al*. (2017), who illustrated that Nano-Zn supplemented the diet of broiler non significantly decreased serum TG while increased HDL than the control.

Table 6. Glucose level and lipid profile of rabbits as affected by treatments

Traits	Experimental groups							±SE	Sig.
	G1	G2	G3	G4	G5	G6	G7		
GLU (mg/dL)	58.94^{d}	68.14^{c}	78.51^{b}	71.77^{c}	85.84^{a}	81.33^{ab}	85.23^{a}	2.038	***
% change	----	15.604	33.20	21.77	45.64	37.99	44.61		
TC (mg/dL)	171.14^{bc}	179.80^{a}	154.00^{e}	158.17^{de}	163.50^{cd}	168.50^{cd}	175.42^{ab}	2.424	***
% change	----	5.06	-10.02	-7.58	-4.46	-1.54	2.50		
TG (mg/dL)	62.50^{a}	63.00^{a}	52.00^{b}	52.00^{b}	55.50^{ab}	55.50^{ab}	60.50^{a}	2.469	*
% change	----	0.80	-16.80	-16.80	-11.20	-11.20	-3.20		
HDLc (mg/dL)	47.50	47.50	44.50	42.00	52.50	45.00	49.00	3.748	NS
% change	----	0.00	-6.32	-11.58	10.53	-5.26	3.16		
LDLc (mg/dL)[1]	111.14	119.70	99.10	105.77	99.90	112.40	114.32	4.912	NS
% change	----	7.71	-10.83	-4.83	-10.11	1.13	2.86		
VLDLc (mg/dL)[2]	12.50^{a}	12.60^{a}	10.40^{b}	10.40^{b}	11.10^{ab}	11.10^{ab}	12.10^{a}	0.494	*
% change	----	0.80	-16.80	-16.80	-11.20	-11.20	-3.20		

$^{a, b, c, d \text{ and } e}$: Means within each row with different superscripts are significantly differ ($P< 0.05$). Sig= Significant; NS= Not significant; * = $P< 0.05$; *** = $P< 0.001$; TC= Total cholesterol; TG= Triglyceride; GLU= Glucose.

[1] LDL-cholesterol level was calculated by using the formula: LDLc= total cholesterol - HDLc - (TG/5), where (TG/5) = [2] VLDL-cholesterol

Hematological parameters are presented in Table (7). There are highly significant increases in Hgb concentration, RBCs count, HCT value, and total leukocytic (TLC) count in treated experimental groups. These parameters' levels increased with increasing ZnONPs levels until they reached the highest value in G6 and began to decline thereafter in G7. These findings are in disagreement with Ismail and El-Araby (2017). They reported that there were no significant differences in Hgb, RBCs, and HCT values in all groups, while TLC and lymphocytic counts were significantly increased in ZnONPs and mixed ZnO and ZnONPs supplemented rabbits groups in comparison with the control group.

Hematological analyses indicate the types and counts of blood cells as well as measure the toxicity of many factors on the hematopoietic system. In the current study, hematological parameters revealed no abnormal results in all experimental groups, indicating that ZnONPs negatively impacted the haemogram. However, the leukocytosis and lymphocytosis seen in the ZnONPs groups may be attributable to an accumulation of NPs that escaped phagocytic uptake and entered the lymph nodes, causing inflammation and a rise in lymphocytes, eventually leading to leukocytosis (Mahdieh *et al*., 2015).

Table 7. Hematological parameters of rabbits as affected by treatments

Traits	Experimental groups							±SE	Sig.
	G1	G2	G3	G4	G5	G6	G7		
Hgb (g/dl)	7.767^{b}	7.767^{b}	8.767^{b}	8.500^{b}	12.000^{a}	12.000^{a}	8.767^{b}	0.708	***
RBCs (10^6/ul)	3.400^{d}	3.667^{cd}	4.000^{cd}	3.800^{cd}	5.767^{b}	6.867^{a}	4.100^{c}	0.200	***
HCT (%)	24.367^{b}	24.400^{b}	27.567^{b}	27.167^{b}	39.367^{a}	39.367^{a}	26.967^{b}	2.709	***
TLC (10^3/ul)	5.267^{bc}	5.800^{a}	5.100^{bc}	4.967^{bc}	5.300^{b}	5.333^{b}	4.867^{c}	0.128	***
Differential leucocyte count									
NEU (%)	26.167^{b}	27.267^{ab}	28.567^{a}	27.400^{ab}	25.100^{bc}	25.100^{bc}	23.067^{c}	0.724	***
LYM (%)	61.500^{c}	62.800^{bc}	60.067^{c}	62.700^{bc}	67.367^{ab}	67.367^{ab}	69.800^{a}	1.604	***
MON (%)	7.000^{ab}	8.067^{ab}	8.600^{a}	7.967^{ab}	5.467^{b}	5.467^{b}	5.567^{b}	0.898	*
EOS (%)	0.300	0.300	0.167	0.167	0.367	0.367	0.367	0.060	NS
BAS (%)	1.567	1.600	2.667	1.800	1.767	1.767	1.433	0.586	NS

[a, b, c and d]: Means within each row with different super scripts are significantly differ (*P*< 0.05). Sig= Significant; NS= Not significant; * = *P*< 0.05; * = *P*< 0.001; Hgb, Haemoglobin; RBC, Erythrocyte count; HCT, Hematocrit; TLC, Total leucocyte count; NEU., Neutrophil (%); LYM., Lymphocyte (%); MON., Monocyte (%); EOS., Eosinophil (%); BAS., Basophil (%).**

Antioxidant enzymes play a critical role in defending against free radical damage. Furthermore, malondialdehyde (MDA) serves as a reliable indicator of lipid peroxidation (LPX) in cells. Results in Table (8) reported that all experimental levels of ZnONPs grape juice reduced serum LPX level and increase serum glutathione (GSH) and superoxide dismutase (SOD) values. These results are in agreement with other previous studies (Mohamed *et al*., 2017; Ismail and El-Araby, 2017).

Findings indicated that ZnONPs grape juice administration had significantly (*P*<0.0001) raised the serum GSH content and SOD activity compared to the control group. The highest values were in G7 at 480 $mgL^{-1}$of ZnONPs (percentage change = 58.123 & 15.842 % of GSH and SOD, respectively) and the lowest ones were in G2at 60 mgL^{-1} of ZnONPs (percentage change = 14.982&7.759% of GSH and SOD, respectively). However serum LPX levels were significant (*P*<0.001) decreased with increasing ZnONPs levels until reached below in G7 (percentage change = -64.972).

These results are in agreement with the results of Walsh *et al*. (1994), Berg and Shi (1996), and Mohamed *et al*. (2017), who found a negative correlation between MDA value and ZnONPs. Also, Fatma *et al*. (2016) reported that serum total antioxidant capacity (TAC) significantly increased in rabbits that eat selenium or zinc-NPs in their diet. Ahmadi *et al*. (2014) said that rising ZnONPs level from 60 to 90 mg/kg diet enhanced broiler antioxidant status and serum enzymes. Burman *et al*. (2013) mentioned that since zinc is found in SOD, it aids in the balance of reactive oxygen species (ROS) generation and scavenging, which is essential for the stability of bio-membranes, and proteins. Zn is a cofactor and ingredient of over 240 enzymes, and it can affect oxidative reactions. Cunningham-Rundles *et al*. (1990) discovered that zinc acts as an antioxidant to protect cell membranes from free radical damage.

Furthermore, they found that Zn is an essential component of Cu-Zn-SOD and that dietary Zn levels are related to Cu-Zn-SOD activity. It could be proved that Cu-Zn-SOD is implicated in the cellular scavenging of ROS and free radicals (Prasad, 2008).Serum Cu-Zn-SOD activity was significant affected by ZnONPs. Still, higher ZnONPs levels were not related to an increase in serum Cu-Zn-SOD activity implying that excess ZnONPs do not contribute to the biological process. Sufficient concentrations of ZnONPs may stimulate Cu-Zn-SOD activity, and the increased Cu-Zn-SOD will inhibit ROS production (Zhao *et al*., 2014).

Table 8. Serum oxidative stress markers and antioxidant enzymes of rabbits as affected by treatments.

Traits	Experimental groups							±SE	Sig.
	G1	G2	G3	G4	G5	G6	G7		
GSH (µmol/L)	0.554^{e}	0.637^{de}	0.765^{bc}	0.710^{cd}	0.853^{b}	0.876^{ab}	0.982^{a}	0.035	***
% change	----	14.982	38.087	28.159	53.971	58.123	77.256		
SOD (U/ml)	115.318^{e}	124.265^{d}	128.860^{bc}	125.665^{cd}	130.105^{ab}	133.587^{a}	133.830^{a}	1.182	***
% change	----	7.759	11.743	8.973	12.823	15.842	16.053		
LPX (µmol/L)	3.560^{a}	2.881^{b}	2.275^{c}	2.908^{b}	1.989^{c}	1.831^{c}	1.247^{d}	0.140	***
% change	----	-19.073	-36.096	-18.315	-44.129	-48.567	-64.972		

[a, b, c and d]: Means within each row with different super scripts are significantly differ (*P*< 0.05). Sig= Significant; * = *P*< 0.001; SOD= Superoxide dismutase; GSH= Glutathione; LPX= Lipid peroxidation.**

CONCLUSION

According to the finding of the present investigation, it was preferable to use grape juice obtained from Flame Seedless grapevines vineyard that treated with zinc in both forms (chelated and nanoparticles) during their cultivation for its significance in enhancing liver and kidney function without negative influences on cell structure, reducing serum lipids and improving the antioxidant status of the growing rabbit. On the other hand, using the grape juice treated with ZnONPs at 480mgL^{-1} has adverse effects on rabbits' general health.

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تأثير عصير العنب المعامل بمركب النانو زنك على أداء وصحة الأرانب

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تم إجراء هذه الدراسة لتقييم تأثير عصير العنب المعامل بصورتين مختلفتين من الزنك (الزنك المخلبي ، وجزئيات النانو زنك) , على أداء الارانب. أستخدمت في هذه الدراسة 42 أرنب ذكر ذو أعمار ثلاثة شهور حيث تم تقسيم هذه الارانب الي سبعة مجموعات عشوائياً (6 / مجموعة) . المجموعة الاولى أخذت 20 مل /كجم من وزن الجسم ماء يومياً (كنترول سلبي) ، المجموعة الثانية أخذت 20مل/كجم من وزن الجسم من عصير العنب الغير معامل (بدون زنك) يومياً (كنترول ايجابي) ، المجموعة الثالثة أخذت 20مل/كجم من وزن الجسم من عصير العنب المعامل بالزنك المخلبي بتركيز 1.5جم/ اللتر يومياً ، بينما مجموعات الارانب بالمجموعة الرابعة ، الخامسة ، السادسة والسابعة أخذت 20 مل /كجم من وزن الجسم من عصير العنب المعامل بالنانو زنك بتركيز 60 ، 120 ، 240 و 480 ملجم / اللتر يومياً. أظهرت النتائج أن أداء النمو والكفاءة الغذائية وبروتينات السيرم ومؤشرات الهيماتولوجي لذكور الأرانب المعاملة بعصير العنب المعامل بالزنك سواء النانو زنك أو الزنك المخلبي تحسناً معنويا حتى وصلت إلى الحد الأقصى في المجموعة السادسة ثم بدأت في الانخفاض بعد ذلك في المجموعة السابعة. اتبعت مستويات السكر في الدم نفس الاتجاه. استمرت مؤشرات وظائف الكبد والكلى في الزيادة تدريجياً مع زيادة معدل النانو زنك. أظهرت معاملات النانو زنك انخفاضاً معنوياً في الكوليسترول الكلي والدهون الثلاثية ، بينما لم تتأثر البروتينات الدهنية عالية ومنخفضة الكثافة مقارنةً بالكنترول. أدى تناول عصير العنب المعامل بالنانو زنك إلى زيادة كبيرة في محتوى الجلوتاثيون في الدم ونشاط السوبر اكسيد ديسموتيز مقارنة بالكنترول. يمكن الاستنتاج أن استخدام عصير العنب المعامل بـ بالنانو زنك حتى تركيز 240 ملجم/اللتر ليس له أي آثار ضارة على صحة الأرانب. وعلى العكس ، فإن استخدام عصير العنب المعامل بالنانو زنك عند تركيز 480 ملجم/اللتر كان له آثار ضارة على صحة الأرانب.