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EFFECT OF SELENIUM NANOPARTICLES AND/OR *spirulina platensis* ON GROWTH, HEMATOBIOCHEMICAL, ANTIOXIDANT STATUS, HORMONAL PROFILE, IMMUNITY, AND APOPTOSIS OF GROWING RABBITS EXPOSED TO THERMAL STRESS

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ABSTRACT

This research was conducted to reconnoiter the influence of dietary supplementation of Spirulina platensis *(SP) or/and selenium nanoparticles (SeNPs) on the growth, blood metabolites and antioxidants capacity of heat-stressed rabbits. Rabbits (n = 120) were divided into 4 experimental groups as the following: the 1st group fed basal diet without supplementation and sarved as a control (CON). The 2nd group fed basal supplemented with 1 gm/kg diet of SP (SP), the 3rd group fed basal diet supplemented with 50 mg/kg diet SeNPs (SeNPs**)** and 4th group fed basal diet supplemented with 1 gm/kg diet of SP (SP) plus50 mg/kg diet SeNPs (SeNPs**) .***

Dietary inclusion with SeNPs and SP significantly (P < 0.05) improved all growth indices in heat stressed rabbits. Circulating hemoglobin, platelets, total protein, albumin and globulins were significantly improved in SeNPs and SP+SeNPs groups compared with CON group; however, WBCs were significantly decreased in all treated groups. Significant reduction in ALT, total bilirubin, direct bilirubin, total glycerides, creatinine, and uric acids were observed in treated groups compared with the CON group.

All experimental additives significantly improved the levels of IgG relative to CON group (P<0.001).

Dietary feed inclusion resulted in lower levels of oxidative stress (MDA and PCO; P < 0.001), while all treatments showed significant enhancement in the level of GSH.

The mixture of SP+SeNPs had higher level of T3 (P <0.001), while both SP and SP+SeNPs treatments had lower values of plasma corticosterone (P < 0.001).

The morphologic of caspase3 immune expression of heat stressed rabbits presented moderate of intracytoplasmic immunoreactivity that existing by a substantial rise in part ratio of caspase 3

immunoreactivity related to another group.

Conclusively, *the present results manifest that SeNPs and/or SP can be possibly to mitigate the negative influences of heat stressed rabbit by enhancing the antioxidant, immunological and reducing the apoptosis process.*

Keywords: Heat stress; rabbit; growth indices; blood metabolites; antioxidant; apoptosis

INTRODUCTION

Global warming exemplifies a real challenge to the livestock sector, mainly in arid and semi-arid areas. Heat stress (HS) is a substantial issue influencing domestic animal production specially in the tropical climate of Egypt (Gitz *et al.*, 2016; Amber *et al.*, 2021). At present, rabbit production is a quickly increasing at the level of commercial sector, due to its rapid growth, a short reproductive cycle, and its meat has highly nutritional value (Ayyat *et al.*, 2021).

Studies have shown that HS induced reduction in the growth performances such growth rate, feed intake, and feed efficiency, as reported in many species of animals (Abdelnour *et al.*, 2020a; Sheiha *et al.*, 2020; Amber *et al.*, 2021). As well known that rabbits have a few numbers of sweat glands that contributing to mitigating HS impacts, thus rabbits are suffering from HS. Rabbits are susceptible to high ambient temperature, and their heat resistance is very low (Abdelnour *et al.*, 2020a). Marai *et al.* (2002) suggested that the temperature-humidity index (THI) must be <30 THI for the optimal growth, thermoregulatory, homeostasis and welfare of rabbits (Abdelnour *et al.*, 2020a). However, through the hot season and the climatic change influences the THI rises >30, impeding the immune, and antioxidant system as well as the thermoregulation ability of rabbits **(**Abdelnour *et al.*, 2020a; Madkour *et al.*, 2021). HS could alter the metabolic functions, protein/energy levels, as well triggering irregular hormonal status and enzymatic excretions in the circulatory system (Ayyat *et al.*, 2021; Hassan *et al.*, 2021). The destroyed physiological responses of heat stressed rabbits is principally associated with the imbalance antioxidant capacity, increase the inflammatory cytokines execrations, and oxidative stress (Abdelnour *et al.*, 2020a; Madkour *et al.*, 2021). It is suggested to integrate microalgae (Spirulina platensis; SP) as nutraceutical agent with using developed mineral nanoparticles (such selenium nanoparticles; SeNPs) in rabbit feeds. Consequently, it is of significant attention to novelty practical tactics to improve heat tolerance in rabbits.

In the last decades, the emergence of nanotechnology has altered the insight of drug discovery and progress by opening many hidden doors in enhancing the productivity and sustainability of livestock. In this sense, selenium (Se) is vital trace component in the diet, needed for maintenance of growth, health, and reproduction. Recently, the developed new source form of Se is SeNPs has a noteworthy impact for environmental, sustainability and nutritional aspects owing to their lower toxicity and capability to slowly release Se after ingestion (Khurana *et al.*, 2019).

Several studies have indicated that the SeNPs exhibited an improvement in the growth indices, hematobiochemical, immunity, and antioxidant status of broilers (Abdel-Moneim *et al.*, 2021) and rabbits (Sheiha *et al.*, 2020) exposed to HS. forms (Sheiha *et al.*, 2020; Dawood *et al.*, 2021). *Spirulina platensis* (Moreover, SeNPs also presented enhancement of the removal of the diabetes-induced oxidative stress damages by declining the pancreatic lipid peroxidation and nitric oxide levels (El-Borady *et al.*, 2020). It has been clarified that the SeNPs are elaborated as more stable, eco-friendly, soluble, and bioavailable than the organic and non-organic SP), a dietary complement and food additive, is a photosynthetic cyanobacterium. Due to its unique bioactive compounds including, β-carotene, phycocyanin, and allophycocyanin, which provide it with anti- strong antioxidant, ant-inflammatory, and immunostimulatory effects (Abdel-Daim *et al.*, 2018).

Moreover, those features of SP is associated with stimulating cytokines and antibodies synthesis, constraining lipid peroxidation, successfully scavenging free radicals, and thus improving livestock health and their productivity and attaining high profitability (Aladaileh *et al.*, 2020; Khalil *et al.*, 2020; Alwaleed *et al.*, 2021). Up to date, there are a few studies on the using of SP and/or SeNPs incorporated into the diets of heat stressed rabbits. Abdel-Moneim *et al.* (2021) reported that dietary inclusion of SP and SeNPs have reported a strong antioxidant potency and attenuated the detrimental effects of HS via boosting the immune and antioxidant capability in broiler chickens. Even though the potential synergistic impacts of SP and SeNPs incorporated to the diets of heat stressed growing rabbits are remain largely uninvestigated. Take into the previous considerations; anti-inflammatory and antioxidative properties of SP and SeNPs, we assumed that dietary inclusion of SP and SeNPs can improve the immunity, antioxidative capacities and reducing the apoptosis of rabbits induced by HS.

Therefore, the current research aims to examine the anti-heat stress property of SP or/and SeNPs on the growth performance, blood indices, endocrine hormones, and antioxidative status of rabbits reared under HS conditions.

MATERIALS AND METHODS

1. Ethics statement

The current trial was carried out at Animal Production Department, Faculty of Agriculture, Zagazig University, Egypt. This experiment was conducted in firm agreement with animal ethics and approved by the Zagazig University Institutional Animal Care and Use Committee (Approval no. ZU-IACUC/2//F/61/2016).

2.Selenium nanoparticles (SeNPs) preparation

Lactobacillus casei ATCC 393 was identified at Agricultural Microbiology Department, Faculty of Agriculture, and used for the synthesis of biogenic SeNPs according to the method of (Xu *et al.*, 2018). Figure 1 debits the synthesized SeNPs was characterized by transmission electron microscope (type JEOL-JEM 2100). The size of SeNPs arranged between 15.97-21.6nm, with well dispersed and spherical in shape.

3. Animals, experimental design, and diets

In the current research a total of one hundred and twenty weaned males of growing New Zealand White rabbits (6 weeks of age and weighting 838.75 ± 21.91g) were used. Rabbits were hired in suitable galvanized wire battery cages with typical dimensions (50 × 45 × 40 cm3) in a well-ventilated rabbitry. The rabbit cages were supplied with manual feeders and an automatic system of nipple drinkers to offer fresh water *ad libitum.* Spirulina platensis (SP) was obtained from the Agricultural Microbiology Department, Faculty of Agriculture, Zagazig University. Animals were randomly allocated into equal four treated groups, each of which (10 cages/group and 3 rabbits/cage) for eight consecutive weeks during the Egyptian summer conditions. The rabbits in the 1st group fed with a basal diet without addition served as the control group (CON). The 2nd group fed basal supplemented with 1 gm/kg diet of SP (SP), the 3rd group fed basal diet supplemented with 50 mg/kg diet SeNPs (SeNPs**)** and 4th group fed basal diet supplemented with 1 gm/kg diet of SP (SP) plus50 mg/kg diet SeNPs (SeNPs**) ..** Animals were received a basal diet formulated to meet the nutrient requirements of growing rabbits (De Blas and Mateos, 2020), and chemical analysis of this diet of rabbits are offered in Table 1.

Growing rabbits were reared under the same environmental conditions, hygienic and management environments. For determining the severity of HS on rabbits under natural conditions, the calculation of relative humidity (RH), ambient temperature (TM), and temperature-humidity index (THI) were

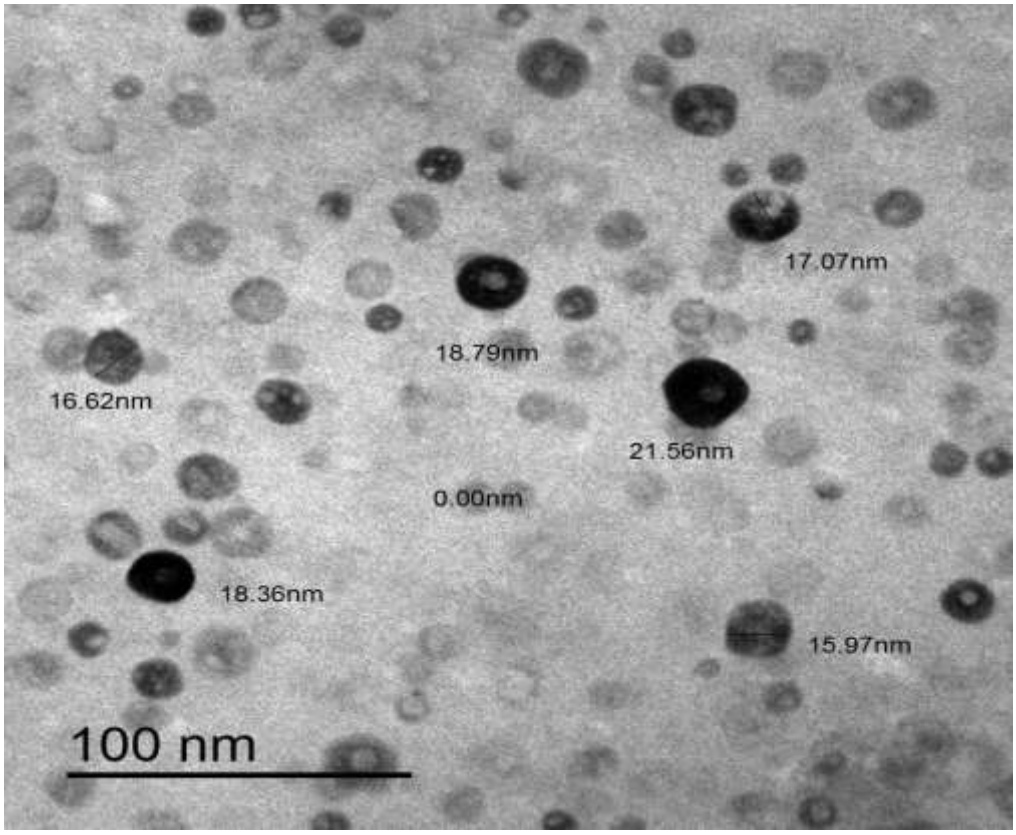


Figure 1. The synthesized SeNPs was characterized by transmission electron microscope (type JEOL-JEM 2100). The size of SeNPs arranged between 15.97-21.6nm, with well dispersed and spherical in shape

documented inside the farm based on a natural climate of HS in Sharkia Province, Egypt conferring to (Marai *et al.*, 2002). Figure 2 show the weekly of THI (31.33), during the experimental period which reflecting the thermal heat stress.

Growth performance

To assess the growth performance of growing rabbit during the high environmental temperature, we recorded weekly the body weight (BW), average daily gain (ADG), as well as, the feed utilization (FI) was determined daily in each treatment to analyze average daily feed intake (ADFI). Feed conversion ratio (FCR; g feed/ g gain) was calculated at the termination of the experiment.

Table 1. Ingredients and nutrient contents of the basal diet of growing rabbits (as fed).

Items	**Basal diet**
Ingredient	***%***
Maize	20
Soybean meal (CP 44%)	20
Wheat bran	16
Berseem hay	30
Barley grain	10
Molasses	2.0
Limestone	1.0
NaCl	0.5
Premix*	0.5
Total	100
Calculated composition, % **	
ME, MJ/kg	7.95
Crude protein	17.50
Calcium	0.88
Available phosphorus	0.20

*Each 1 kg of premix (Minerals and Vitamin mixture) contains: Vit. A, 20,000 IU; Vit. D3, 15,000 IU; Vit. E, 8.33 g; Vit. K, 0.33 g; Vit. B1, 0.33 g; Vit. B2, 1.0 g; Vit. B6, 0.33 g; Vit. B5, 8.33 g; Vit. B12, 1.7 mg; Pantothenic acid, 3.33 g; Biotin, 33 mg; Folic acid, 0.83 g; Choline chloride, 200 g.
**Calculated according to NRC 1977.

Blood metabolites

At the end of the experimental period, six animals from each treatment were randomly selected, for collecting blood samples. The blood samples were collected from the marginal ear vein through heparinized tubes as previously reported by (Abdelnour *et al.*, 2020a). The blood collected was sub-grouped into two tubes; the first once was hired to determine the hematological criteria using blood haematology analyser (HB 7021). While, the second one was used to obtain the plasma. For extraction of plasma from the blood, blood cells removed by centrifugation (at 2000 × g for 15 min at 4°C), and then kept (at -20°C) for pending investigation. Blood plasma metabolites, including total protein (TP), Albumin (ALB), globulins (GL), A/G ration, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, total bilirubin (TB), direct bilirubin (DB), glucose, and triglycerides (TG) were assessed by spectrophotometric method conferring to the manufacturer's

Figure 2. Demonstrate the weekly values of relative humidity (RH), ambient temperature (TM), and temperature humidity index (THI), during the experimental period.

protocol using commercial kits acquired from Biodiagnostic Company (Giza, Egypt).

Immunity

The levels of interferon gamma (IFNγ) in rabbit plasma were quantitated using enzyme-linked immunosorbent assays (ELISA) kit assay (Cat No. MBS2601171) with sensitivity (5 pg/ml), detection range (15.6 -1000pg/ml), intra and inter-assay precision (8-12%) conferring to the producer's instructions (MyBioSource, San Diego, CA, USA). Immunoglobulins levels (IgG and IgM) in blood plasma were evaluated by using ELISA kits (Shang Hai Lengton Bioscience Co., China) according to the manufacturer's instructions.

Redox status

The levels of antioxidant enzymes including reduced glutathione (GSH) and superoxide dismutase (SOD), and the oxidative stress including malondialdehyde (MDA) and protein carbonyl (PCO) in the rabbit plasma were measured according to the hemanufacturer instructions via commercial ELISA kits (Shimadzu, Kyoto, Japan).

Hormonal profile assessment

Plasma thyroid hormone such as triiodothyronine (T_3) was assessed using commercial ELISA competitive kit (Catalog Number=MBS730536; MyBioSource, San Diego, USA), with sensitivity up to 3.125 ng/ml. For detection the stress hormone, corticosterone (CORT) was assessed using a specialized rabbit competitive ELISA Kit by quantitative method (Catalog Number= MBS704261; MyBioSource, San Diego, USA) with detection range 6.25 -100 ng/ml and sensitivity up to 3.125 ng/ml.

Immunohistochemical study

Immediately following slaughtering at the end of experiment, small specimens of liver were collected and directly fixed in neutral buffered formalin solution (10%) for two days. Approximately, a 4µ of the thick segments were taken out from paraffin blocks; deparaffinization and rehydration in a descending sequence of ethyl alcohol were preceded. The antigen recovery was used via phosphate buffered saline (PBS; 0.01 mol/L; pH 6.0) and quenching of endogenous peroxidase activity was achieved by using H2O2 (0.3%) in PBS. Following that, blocking of the non-specific binding of immunological reagents was completed via incubation for one hour with normal goat serum 10% (v/v). After that, the specimens of liver were incubated with Rabbit Caspase 3 Polyclonal Antibody (MBS9601080, MyBioSource, San Diego, USA) overnight at 4°C. Next, the liver sections were washed twice with PBS and incubated for 30 minutes with streptavidin-peroxidase conjugate (Histofine kit, Nichirei Corporation, Japan). Visualization of the streptavidin-biotin complex was performed via 3,3′-diaminobenzidine tetrahydrochloride (DAB)-H2O2 solution (pH 7.0, for 3 minutes). Conclusively, sections were counterstained with Mayer's hematoxylin solution (Aladaileh *et al.*, 2020). The assessment of histomorphometric indices was detected on original micrographs gotten from five random fields of each immune-stained sections in each group (X100). By the image J software (v1.46r, NIH, Bethesda, MD, USA), the part proportion of caspase3 immuno-staining in hepatic cells were assessed of all experimental groups (Schneider *et al.*, 2012).

Statistical analysis

The obtained data were analyzed by the SPSS program version 16.0 (SPSS Inc., Chicago, IL, USA). All results were examined with a one-way ANOVA (with the diet as the fixed factor) via the post hoc Tukey's test (level of significance was recognized at $P < .05$).

RESULTS AND DISCUSSION

1. Growth performance

The feed additive supplementation significantly enhanced ($P < 0.01$) cumulative body weight gain compared to the heat stressed group (Table 2). Both SP and SP+SeNPs treatments improved significantly the cumulative daily feed intake (CDFI), while the lowest values were observed in the control group ($P < 0.01$). SeNPs exhibited intermediate values of CDFI compared with other groups. For cumulative feed conversion ratio (CFCR), all experimental feed additives decreased significantly the CFCR in related to the control ($P < 0.05$). The existing work confirmed that SP or SeNPs or their combination enhanced the FBW at marketing, CBWG and CFCR of heat stressed rabbits, while these supplements significantly reduced average CDFI. To the best of our knowledge, the existing explore is the first one that illustrate the synergistic effect of SeNPs and SP in attenuating the detrimental effects of heat-stress of growing rabbits. We incorporated between the use of nanotechnology (such using SeNPs) and the natural green antioxidant modulator such as *spirulina platensis* in growing rabbit diets during hot conditions. Heat stress (HS) is one of the major encounters facing animal agriculture as it jeopardizes, welfare, impends sustainability, and restrictions profitability. Reduction in growth performance, alters physiological responses, decline the immune-capacity, disturbance in hormonal profile and increase the oxidative stress as well as reduced the reproductive performance are the main consequences of environmental hyperthermia in rabbit productivity in hot seasons (Abdelnour *et al.,* 2020a; Amber *et al.,* 2021; Hassan *et al.,* 2021; Sirotkin *et al.,* 2021). Studies have shown that high environmental temperatures induced a wide array of metabolic, physiological, endocrine, and changes, a process that appears mediated by reduced immune function and antioxidant capacity in rabbits (López *et al.,* 2018; Sejian *et al.,* 2018; Oladimeji *et al.,* 2021).This inquiry seems to be the first to validate those synergistic effects of SP and SeNPs supplementation in rabbit diets can attenuate the thermoregulatory response to heat environmental temperature. These findings are in accordance with those of (Hassan *et al.*, 2015), who reported that dietary inclusion of zinc (50, 75 and 100 mg/kg) and SP in rabbit diets improved the growth performance such CBWG, CFCR and FBW. Se enriched with SP had a constructive influence on growth indices of growing rabbits (Hassan *et al.*, 2015). Sheiha *et al.* (2020) indicated that the dietary addition on biogenic selenium (25 or 50mg/kg diet) exhibited gradual enhancement in FBW, CFI and reduced the CFCR in heat stressed rabbit. The

Table 2. Effect of selenium nanoparticles, spirulina and their combination on growth performance of male rabbits exposed to high environmental temperature.

Item[1]	Treatment[2]				Pooled SEM	*P* value
	CON	SP	SeNPs	SP+SeNPs		
IBW (g/day)	832.73	816.82	871.36	834.1	9.71	0.240
BWG (g)	979.54^{b}	1156.81^{a}	1128.63^{a}	1182.72^{a}	14.90	<0.001
DFI (g/day)	65.50^{c}	72.68^{a}	69.429^{b}	72.91^{a}	0.68	<0.001
FCR (g feed/g gain)	4.69^{a}	4.41^{b}	4.31^{b}	4.33^{b}	0.050	<0.001
FBW (g)	1812^{c}	1970^{b}	2000^{ab}	2016.18^{a}	6.58	0.026
Mortality (%)	0	0	0	0	--	--
GPR (%)	38.72^{b}	44.86^{a}	46.55^{a}	46.87^{a}	0.689	<0.001

^{a,b,c} Mean values followed by different superscript letters in the same row are significantly different ($P < 0.05$).

[1] IBW; Initial body weight: FBW: Final body weight; BWG; Daily body weight gain, g: DFI; Daily feed intake, g: FCR; feed conversion ratio; Growth performance rate: GPR.

[2] CON= Basal diet without additive; SP=Basal diet with 1g/kg spirulina; SeNPs= Basal diet with 50mg/kg selenium nanoparticles; and SP+SeNPs = Basal diet with spirulina (1g/kg diet) plus selenium nanoparticles (50 mg/kg diet).

dietary inclusion of SP (1g/kg) decreased the detrimental impact of lead in rabbits and authors suggested that this attenuation may be accredited by its anti-inflammatory, anti-oxidative, as well as its immune-modulatory property (Aladaileh *et al.,* 2020). Broiler chickens fed diets with SP (0.1%) had higher activities of superoxide dismutase and glutathione peroxidase and lower concentrations of cholesterol and triglyceride in serum (Joya *et al.*, 2021). In broiler chickens' studies, the SP addition had a constructive impact on performance indices and carcass traits. Authors suggested that SP could resulting in enhancement of epithelial morphology in the small intestine (Joya *et al*., 2021), which may improve the absorption and thereby feed efficiency and overall the growth performance of broiler. Dietary inclusion of SeNPs (0.6 mg SeNPs head/day) in goat diets significantly augmented the Se level (Kachuee *et al.,* 2019). In earlier study, it clear that presenting SeNPs during the late stage of pregnancy of goats significantly enhanced the iron levels in the serum and blood of kids or goats and also the colostrum (Zhang *et al*., 2008). In our study, we did not investigate the Se level and its association with iron in the blood or plasma, while the most blood variables studied were improved by dietary SeNPs and/or SP inclusion during HS conditions. A better iron homeostasis ability was detected after the adding of SeNPs in comparison with other Se forms (Khurana *et al*., 2019; Sheiha *et al*., 2020). This pattern could be attributed to the notable physicochemical structures of SeNPs such as large surface area, small size, improved absorption through epithelial cells, and other functional pathways.

2. Blood hematology and biochemical analysis

Circulating hemoglobin and platelets were significantly improved in SeNPs and SP+SeNPs groups compared with control group; however, WBCs were significantly decreased in all treated groups (Table 3). No significant differences in RBCs, MCV or MCH were observed among all treatments and the control group. Rabbits fed with SeNPs or SP+SeNPs enhanced hemoglobin levels by 10.7 and 15.4%, respectively compared with the control. Table 4 shows the impacts of dietary SP, SeNPs or their combination supplement on some blood biochemical in heat stressed rabbits. Rabbits fed with SP+ SeNPs had higher total protein, albumin and globulins compared to other treatments and the control groups. Significant reduction in ALT, total bilirubin, direct bilirubin, total glycerides, creatinine, and uric acids were observed in treated groups compared with the heat stress group.

Dietary inclusion of SP or in combined with SeNPs exhibited lowest values of uric acid and direct bilirubin in relative to other groups. Creatinine ($P<0.001$)

Table 3. Effect of selenium nanoparticles, spirulina and their combination on blood hematology of male rabbits exposed to high environmental temperature.

Item[1]	Treatments groups [2]				Pooled SEM	*P* value
	CON	SP	SeNPs	SP+ SeNPs		
Hemoglobin (g/dl)	11.04^{c}	11.82^{bc}	12.22^{ab}	12.74^{a}	0.182	0.001
RBCs (10^6/ml)	5.16	4.97	5.25	5.38	0.096	0.536
MCV (pg)	63.57	65.07	64.38	62.40	0.570	0.413
MCH (pg)	22.23	22.62	22.56	22.09	0.219	0.820
Platelets (mcl)	174.6^{c}	206.4^{b}	195.4^{b}	227.2^{a}	5.058	<0.001
WBC 10^3/ml)	7.64^{a}	5.642^{c}	6.48^{b}	5.08^{c}	0.251	<0.001

^{a,b,c,d} Mean values followed by different superscript letters in the same row are significantly different ($P < 0.05$).

[1] RBCs= Red blood cells; MCV= Mean corpuscular volume; MCH= Mean corpuscular hemoglobin; WBCs=White blood cells.

[2] CON= Basal diet without additive; SP=Basal diet with 1g/kg spirulina; SeNPs= Basal diet with 50mg/kg selenium nanoparticles; and SP+SeNPs = Basal diet with spirulina (1g/kg diet) plus selenium nanoparticles (50 mg/kg diet).

and ALT (P<0.006) were reduced in all supplemented groups compared with the control. Plasma glucose (*P*< 0. 0.189) and AST (*P*> 0. 0.763) were similar across treatments (Table 4).

Blood biochemical analysis could reflect the health status and welfare of an animal. In the present study, significant constructive influences of the incorporated diets with SP and SeNPs on plasma biochemical catalogues. These upshots are consistent with those of (Hosny *et al*., 2020; Sheiha *et al*., 2020), who documented that SeNPs (25 or 50mg/kg diet) added to heat stressed rabbit, significantly reduced the kidney function (uric acid and creatinine), bilirubin and total bilirubin and, while significantly in blood proteins such total protein and albumin. Several reports indicated that the addition of SP in rabbit diets did not have beneficial effects on protein fractions and activities of ALT and AST in rabbits (Hassan *et al.*, 2021).

Immunological responses

Circulating immunoglobulins G (IgG) increased in SeNPs group (Table 5). All experimental additives significantly improved the levels of IgG relative to CON group (P<0.001). The level of IgA was not affected by dietary inclusion; all treated additives tended (P=0.545) to boost the IgA relative to CON group. The mixture of SP and/SeNPs exhibited the lowest values of interferon gamma (IFNγ) in heat stressed rabbits compared with CON group.

The present results observed that all experimental additives significantly improved the levels of IgG relative to HS group (P<0.001). The potentiality impact of SeNPs as anti-inflammatory agent is needed to be clarifying in various animal's species. The level of IFN-γ was reduced (P<0.001) in all experimental clusters when comparison with those in the HS group. Interleukins are a group of cytokines that were first apparent in leukocytes and have a significant role in the cellular defensive (Madkour *et al*., 2021). HS induced a greater expression of IL-4 in the plasma of stressed rabbits (Sheiha *et al.*, 2020), representing repression of immunity. Studies have reported that the dietary intake of immunity enhancer compounds such as microalgae or phytochemicals enhance the health status, and welfare as well as supporting in avoiding environmental issues. Furthermore, literature from earlier trails have revealed fucoxanthin and phycocyanin as natural pigments have potential anti- ammatory feature that happens via the suppression of histamine production and decreasing apoptosis in human tumor cells (Zhao *et al.*, 2020).

Hence, the present study shows that dietary combined of SP and SeNPs exhibited positively the health status, decrease inflammatory characters, and improve immunity.

Table 4. Effect of selenium nanoparticles, spirulina and their combination on blood biochemical of male rabbits exposed to high environmental temperature

Item[1]	Treatment groups[2]				Pooled SEM	P value
	CON	SP	SeNPs	SP+ SeNPs		
Total Protein (g/dl)	5.89^{d}	2.7^{b}	6.74^{c}	8.9^{a}	0.360	<0.001
Albumin (g/dl)	3.89^{b}	4.60^{a}	4.04^{b}	4.72^{a}	0.128	0.018
Globulins (g/dl)	2.0^{c}	3.29^{b}	2.67^{b}	4.18^{a}	0.258	<0.001
ALT (U/L)	89.88^{a}	35.11^{b}	60.94^{b}	41.79^{b}	7.305	0.006
AST (U/L)	36.62	37.17	34.91	35.91	0.712	0.763
Total Bilirubin (mg/dl)	1.13^{a}	0.87^{b}	0.46^{c}	0.48^{c}	0.086	<0.001
Direct Bilirubin (mg/dl)	0.22^{a}	0.14^{c}	0.18^{b}	0.13^{c}	0.011	<0.001
Glucose (mg/dl)	14.16	22.74	22.95	23.65	1.786	0.189
Total Glycerides (g/dl)	89.26^{a}	83.62^{b}	62.93^{c}	47.74^{d}	5.043	<0.001
Creatinine (mg/dl)	1.81^{a}	1.36^{b}	1.43^{b}	1.36^{b}	0.061	<0.001
Uric acid (mg/dl)	5.75^{a}	4.74^{c}	5.28^{b}	4.63^{c}	0.143	<0.001

^{a,b,c,d} Mean values followed by different superscript letters in the same row are significantly different ($P < 0.05$).

[1] ALT; Alanine aminotransferase: AST; Aspartate aminotransferase.

[2] CON= Basal diet without additive; SP=Basal diet with 1g/kg spirulina; SeNPs= Basal diet with 50mg/kg selenium nanoparticles; and SP+SeNPs = Basal diet with spirulina (1g/kg diet) plus selenium nanoparticles (50 mg/kg diet).

Table 5. Effect of selenium nanoparticles, spirulina and their combination on immunological responses and interferon gamma (IFNγ) level of male rabbits exposed to high environmental temperature.

Item[1]	Treatment groups[2]				Pooled SEM	*P* value
	CON	SP	SeNPs	SP+ SeNPs		
IgA (g/ml)	233.67	245.33	254.67	258	6.031	0.545
IgG (g/ml)	258.33^{c}	318^{b}	384.33^{a}	327.67^{b}	10.401	<0.001
IFNγ (pg/ml)	64.97^{a}	32.44^{c}	51.18^{b}	26.8^{c}	4.700	<0.001

^{a,b,c} Mean values followed by different superscript letters in the same row are significantly different ($P < 0.05$).

[1] IgA: immunoglobin A: IgG: immunoglobin G and IFNγ: interferon gamma.

[2] CON= Basal diet without additive; SP=Basal diet with 1g/kg spirulina; SeNPs= Basal diet with 50mg/kg selenium nanoparticles; and SP+SeNPs = Basal diet with spirulina (1g/kg diet) plus selenium nanoparticles (50 mg/kg diet).

Redox status

The results presented in Table 6 show that the SP or SeNPs or their combination resulted in lower levels of oxidative stress (MDA and PCO; $P < 0.001$), while all treatments showed significant enhancement in the level of GSH. The SOD level was similar across all experimental groups (P=0.736). The enhancing of antioxidant status is generally connected with precise enhancements, the health status, welfare, and production of animals. Male goats
received SeNPs had a greater level of SOD, CAT, and GSH in related to other given sodium selenite or Se yeast (Shi *et al.,* 2011).

All the antioxidant system including the enzymes play a critical role in removing the freed radical form the cellular system. Our results shown that the dietary supplement with SP or/and SeNPs enhanced the antioxidant capacity and attenuating the oxidative stress such as MDA and PCO. Similar to our data studies of (Zhang *et al.,* 2008; Khurana *et al.,* 2019) presented that SeNPs revealed an excellent bioavailability owing to their great catalytic efficacy, absorbing solid capacity and low toxicity. These detailed properties and the various absorption forms may highlight how SeNPs is more bioavailable than other sources (organic or inorganic form) (Khurana *et al.,* 2019). Recently, several have been reported that SP exhibited promising impact in reducing the risks of severe health complications in respect of their antioxidant properties (Khurana *et al.,* 2019), and improving overall the productivity of animals as reviewed by (Abdelnour *et al.,* 2021). In a previous study of our work, we demonstrated that the dietary inclusion of SeNPs has a positive effect in reducing the HS challenges and sustain rabbit health and productivity (Sheiha *et al*., 2020). This previously effect might be related to the enhancement of antioxidant capacity and reduce the pro-inflammatory cytokinesis synthesis in rabbit. Furthermore, (Hassan *et al*., 2021), dietary addition of zinc nanoparticles (100 mg/kg diet), SP (0.5 mg/kg diet) or their mixture could improve growth indices, nutrient efficiency and antioxidant status of heat stressed growing rabbits. In this sense, the combination of SeNPs and SP in heat stressed rabbit has been not explored.

Numerous studies recently reported the valuable effects of nanoparticles, especially nano minerals on animal health and immunity (Shi *et al.,* 2011; Sheiha *et al.,* 2020; El-Desoky *et al*., 2021). In pigs, (Liu *et al.,* 2021b) found that dietary addition of organic Se alleviated the negative influences of HS via enhancing the antioxidant responses, and regulation of selenoproteins in skeletal muscle. Regulating of selenoprotiens and its genes is a critical strategy for mitigate the detrimental effects of HS in animals. Enhancing the antioxidant status of an animal could sustain the normal cellular, molecular and physiological functions.

Table 6. Effect of selenium nanoparticles, spirulina and their combination on redox status of male rabbits exposed to high environmental temperature.

Item[1]	Treatments[2]				Pooled SEM	*P* value
	CON	SP	SeNPs	SP+ SeNPs		
Oxidative stress biomarkers						
MDA (nmol/ mL)	0.40[a]	0.20[c]	0.27[b]	0.21[c]	0.025	<0.001
PCO (ng/mg)	2.65[a]	1.42[c]	1.83[b]	0.91[d]	0.179	<0.001
Antioxidant biomarkers						
SOD (u/ml)	0.25	0.24	0.24	0.24	0.003	0.736
GSH (u/mL)	0.14[c]	0.22[b]	0.20[b]	0.27[a]	0.015	<0.001

[a,b,c,d] Mean values followed by different superscript letters in the same row are significantly different ($P < 0.05$).

[1] MDA; malondialdehyde: PCO; Protein carbonyl: SOD; Superoxide dismutase and GSH: Reduced glutathione.

[2] CON= Basal diet without additive; SP=Basal diet with 1g/kg spirulina; SeNPs= Basal diet with 50mg/kg selenium nanoparticles; and SP+SeNPs = Basal diet with spirulina (1g/kg diet) plus selenium nanoparticles (50 mg/kg diet).

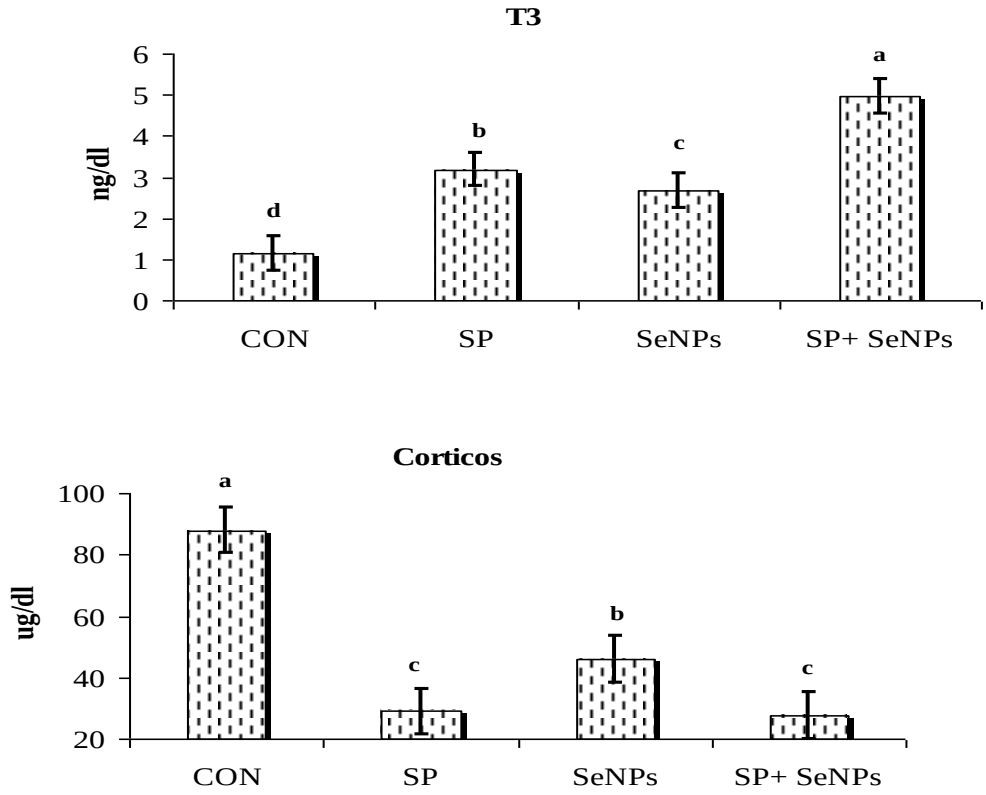
As described above, HS induces suppression in the animal immunity and antioxidant capacity resulting in higher lipid (MDA) and protein oxidation (such PCO), and thus reduce the performance potential of rabbits (Amber *et al.,* 2021; Hassan *et al.,* 2021). Park *et al.* (2018) recommended that dietary SP supplement in broiler diets (0.25-1.0%) triggered an augment in the activities of SOD and GSH-Px. In heat stressed broilers, Liu *et al.* (2021a) dietary algae-derived polysaccharide improved the total antioxidant capacity (T-AOC) and GSH-Px, while reducing the MDA levels of the duodenum ($P < 0.05$). Results though were consistent with other animal studies that have observed a significant inflammatory response to exposure to acute heat stress.

It was documented that heat stressed broiler exhibited lower T- AOC, that is associated with the destruction of Nrf2 expression (Park *et al.,* 2018; Abdel-Moneim *et al.,* 2021; Alwaleed *et al.,* 2021). Higher levels of MDA were detected in the duodenum of heat stressed broiler, however, this elevation was restored after algae-derived polysaccharide feeding (Qiao *et al.,* 2020). Altered intestinal integrity and the ensuing passage of bacterial antigens across the gut barrier elicit an immune response that is energetically expensive to initiate and maintain; consequently, animal efficiency is compromised as nutrients are reallocated away from productive processes (*i.e*., growth, reproduction) to sustain an immune response(Oladimeji *et al.,* 2021).

Hormonal profile:

The effects of dietary SP, SeNPs or their combination (SP+SeNPs) supplementation on hormonal profile T3 and corticosterone of heat stressed rabbits are shown in Figure 3. The mixture of SP+SeNPs had higher level of T3 ($P< 0.001$), while both SP and SP+SeNPs treatments had lower values of plasma corticosterone ($P< 0.001$). Compared with the feed additive groups, the heat stress rabbit exhibited the lowest values of T3 and highest values of plasma corticosterone.

Heat stress (HS) impairs several physiological processes and its need critical component to combat and preserve these functions in the face of HS (Sejian *et al.*, 2018). The high levels of stress hormones such corticosterone are considering an important feature for determining the severity of HS on rabbits (Abdel-Latif *et al.*, 2018). Stress hormone such as corticosterone may also be a plausible hormonal arbitrator of HS effect on growth and immunity. High ambient temperatures are able to promote corticosterone release in animals, which in turn may suppress immunity functions in an organism (Abdel-Latif *et al.,* 2018; Sejian *et al.*, 2018). In this sense, the results mentioned that the capability of all treated groups; SP or SeNPs or their combination can significantly reduce the HS severity though



. **Figure 3.** Effect of selenium nanoparticles (SeNPs; 50 mg/kg), Spirulina (SP; 1 g/kg) and their combination (SeNPs; 50 mg/kg + SP 1g/kg) on hormonal profile of male rabbits exposed to high environmental temperature.

restoring the hormonal balance in the body (Sirotkin *et al.,* 2021) clarified that the HS induced a significant augmentation in corticosterone level of young in rabbits. Recently, different nanoencapsulation phytogenic improve rabbit productivity (El-Desoky *et al.,* 2021) and semen cryopreservation (Abdelnour *et al.,* 2020b). SeNPs derived from biogenic method has displayed eco-friendly agents, higher biodegradability, and promising potentials for industrial pharmacological and numerous uses (Khurana *et al.,* 2019; Sheiha *et al*., 2020). (Hosny *et al.,* 2020) reported that the presence of Se (0.3 mg/kg diet) of naturally heat-stressed rabbit bucks contradicted the undesirable influences of HS on homeostasis, and redox status.

Immunohistochemical findings

The morphologic of caspase3 immune expression of HS-exposed rabbits presented moderate of intracytoplasmic immunoreactivity that existing by a substantial rise in area percentage of caspase 3 immunoreactivity related to other groups {Figure 4 (1)}. However, the co-treatment with SP, SeNPs or their combination induced a dose-dependent enhancement in caspase morphologic intensity and area proportion of immunoexpressing related to HS-exposed rabbits {Figure 4 (2, 3 and 4)}. HS could induce apoptosis process in the liver tissues via reducing the antiapoptotic gene such BCL2 (Yasoob *et al.,* 2022), whereas the phytogenic improved the IL-6 expression clarifying it immune, survival response, and thermo-tolerance resistance. Caspase-3 contributes in both the death receptor apoptotic and mitochondrial pathways and is the ending executioner of apoptosis process (Sudo and Minami, 2010). Our findings discovered that HS encouraged the relocation of caspase-3 from the cytoplasm into the nucleus, which in turn activated apoptosis, follow-on DNA injury in the liver. Similar with our data, (Madkour *et al*., 2021) indicated that HS induced a substantial ($P < .05$) increase in caspase-3 levels in rabbits. In rats exposed to HS, (Khafaga *et al.,* 2019) showed that the use phytochemical presented significantly reduced levels of apoptotic marker caspase 3, along with the logical decrease of oxidative stress and histopathologic lesions. Further studies are desired to explore the impacts of nanoparticles and/or marine algae on whole physiologic parameters, transcription and deep proteomic scrutiny in animals exposed to HS.

Conclusion

The existing results suggested the valuable roles of SeNPs or in combined with SP in improving the growth performance, blood metabolites, antioxidant and immunological responses and reduced the hormonal profile in HS rabbits. Dietary inclusion of the mixture (SeNPs and SP) could ameliorate HS-induced apoptosis and stress biomarkers via modulating Caspase3 protein and MDA and PC. These results show that use of nano minerals or green algae (SP) is an effective nutritional approach to mitigate the detrimental effects of HS in rabbits. However, the use of nanotechnology has been partially explored in some ruminants reviewed by (Abdelnour *et al.*, 2021), further explorations regarding to the potential using of nano elements as an anti-HS agent are needed.

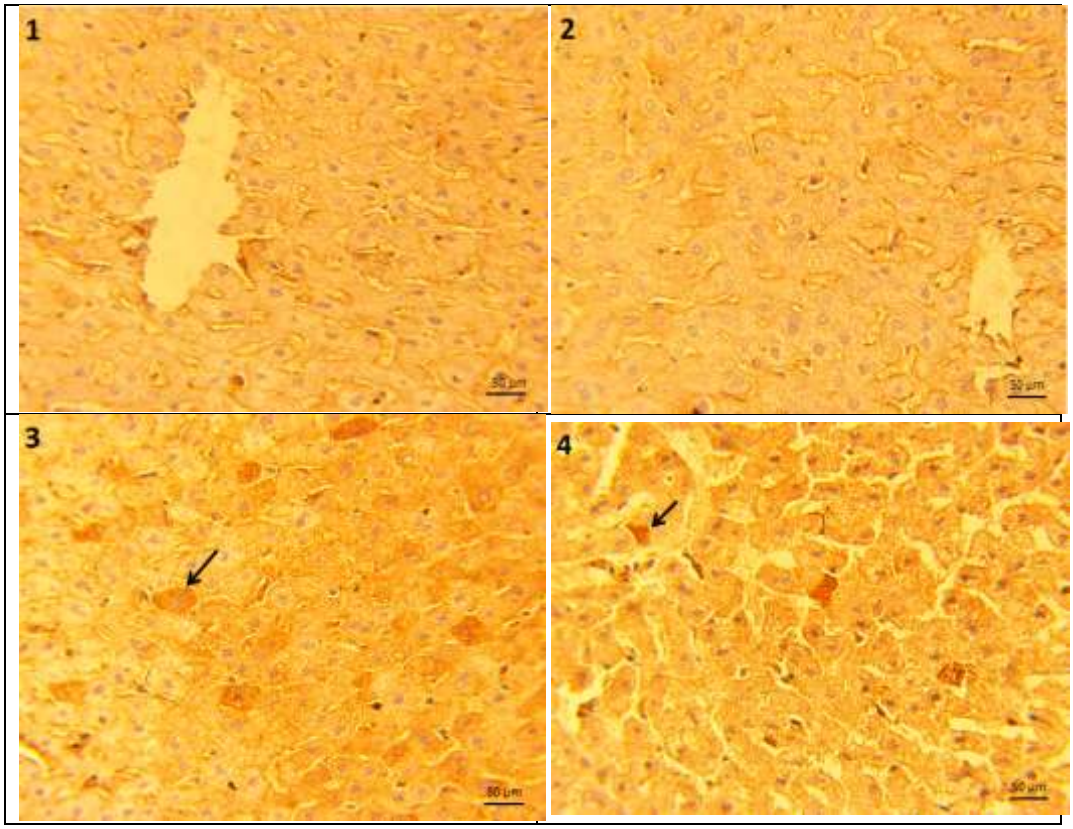


Figure 4. Representative photomicrograph of caspase-3 immuno- stained (1,2,3,4) liver sections×100µm.

1) Liver tissues from HS-exposed rabbits presented moderate expression of intracytoplasmic caspase-3 protein immunostaining.
2) Liver tissue from HS-exposed rabbits co-treated with SP (1g/kg) showed intensive the expression of intracytoplasmic caspase-3 protein.
3) Liver tissue from HS-exposed rabbits co-treated with SeNPs (50mg/kg showed moderate to a mild immune expression of caspase-3.
4) Liver tissue from HS-exposed rabbits co-treated with SeNPs (50mg/kg) and SP (1g/kg diet) showed intensive immune expression of caspase-3.

CRediT authorship contribution statement

A.M.B., S.A.A.; Conceptualization, Methodology, Software, Data curation, Visualization. A.A.E.; Writing, original draft. A.M.S.; Formal analysis, Writing - original draft. S.A.A.; Project administration, review & editing.

Declaration of Competing Interest

No potential conflicts of interest declared.

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تأثير النانو سيلينيوم و/ أوالأسبيروليـنا بلاتنسيس على النمو وخصائص الدم وحالة مضادات الأكسدة والمظهر الهرموني ، والمناعة ، وموت الخلايا المبرمج للأرانب النامية المعرضة للإجهاد الحراري.

عمرو محمد بشار[1], سامح عبد النور[1], عبدالحليم على الدروانى[1], أسماء محمد سعيد شيحه[1]
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تم إجراء هذه الدراسة لمعرفة تأثير اضافة الأسبيروليـنا بلاتنسيس (SP) أو / النانو سيلينيوم (SeNPs) كمكملات غذائية على النمو، ومستقلبات الدم ومضادات الأكسدة في الأرانب النامية التي تقع تحت الإجهاد الحراري في فصل الصيف. تم تقسيم الأرانب (ن = 120) عشوائيًا إلى أربع مجموعات معاملة مكونة من 30 أرنباً لكل مجموعة. وكانت مجموعات المعاملة على النحو التالي: العليقة الأساسية بدون مكملات (CON), أو مع 1 جم SP / كجم من العليقة ، أو 50 مجم SeNPs / كجم من العليقة ، أو مزيج منها (50 مجم SeNPs + 1 جم SP).

وكانت أهم النتائج كالتالي:-

- أدت أضافة SeNPs و SP بشكل ملحوظ ($P < 0.05$) إلى تحسن جميع مؤشرات النمو في الأرانب المجهدة بالحرارة.
- تم تحسين الهيموجلوبين والصفائح الدموية والبروتين الكلي والألبومين والجلوبيولين بشكل ملحوظ في مجموعات SeNPs و SP + SeNPs مقارنة مع مجموعة .CON ومع ذلك، انخفضت كرات الدم البيضاء بشكل ملحوظ في جميع المجموعات المعاملة.

- لوحظ انخفاض كبير في ALT، وإجمالي البيليروبين، والبيليروبين المباشر، وإجمالي الجلسريدات، والكرياتينين، وأحماض البوليك في المجموعات المعاملة مقارنة مع مجموعة CON
- مستويات IgG تحسنت بشكل معنوي (P <0.001) في جميع المجموعات المعاملة مقارنة بمجموعة CON
- أدت الإضافات التجريبية في الغذاء إلى انخفاض مستويات الإجهاد التأكسدي (MDA and PCO; (P<0.001) و تحسنًا كبيرًا في مستوى GSH.
- وكان للمجموعة المعاملة ب SP + SeNPs مستوى أعلى (P <0.001) من T3 ، بينما كان لكل من معاملتي SP و SP+SeNPs قيم منخفضة (P <0.001) من الكورتيكوستيرون في البلازما.
- أظهر الشكل المورفولوجي للتعبير المناعي لـ caspase3 للأرانب المجهدة بالحرارة نشاط مناعي معتدلاً داخل السيتوبلازم والذي يظهر من خلال ارتفاع كبير في نسبة النشاط المناعي لل caspase 3

التوصية:نستخلص مما سبق وفقا للنتائج التي تم الحصول عليها، أن اضافة ال SeNPs و / أو SP يمكن أن تخفف التأثيرات السلبية للإجهاد الحراري علي الأرانب من خلال تعزيز مضادات الأكسدة والمناعة وتقليل عملية موت الخلايا المبرمج.