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The Role of Nano-Spirulina Platensis in Enhancement of Female Rabbit Puberty and Amelioration of the Heat Stress Adverse Effect

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Abstract

Spirulina platensis alga is an extreme protein source that enhances ovarian function and diminishes heat stress impacts. This work aims to study the enhancement effect of *Nano-Spirulina-platensis* alga on female rabbit puberty and its ameliorative effect against adverse effects of heat stress (HS). HS impairs growth and delays the puberty of rabbits. *Spirulina*-nanoparticles were prepared, and then; HPLC, antioxidant DPPH, total phenolic, and flavonoids were estimated. 24 New Zealand female rabbits were divided into 4 groups (n=6). G1 negative control group under ambient temperature. G2 was exposed to heat stress (35°C). G3 and G4 were dietary supplied with *Nano-Spirulina* (15mg and 35mg/Kg bwt/30 days under heat stress respectively. Rabbits' body temperature, respiration rate, body weight gain, feed intake, food conversion rate (FCR), feed efficiency ratio (FER), and puberty behavior observation were recorded. Blood plasma was collected and analyzed for the reproductive hormones; follicle- stimulating hormone (FSH), luteinizing hormone (LH), and progesterone (P4). Also the proliferative marker Transforming- Growth-Factor-Beta-2 (TGFβ2), and the cytokines as; Gamma Interferon (INFγ), Interlukine-1(IL-1), and Tumor-Necrosis- Factor α(TNFα). In addition to the liver and renal function markers Albumin, Alanine-Transaminase (ALT), Aspartate-aminotransferase (AST), and Creatinine. Also, the oxidative stress marker Malondialdehyde(MDA) and antioxidant markers Catalase and Superoxide Dismutase(SOD) were measured. Finally; rabbits were slaughtered and histopathological investigations were performed for the ovary, small intestine, liver, and spleen. Our findings showed that; *Nano-Spirulina* improved the oxidative status, elevated the reproductive hormones, and protected the examined tissues from heat stress effects. *Spirulina* stimulated folliculogenesis earlier than control rabbits. It could be concluded that; *Nano-Spirulina* enhanced puberty in female rabbits during heat stress.

Keywords: Nano-Spirulina; puberty; heat stress; ovary; rabbit.

1. Introduction

Rabbit puberty was reported to be delayed as the age at first mating increased with elevated environmental temperature and humidity. Egyptian native breeds reached puberty at about 6 months of age this may be due to the negative effect of heat stress on feed intake and body weight gain. The enforced mating practice resulted in very low conception rate and an increase in neonatal loss [1].

Plenty of nutritional studies were done to improve female reproductive traits like ovarian follicular activity and conception rate. *Spirulina platensis* is emerging as a potential candidate to meet these criteria. It is rich in vitamins, minerals, antioxidants, and phenolic compounds [2]. It was reported that; when Spirulina platensis was fed to animals, it showed a high feed conversion rate and optimized animal product quality and quantity. Dietary Spirulina had immunogenic activity, increased the natural killer cell activity (NK), and enhanced the disease resistance [3]. A previous study reported that administration of Spirulina platensis stimulated sex hormones and enhanced ovarian function and follicle formation in females [4].

It is well-known that nanoparticles are more targeted, can persist in the bloodstream for a longer time, and give a more favorable effect than conventional particles [5].

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The current study aimed to explore the effect of supplementing different doses of *Nano-Spirulina* on the general health of female rabbits with special emphasis on the onset of puberty.

2. Materials & methods

2.1. Identification of active component of the microalgae

The microalgae were provided by the Alga Biotechnology Unit/National Research Center (NRC)/ Dokki/Egypt. Nutritional analysis and active composition were identified by atomic absorption and HPLC units in NRC according to [6].

2.2. Preparation of the nanoparticles

The particle size of dried algae was reduced by mechanical sonication. The z-average hydrodynamic diameter was determined, in a study of samples at 25°C by photon correlation spectroscopy using a Zeta Sizer Nano (ZS) (Malvern Instruments Inc., Southborough, MA) in the Central Lab in NRC).

2.2.1. Morphology of nanoparticles using Transmission Electron Microscope (TEM)

The examined samples' z-average hydrodynamic diameter was estimated at 25 ± 0.1°C by photon correlation spectroscopy using a Zeta Sizer Nano ZS (Malvern Instruments Inc., Southborough, MA). Morphology of sprayed powder was done using TEM.

2.2.2. Cytotoxic effect of *Nano-Spirulina* on human normal fibroblast cell line (BJ1)

Cell viability was assessed by the mitochondrial- dependent reduction of yellow MTT (3-(4, 5-dimethyl thiazol2-yl)-2, 5-diphenyl tetrazolium bromide) to purple formazan [7].

2.3. Estimation of *Nano-Spirulina* total phenolic and flavonoid contents

The total phenolic and flavonoid content was determined using gallic acid and rutin as standard [8- 9].

2.4. Determination of the antioxidant activity of *Nano-Spirulina*

The free radical scavenging capacity of microalgae was determined using the stable 1, 1-Diphenyl-2- picryl hydrazyl (DPPH) compared to ascorbic acid as standard [10].

2.5. Polyphenol profile of *Nano-Spirulina* by HPLC

Polyphenols HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using the Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm) [11].

2.6. Biological study

2.6.1. Ethics

The biological experiment was designed and done under the regulation of the ethical committee of NRC (project no.13050419). Female rabbits were kept in clean well-aerated chambers and had free access to feed and water. 12 hours light period. Kept in a naturally ventilated and lighted rabbitry. Rabbits were housed individually in stainless steel cage batteries (40× 50 × 35 cm) supplied with feeders and automatic fresh-water nipples.

2.6.2. Experimental design

A total number of 24 Healthy New Zealand white female rabbits 2 months old weighing 1.5 kg bwt were used in this study. The experimental rabbits were kept in a temperature-controlled room at 35°C to keep rabbits under heat stress (HS) except the control group and were fed on a commercial pelleted diet (Table 1) covering their daily nutritional requirements [12]. Rabbits were equally divided into four groups (n=6). G1 was fed only on the basal diet and kept at ambient temperature. G2 was fed on the basal diet and kept under HS. G3 and G4 were fed on the same diet besides oral administration with *Spirulina platensis* nanoparticles (15mg and 35mg / Kg bwt respectively) and kept under heat stress. Rabbits' body temperature, body weight gain, feed intake, feed conversion rate(FCR), feed efficiency ratio (FER), and vitality of rabbits were recorded. The treatment period was 30 days. Females were observed for the onset of puberty behavior.

Table 1: Formulation of basal diet

Ingredient	%	Chemical analysis	%
Berseem hay	30.0	Dry matter (DM)	87.7
Barley grain	24.6	Organic matter (OM)	88.9
Wheat brain	21.5	Crude protein (CP)	17.8
Soybean (44% CP)	17.5	Crude fiber (CF)	14
Molasses	3.0	Ether extract (EE)	2.23
Limestone	1.0	Nitrogen free extract (NFE)	59.5
Di-calcium phosphate	1.6	Ash	8.6
Sodium chloride	0.3	Metabolizable energy (ME, kcal/kg)2	23.14
Mineral – vitamin premix1	0.3	Calcium2	1.24
Dl- Methionine	0.2	Phosphorus2	0.81
Total	100	Methionine2	0.45

2.6.3. Heat stress room environmental condition

The heat stress room was equipped with thermometers and humidity-measuring equipment, the minimum and maximum average temperatures were measured and recorded in Table (2).

Table 2: Ambient temperature (C°), relative humidity (RH %) and THI values in different week of of the experimental period under heat stress

	Heat stress conditions for G2, G3 and G4					
Week	**C°**		**RH%**		**THI**	
	Minimum	**Maximum**	**Minimum**	**Maximum**	**Minimum**	**Maximum**
1	19.29±0.42	35.14±0.5	22.86±2.20	61.86±1.4	29.38±0.55	32.51±0.55
2	19.86±0.96	40.86±1.79	24.57±3.83	66.43±1.97	28.36±1.54	37.06±1.54
3	18.86±0.26	36.29±0.89	31.43±4.63	72.71±3.51	28.65±0.72	32.38±0.72
4	17.14±0.34	34.86±0.26	31.29±0.28	66.86±2.25	30.14±0.16	31.08±0.16
	Normal conditions for G1					
	Minimum	**Maximum**	**Minimum**	**Maximum**	**Minimum**	**Maximum**
1	19.4±0.44	29.14±0.52	35.86±2.20	61.86±1.42	24.38±0.54	27.14±0.57
2	21.86±0.85	29.86±1.75	32.57±3.81	68.43±1.95	23.94±1.57	27.45±1.52
3	17.86±0.24	29.29±0.86	32.43±4.62	74.71±3.54	26.39±0.70	27.83±0.73
4	17.14±0.32	29.86±0.27	32.29±0.27	68.86±2.26	26.44±0.14	27.14±0.15

3. Results & Discussion

3.1. Analysis of nutrients of microalgae

Analysis of *Nano-Spirulina* (Table 3, 4) showed that; *Nano-Spirulina* is rich in protein, carbohydrate, phosphorus, calcium, magnesium, iron, zinc, selenium, copper, and vitamins A, D3, and E.These findings were clarified by a previous study that reported a high nutritive value of Spirulina platensis [17].

Table 3: Analysis of *Nano Spirulina* nutrients and vitamins

Chemistry	(% of dry matter basis)
Carbohydrate	39.92
protein	56.2
Fat	0.72
Ash	1.9
Fiber	1.26
Dry matter	90.45
Organic matter	98.1
Vitamins	(IU)
Vit A	4550
Vit D3	1427
Vit E	58

Table 4: Estimation of mineral and traces in nano spirulina

Minerals and traces	Mg/kg
Phosphorus	3500
Calcium	1875
Magnesium	1050
Iron Selenium	220
Copper	1.25
zinc	1.5
	14.5

3.2. Morphology of nanoparticles using Transmission Electron Microscope (TEM)

The morphology and size of the nanoparticles were estimated by transmission electron microscope which was 33 nm (Fig.1)

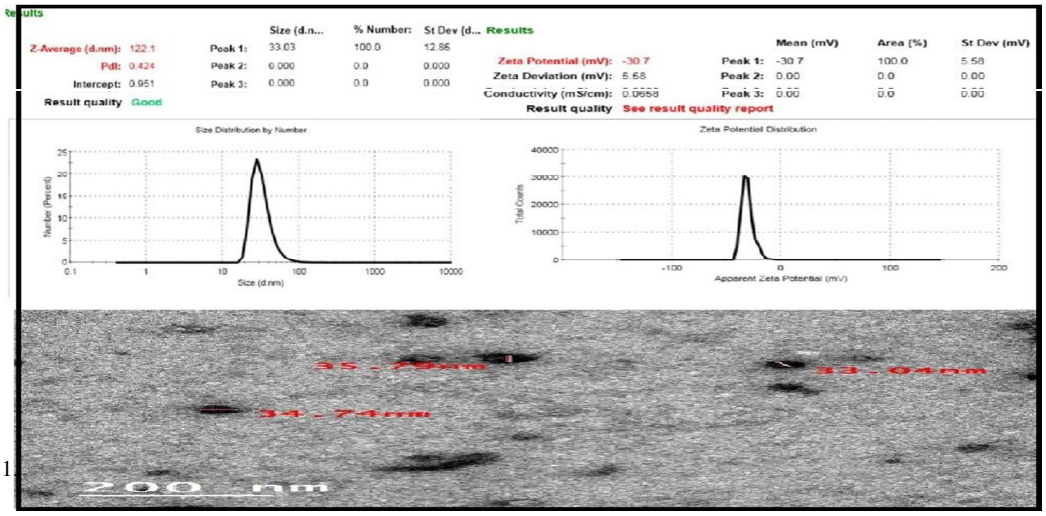

Figure 1: Zeta sizer and morphology of nano particles using TEM

The physical stability of the particles depends on the size of a colloidal system's ζ- potential (Zeta potential: which is an electro-kinetic potentiality for the particles in colloidal dispersions). If all of the particles had strong ζ-potential (either positive or negative), they repel each other and did not form aggregations [18]. The ζ-potential value was 30.7 mV (millivolts) in *Spirulina* which indicated repulsive interactions between charged molecules as if the ζ- potential of the particles were greater than 30 mV they are physically constant [19-20-21]

3.3. Cytotoxic effect of *Nano-Spirulina* on human fibroblast cell line (BJ1)

The present study revealed that; 100 ug/ml or ppm conc. of the *Nano-Spirulina* killed 13.5% of the normal human epithelial

cell line BJ1 (normal skin fibroblast) and this is the normal physiological range. That means; the *Nano-Spirulina* has a great biosafety on somatic cells [22].

3.4. Estimation of the total phenolic and flavonoid contents in the *Nano-Spirulina* alga

The illustrated findings in Fig (2) exhibited that; the *Nano-Spirulina* extract is rich in flavonoids (3.9) as matched to gallic acid (2.59), rutin (2.7). In addition, the estimated total phenolics is (36.9) as matched to gallic acid (36.5) and rutin (40) All these records were standardly expressed as mg/gm dry weight [22].

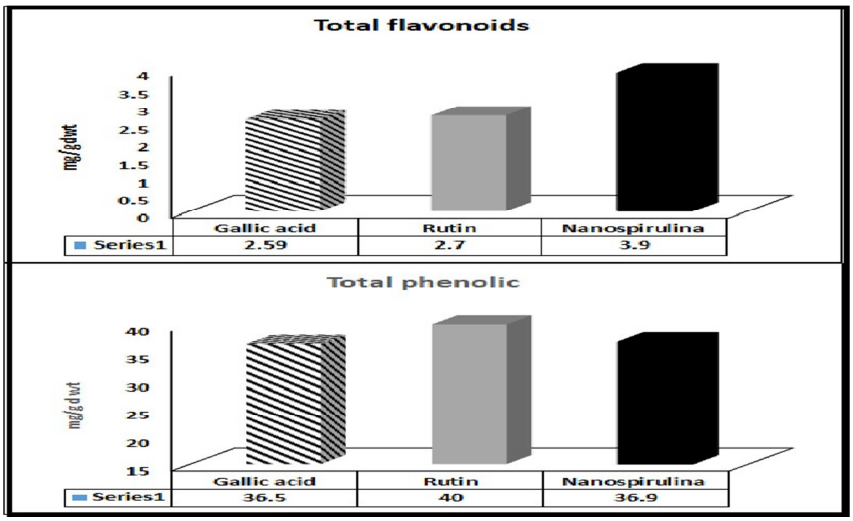

Figure 2: Total flavonoid, total phenolic content of nano spirulina extract

3.5. Antioxidant activity

The antioxidant activity of the *Nano-Spirulina* extract which is estimated in Fig (3) has a high antioxidant activity (DPPH) as compared with ascorbic acid. This finding could be clarified as, Spirulina is rich in vitamins, and minerals (Table 4) and also gallic acid, and ferulic acid (Table 5) these components the strong antioxidant activity as confirmed by Enyidi [24].

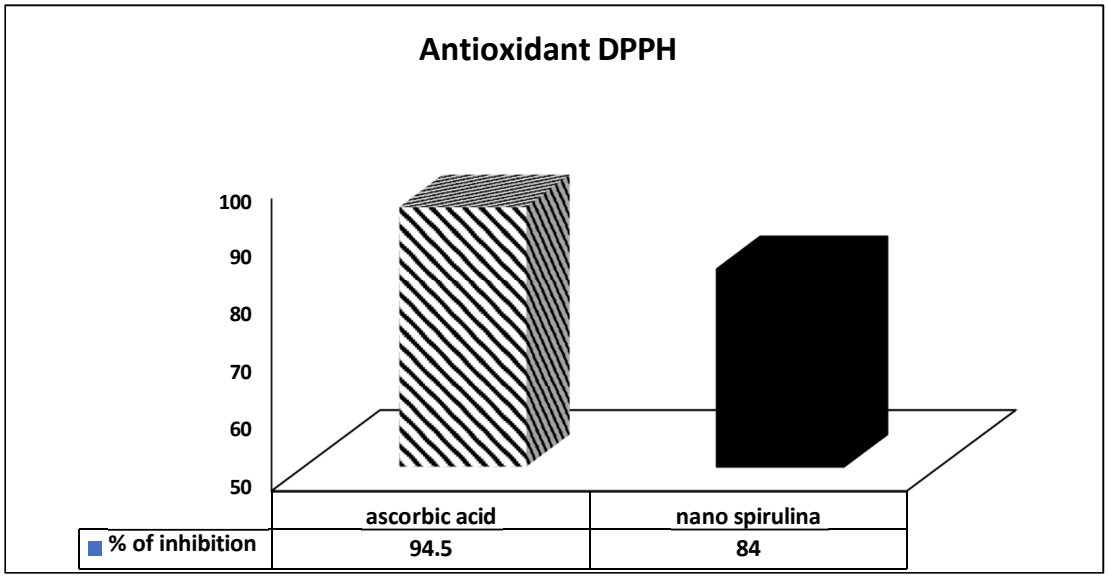

Figure 3: Antioxidant activity (DPPH) of nano spirulina extract

3.6. Polyphenol profile (HPLC)

The screening process of the polyphenolic compounds of the Nano-Spirulina extract using HPLC and displayed in Table (5) showed that; Gallic acid is the dominant compound which is previously reported and well established to be a strong antioxidant [25].

Table 5: Polyphenol profile (HPLC) of *Nano spirulina* extract

Polyphenol compound	Spirulina platensis (ug/ml)	Slandered conc.(ug/ml)
Gallic acid	9.40	8.4
Coffeic acid	0.25	9
Ferulic acid	0.20	6.0
Naringenine	0.21	7.5
querectin	0.34	6.1
Cinnamic acid	0.09	2.3
Kaempferol	0.34	6
Chlorogenic acid	ND	14
Rutin	ND	30.5
Daidazin	ND	10
Vanillin	ND	6.15
Ellagic acid	**ND**	**17.15**

3.7. Growth performance

The growth performance of the present work as in Fig (4) revealed that; animals kept under heat stress (G2) showed a decrease in feed intake, body weight gain, and feed efficiency ratio, in addition to the histopathological picture of the small intestine in fig. (6) Which showed a destruction and degeneration of the cell lining of intestinal that leads to the limitation of the nutrient absorption in this group. Administration of the Nano Spirulina led to an improvement in the parameters of feed intake, body weight gain, and Feed Efficiency Ratio (FER), especially at (G3) (15 mg/kg bwt) which recorded the highest values of these parameters.

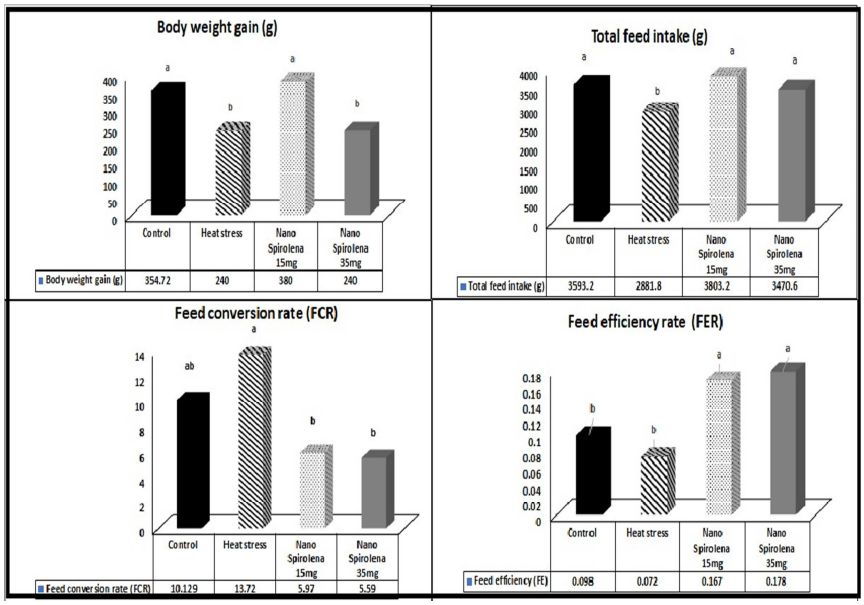

Figure 4: Effect of using nano spirulina on growth performance of rabbit

These results were supported by our histological findings of the intestinal villi (Fig 6). These findings illustrated the reverse effect that was clarified in (G3) and (G4) due to the antioxidant effect of *Nano- Spirulina. Spirulina* was riched in phenolic compounds that have antioxidant effects [25] and so lowered the animals' body temperature and enhanced the antimicrobial activity which leads to clearing of the alimentary 5 tract from pathogens therefore increasing the nutrient absorption and feed utilization. It is also rich in protein, minerals, and trace elements necessary for anabolism [26].

3.8. Plasma chemistry

The analytical findings of plasma chemistry in the current study displayed in Table (6) showed that; Rabbits kept under heat stress recorded low levels of both antioxidant markers (SOD and Catalase), LH hormone, and TGFβ2. Also recorded a high levels of liver and kidney function markers (ALT, AST, and creatinine), oxidative markers (MDA), and cytokines (INFγ)

as in comparison with G1 (the control group kept under ambient temperature). These results were supported by our histopathological findings (Fig 7) that showed degenerative changes and periportal congestion among hepatic cords in the liver of G2, subjected to heat stress.These records could be explained as; Heat stress is associated with oxidative stress and elevated inflammatory cytokines [27] and is well known to harm all the body's metabolic processes, showing pathological changes in the liver, kidney, and ovary [28].On the other side; the results of the administration of *Nano-Spirulina* in two levels exhibited in Table (6) showed that; the examined plasma chemistry of rabbits in G3 & G4 recorded high levels of the antioxidant enzymes (Catalase and SOD) as well as high levels of the reproductive hormones FSH, LH, and progesterone indicating early puberty. In addition; administration of the *Nano-Spirulina* slightly elevated ALT, AST, and creatinine, and also elevated IL-1 β and INFγ. Moreover, the histological findings of these two groups of the liver did not show any pathological changes and showed complete protection for tissues due to the administration of *Nano-Spirulina.*

Table 6: Effect of administration of *Nano Spirulina* on Plasma chemistry of rabbits

	Control	Heat stress	Spirulina 1	Spirulina 2	P value
Total protein	3.13 + 0.20	3.19 +0.38	3.03+0.31	3.62+0.17	0.400
Albumin g/dl	2.51+ 0.09ab	2.99+0.39 a	2.132+0.65b	1.957+0.38b	0.011
Globulin g/dl	0.62+0.23	0.57+2.06	1.26+1.12	1.66+0.49	0.508
Cholesterol mg/dl	44.79+15.81	33.42+2.06	29.32+2.15	38.51+2.15	0.613
Catalase	346.15+56.84a	80.70+60.94c	247.60+17.12b	228.37+27.88b	0.000
Creatinine mg/dl	2.60+0.66c	5.20+0.489ab	6.50+1.88a	4.40+0.90b	0.000
Creatinine mg/dl	0.525+0.21b	1.342+0.12a	1.632+0.47a	0.712+0.25b	0.000
AST U/L	14.56+1.04b	24.9+8.56a	26.317+4.54a	20.132+ 6.07ab	0.034
ALT U/L	3.827+0.31	6.817+2.27	7.6+100+4.47	8.620+5.17	0.321
SOD U/ml	187.500+80.68b	437.500+148.77a	250.00+51.03b	291.66+41.66b	0.006
MDA nmol/ml	9.760+0.68a	7.312+0.99b	7.055+0.35b	9.286+0.81a	0.000
P4 ng/ml	0.756+0.19	0.751+0.15	0.878+0.14	1.089+0.23	0.533
LH ng/ml	0.259+0.05b	0.091+0.01b	0.492+0.01a	0.495+0.09a	0.000
FSH ng/ml	1.475+0.14b	1.950+0.41b	72.450+23.32a	18.30+4.28b	0.000
IL-1beta pg/ml	$4.04+0.47^{bc}$	$5.079+0.45^{ab}$	$6.845+1.05^{a}$	$2.711+0.32^{c}$	0.000
TGFbeta2 pg/ml	$45.929+3.51^{a}$	$13.607+2.22^{c}$	$26.076+4.62^{b}$	$14.90+0.57^{c}$	0.000
IF-gamma pg/ml	$2.829+1.05^{b}$	$10.48+1.91^{a}$	$14.484+3.38^{a}$	$0.807+7.73^{b}$	0.000
TNF-alpha pg/m	$1.938+0.31^{b}$	$1.253+0.09^{b}$	$2.586+0.44^{b}$	$5.625+1.06^{a}$	0.000

Data were presented as Means + SE. Different superscripts within row means significance ($P \geq 0.05$).

Also, Fig. (7) showed the normal hepato-portal area with normal healthy hepatic cords. These findings could be supported by [29] who stated that; *Spirulina* is an immunomodulator. It acts on the receptors of the natural killer cells (NK) and induces up-regulation of some cytokines (IL-1 and INF) while other cytokines are down-regulated like TNF and TGF. *Spirulina* is rich in vitamins and minerals as shown in Table (5) so it has high antioxidant activity as mentioned in Fig (3) and is rich in flavonoids and phenolic compounds as mentioned in Fig (2) and this was reflected in plasma chemistry. Similar results in rabbits fed spirulina were recorded by [30]. The histological picture of the ovary (Fig 5) confirmed the hormone profile as the elevated FSH and LH expressed as multiple mature follicles and corpus luteum indicating ovulation and early puberty as compared to control animals.

Previous studies reported that; the administration of an aqueous extract of *Spirulina platensis* affected the reproductive performance of rabbit doe and recorded elevated LH, estradiol, and serum protein concentration [23].

3.9. Histopathological study

The current histopathological study was performed to shed light on the relationship between heat stress and the detected lesions in ovaries, intestine, liver, and spleen.

Ovary

The ovarian tissue of the control rabbits (G1) contained moderate numbers of the different follicular stages as primordial and mature follicles (Fig. 5A). On the other hand; The prominent changes in ovaries of the heat stress-exposed immature rabbit doe (G2) were shown as an inadequate ovarian follicular activity which was exhibited in the form of small numbers of primordial follicles and a great number of atretic follicles (Fig. 5B).

It was reported that; thermal stress causes different changes in the tissue details of several organs [32]. Heat stress causes an elevation in the production of transition metal ions, which can induce electron donations to oxygen-constituting superoxide (H_2O_2) which is afterward reduced to reactive oxygen species (ROS) leading to oxidative stress [32], which consequently leads to metabolic and functional disorders in various cells and directly affects the growth, immunological state, and reproductive efficiency of animals [34]. These stressors effects expressed as; ovarian tissue changes, follicular degeneration

or atresia, and lysis of oocytes [32].

The *Nano-Spirulina Platensis*-treated rabbit doe (G3 and G4) results clarified the safeness effects of Spirulina algae on the rabbit doe tissues and in addition; repulsed the heat stress effects on the ovarian tissue and enhanced their follicular activity where it showed an increase in the follicular number and corpora lutea with a pronounced decline in the follicular atresia (as compared with the G2 kept under heat stress), especially in G3 (15 mg/kg *Nano- Spirulina Platensis*-treated rabbits). (Fig. 5C). On the other hand, G4 (35mg/kg *Nano-Spirulina Platensis*- treated rabbit doe) exhibited the presence of moderate numbers of primordial follicles and a few mature follicles (Fig. 5D).

These findings could be due to the *Spirulina* anti- inflammatory and antioxidant properties that hinder the oxidation process and ameliorate the cell injury [35].

Figure (5)

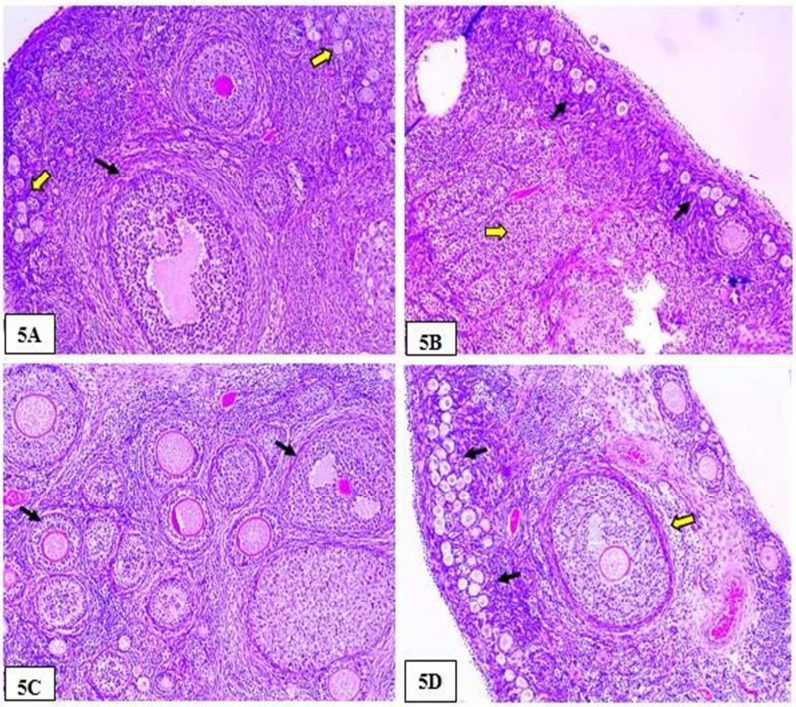

Figure (5): The histopathological changes in the ovary of the four experimental groups: **Fig. 5A:** Ovary of the rabbit doe showed normal tissue architecture containing moderate numbers of the different stages of folliculogenesis as mature follicles (black arrow) and primordial follicles (yellow arrows). **Fig. 5B:** Ovary showed poor follicular activity characterized by the existence of small primordial follicles (black arrows) associated with the existence of large numbers of atretic follicles (yellow arrow). H&E X 100. **Fig. 5C:** Ovary of (G3) showed an FSH-like effect for the *Nano- Spirulina Platensis* algae as the existence of the ovarian follicles in different stages with large numbers adjacent to each other (arrows) H&E X 100**. Fig. 5D:** Ovary of (G4) showed a moderate FSH-like effect for the *Nano-Spirolina Platensis* algae which exhibited the existence of moderate numbers of primordial follicles (black arrows) in addition to a few mature follicles (yellow arrow) H&E X 100**.**

Small intestine

The small intestine of the control rabbit doe (G1) showed normal intestinal villi with intact columnar epithelium and normal tissue cores (Fig. 6A). The heat stress-exposed rabbit doe (G2) exhibited destruction of most of the intestinal villi associated with mild hyperplasia in the lining epithelium of the apical portion of some intestinal villi (Fig. 6B). It was reported that; circulating cytokines may contribute to tissue damage. The changes in concentration of several cytokines and chemokines like GM-cSF, McP-1, MIP-1α, IL-4, IL-1β, Il-2, IL-6, IL-10, IL-12p40, and TNF-α produced in the ileum tissue that induces histopathological changes. If IL-1β is in high levels in the small intestine, it plays an antimicrobial role in protecting the intestinal mucosa, but the overdose of this cytokine causes injury to the intestinal epithelium leading to apoptosis and necrosis [36]. Also, a positive correlation was found between intestinal IL-10, which was elevated during heat stress, and tissue injury [37].

Results of the current study showed that; the administration of 15mg *Nano-Spirulina platensis* to the heat-stressed rabbit doe (G3) illustrated histologically normal intestinal villi lined with healthy intact simple columnar epithelium with active goblet cells, (Fig. 6C). In addition; the 35mg *Nano-Spirulina platensis*-treated group (G4) showed the same picture associated with mild necrosis in the lining epithelium of the apical portion of the intestinal villi (Fig. 6D). These results could be

attributed to the antioxidant and anti-inflammatory effects of *Nano-Spirulina*. Also, these histopathological findings were confirmed by our finding in Table (4) where the cytokines level was lower in *Nano-Spirulina*-treated groups as compared with other groups [38].

Figure (6)

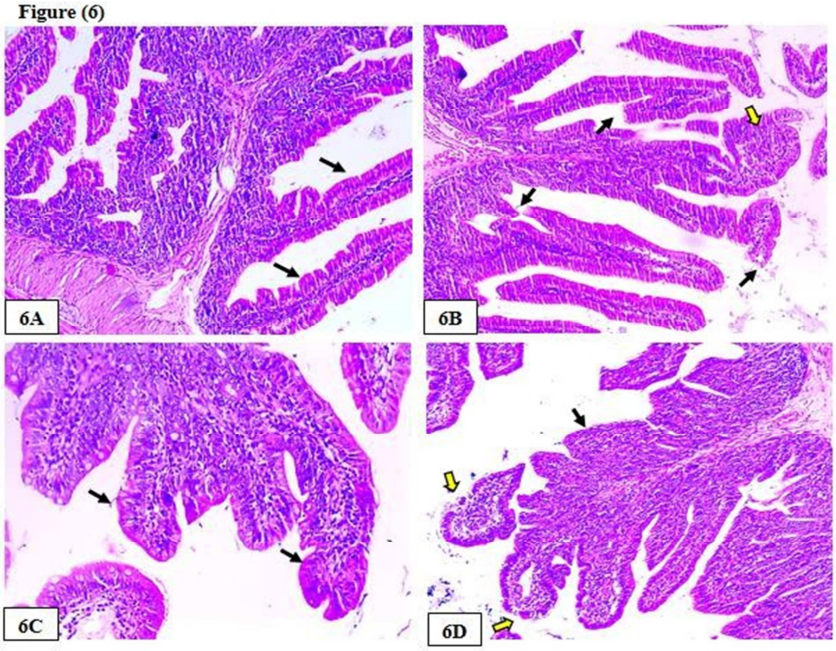

Figure (6): The histopathological changes in the intestine of the four experimental groups: Fig. 6A: Intestine illustrated normal intestinal villi with normal height and intact columnar epithelium with normal tissue cores (arrows). **Fig. 6B:** Intestine showed destruction of most of the intestinal villi (black arrows) associated with hyperplasia in the lining epithelium of the apical portion of some of the intestinal villi (yellow arrow). H&E X 100. **Fig. 6C:** Intestine exhibited healthy intact intestinal villi lined with normal intact simple columnar epithelium with active goblet cells, (arrows) H&E X 200. **Fig. 6D:** Intestine showed healthy intestinal villi lined with normal intact simple columnar epithelium (black arrows). Still, mild necrosis was noticed in the lining epithelium of the apical portion of the intestinal villi (yellow arrows) H&E X 200.

Liver

The hepatic tissue of the control rabbit doe (G1) was illustrated as normal healthy hepatocytes with a noncongested normal portal triad (Fig. 7A). In the heat stress-exposed rabbit doe (G2), periportal hemorrhage adjacent to the bile ductules accompanied by mild congestion among the hepatic cords was observed (Fig. 7B).

It was reported that; the thermal insult causes different histopathological changes in several organs as a result of elevated free radicals causing tissue injury [32]. Previous study mentioned that; hepatic tissue was highly sensitive to heat stress which induced high portal venous radical content, leading to hepatocyte hypoxia [28]. Another study showed hepatic vessel congestion, perivascular leucocytic infiltration, and hepatic degeneration with focal necrosis in heat-stress exposed liver [39 and 44].

The two groups (G3 and G4) of rabbits given *Nano-Spirulina platensis* (Fig. 7C&7D) showed a normal hepato-portal area with a normal noncongested portal vein surrounded by normal healthy hepatic cords.

This amelioration in the tissue architecture may be referred to the antioxidant and anti-inflammatory effect of *Spirulina* [33].

Figure (7)

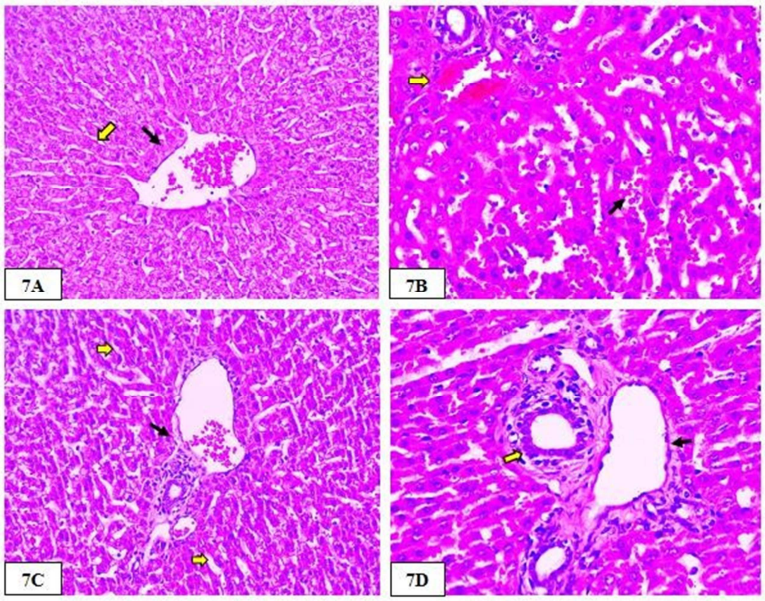

Figure (7): The histopathological changes in the liver of the four experimental groups: Fig. 7A: The Liver exhibited normal non congested non-dilated central veins (black arrow) and normal hepatic cords (yellow arrow).**Fig. 7B:** The liver illustrates periportal hemorrhage adjacent to the bile canaliculi (yellow arrow), which is accompanied by hemorrhages among the hepatic cords (black arrow). H&E X 200. **Fig. 7C:** Liver illustrated normal hepato-portal area with the normal non-congested portal vein (black arrow) Surrounded with normal healthy hepatic cords (yellow arrows).**Fig. 7D:** Liver displayed histologically normal hepato-portal area with the normal non-congested portal vein (black arrow) Surrounded with normal healthy hepatic cords and normal intact bile ductules (yellow arrow) H&E X 400.

Spleen

The splenic tissue of the control rabbit doe (G1) displayed normal lymphoid follicles fully packed with lymphocytes and a normal splenic artery (Fig. 8A). In addition, the heat stress-exposed rabbit doe (G2) exhibited subcapsular lymphocytic necrosis (Fig. 8B). In (G3) the 15mg/kg *Nano-Spirulina platensis*-treated rabbit doe; normal lymphoid follicles in the white pulp (Fig. 8C). While; in the (G4) 35mg/kg *Nano-Spirulina platensis*-treated rabbit doe illustrated normal lymphoid follicles in the white pulp but also, moderate subcapsular lymphocytic necrosis was detected (Fig. 8D).

The amelioration effects of *Nano-Spirulina Platensis* on splenic tissue could be related to that; *Nano-spirulina* has several health advantages on different body organs as a reduction in oxidative stress [27].

Figure (8)

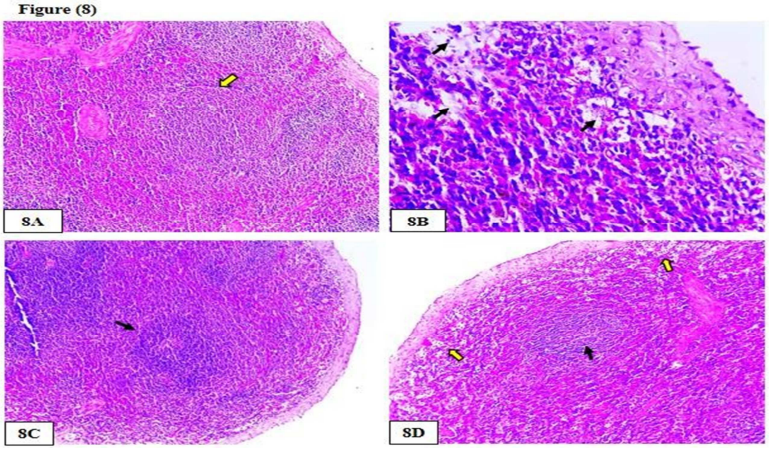

Figure (8): The histopathological changes in the spleen of the four experimental groups: Fig. 8A: Spleen displayed normal active lymphoid follicles with normal splenic artery (arrow). All figures H&E X 100.**Fig. 8B:** Spleen displays lymphocytic necrosis with lymphocytic nuclear pyknosis leaving a white zone around cells. H&E X 200. **Fig. 8C:** Spleen displayed normal splenic tissue with normal lymphoid follicles in the white pulp (arrow). H&E X 100. **Fig. 8D:** Spleen illustrated normal lymphoid follicles in the white pulp (black arrow) but also, moderate subcapsular lymphocytic necrosis was seen (yellow arrow). H&E X 100.

4. Conclusion

It can be concluded that the administration of Nano-Spirulina to young rabbits stimulates the growth rate, food efficiency ratio, improves ovarian function, and induces early puberty. Also, it improves the general condition of rabbits under heat stress and increases the animal vitality. It can be recommended that; dietary supplementation of growing animals with nano-particles of *Spirulina* as a small dose of *Nano- Spirulina* was more effective and targeted than ordinary micro size.

5. Conflict of Interest

The authors declare that there is no conflict of interest.

6. Formatting of funding sources

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7. Ethical of approval

This study fulfilled the requirement and guidelines of good medical and laboratory practice (GCP and GLP) guidelines as well as institutional and animal care and use committee (LACUC) guidelines, recommendations, and rules regarding the ethics of scientific research and approved in July 2020

8. Acknowledgments

Not applicable.

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