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The Protective Role of *Arthrospira platensis* Nanoparticles in Induced Testicular Damage in Male Rats

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Abstract This study was conducted at the College of Veterinary Medicine, Al-Qasim Green University. It was between October 2024 and March 2025 to evaluate the efficacy of *Arthrospira platensis* that encapsulated in chitosan nanoparticles to inspection the mitigating effect of phenyl hydrazine which induces testicular damage in adult male rats. Arthrospira platensis (AP) was procured from selzeal, British AP and was loaded onto chitosan nanoparticles, and the resulting formulation was optimized and characterized.

The study involved 40 adult male rats, divided into four groups of 10 for each. The first group used as control (CG) received only normal saline. Rats of the all other three groups were injected intraperitonealy with 20 mg/kg BW phenylhydrazine (PHZ) to induce testicular damage. Rats in third group received 50 mg/kg BW Arthrospira platensis (AP) daily. Rats in fourth group received 50 mg/kg AP-NPS daily. At the end of the study, the blood of the rats was collected, and all the rats were sacrificed and the testes of them were harvested. The study concluded that the use of AP-NPS effectively treat the induced testicular damage in rats.

Key words: *AP-NPS, ROS, infertility, phycocyanin, chitosan*

Introduction Male infertility can result from multi-factorials caused by lifestyle, infectious diseases, behaviors, environmental exposures, foods, drugs and physical injuries. Essential contributing factors involve huge alcohol intake, tobacco use, and substance abuse; persistent expose with harmful chemicals; high temperature of tests; venereal disease such as sexually transmitted diseases or mumps; hereditary conditions; and trauma or surgical procedures including the reproductive system (1, 2). These elements can generate complications such as reduce production of sperms, low sperm movement, DNA damage, hormonal imbalance, and ROS. The ROS generation can induce damage to mammalian testicular tissue, specially via lipid peroxidation due to the presence of high concentration of polyunsaturated fatty acids in the testes. Such defect may significantly impair testicular performance (3). The development of reliable antioxidant therapies is vital for mitigating oxidative damage following testicular Ischemia–Reperfusion Injury (IRI), thereby ensuring the maintenance of testicular integrity (4), as a widely recognized dietary supplement (5). The blue-green alga *Arthrospira platensis* (AP) provides an exceptional spectrum of nutrients and bioactive molecules, which

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includes: Protein; Vitamins and Minerals and Protective Agents, like Antioxidants for cell defense and anti-inflammatory compounds with therapeutic implications for chronic conditions (6).

The Arthrospira platensis pharmacokinetics are restricted by low oral bioavailability due to rapid gastric metabolism, metabolism by first-pass, and low intestinal absorption of its bioactive compounds (7,5). Additionally, hydrophilic in nature and high molecular weight result in low uptake by cell and rapid renal elimination, resulting in low therapeutic efficacy. Drug delivery by chitosan act protecting spirulina from gastric acid degradation through its mucoadhesive properties and pH-responsive swelling behavior (7). Chitosan NP improve drug bioavailability by promoting intestinal uptake, providing sustained release, and enabling site-specific delivery effectively addressing the pharmacokinetic challenges of conventional drug formulations (2). Phycocyanin represents a significant natural phycobiliprotein and constitutes the predominant bioactive component present in the blue-green AP (8). Multiple animal studies investigating phycocyanin dietary supplementation for combating inflammation and conditions triggered by oxidative stress have demonstrated that this supplementation can reduce inflammatory and apoptotic biomarkers, ameliorate neurodegenerative conditions, and has been examined for its capacity to suppress nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity while promoting aryl hydrocarbon receptor activation. Phycocyanin mitigates oxidative damage in Leydig cells, the primary cells responsible for testosterone biosynthesis (9, 10). Through its protective effect against reactive oxygen species (ROS)-induced cellular injury, phycocyanin supports the maintenance of physiological androgen production and androgen receptor (AR) expression levels. Aim of study: To investigate the therapeutic activity of spirulina nanoparticles in improving experimentally induced testicular damage in rats.

Material and methods

Ethical Considerations

The Ethics Committee of Al-Qasim Green university, reviewed and approved all procedures for this study (Approval Number: qgec/34 /2025).

The experimental animals

Forty albino male rats with weights ranging 150-200 g and 12 weeks of age were used in the experiment. The rats were placed in the animal house of the College of veterinary medicine /University of Al-Qassim Green, with environmental conditions that include moderate temperature, a 12-hour dark and 12-hour light cycle. The animals were treated with the approval of the ethics committee at the department, where they were kept in meshed plastic cages containing sawdust; the pellets were fed (mix of corn, wheat and milk) and they drank tap water throughout the experiment. The animals were left to adapt for 14 days before starting the experiment for the period from December 2024 to February 2025. The study employed forty male albino rats, which were randomly allocated into four groups of ten animals each. One group served as the control, while the remaining thirty rats were distributed across three experimental groups. An intraperitoneal injection of 20 mg/kg phenylhydrazine was applied for two consecutive days and 4th day after the 1st injection, followed by single dose 50 mg\kg triton X100 to 30 male albino rats (11).

Treatments were administered orally via gastric gavage over a 60-day period, with the following group designations:

Control Negative Group (CNG): This group received no treatment and served as the negative control.

Phenylhydrazine Group (PHZ-G): Animals in this group were administered cocktail induced testicular damage and served as the positive control.

Arthrospira platensis Group (SPG): Rats in this group received the administered cocktail induced testicular damage followed by daily oral treatment with spirulina at a dose of 50 mg/kg body weight .

Arthrospira platensis loading chitosan Group (SP-NPS): Animals in this group were administered cocktail induced testicular damage followed by daily oral treatment with chitosan nanoparticle-encapsulated spirulina at a dose of 50 mg/kg body weight. Animals were sacrificed

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24 hours after the last dose administration. Anesthesia was performed by intraperitoneal injection of a ketamine-xylazine mixture (50 mg/kg and 10 mg/kg body weight, respectively) at a total volume of 1.0 ml. Following confirmation of deep anesthesia, approximately 5 ml of whole blood was collected by cardiac puncture using sterile disposable syringes for measuring the levels of LH and Testosterone. The animals were then dissected, and vital organs were harvested for subsequent histopathological and biochemical examinations. Measuring of Rat Malondialdehyde (MDA), and total antioxidant(T-AOC). The above parameters estimated by specific kits used method of Sandwich-ELISA were determined according to (12).

Statistical analysis

The Microsoft Program (SPSS) was used to statistically evaluate the data, and Morgan, Leech (13) showed how the T-test was used to compare the mean of variance at $p<0.05$.

Result

The FESEM image magnification shows chitosan-loaded AP particles with an irregular, aggregated shape and rough surface. Particle sizes range from approximately 40–300 nm, as estimated from the 1 µm scale bar. The surface texture suggests efficient encapsulation and interaction between chitosan and AP.

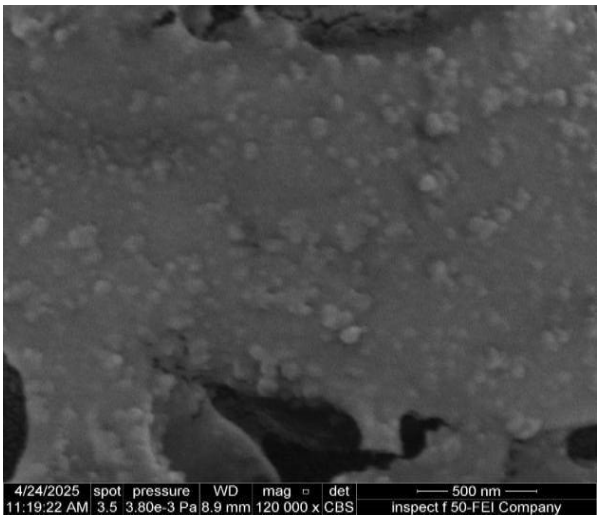

Fig (1): FESEM image magnification less than 100 nm.

Table (1): Effect of Different *Arthrospira platensis* Concentrations on Free Radical Scavenging Activity *In vitro*

Concentration (µg/mL)	% Inhibition (Mean ± SD)	Control (Ascorbic Acid % Inhibition)
25	33.45 ± 1.12	42.87 ± 0.95
50	50.67 ± 1.25	59.34 ± 1.02
100	62.93 ± 1.08	74.21 ± 1.11
200	75.42 ± 1.35	85.10 ± 1.25
400	86.16 ± 0.97	94.87 ± 1.04

- IC_{50} (µg/mL)
- p-value (for statistical comparison)

Total antioxidant capacity (T-AOC)

Our data in (Table 2) appear a significant ($P<0.05$) increase of total antioxidant capacity in treatment group that received CNP-S (77.208±0.4 0.4137) when compared with anemic group PHZ- TD (4.296±0.04 108) also showed significant ($P> 0.05$) differences between normal negative group, *TD-AP extract* a *TD-AP loading chitosan* (6.478 ±0.3602 and 5.286±0.07820).

Table (2): Effect of Different *Arthrospira platensis* on antioxidant and some male hormones

Parameters	CNG	PHZ-TD	*TD-AP extract*	*TD-AP loading chitosan*	*LDS*
TAOC	7.208±0.4137	4.296±0.04108	6.478±0.3602	6.286±0.07B	0.88

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	A	C	A		
MDA	300.5±25 B	620.5± 55A	3540.2±61B	320.7± 20 B	98
LH mIU/mL	3.55 ±0.02 A	1.96 ± 0.02 B	2.87 ± 0.07 AB	2.93 ± 0.069 AB	1.2
Testosterone MIU/mL	1.95 ± 0.02 A	1.31 ± 0.01 C	1.62 ± 0.07 B	1.71 ± 0.02 B	0.29

- The value represents mean ± SE.
- N=10 for each group.
- Different small letters indicated significant ($p \leq 0.05$) among groups.
- *TD- Testicular damage*
- A P - Arthrospira platensis

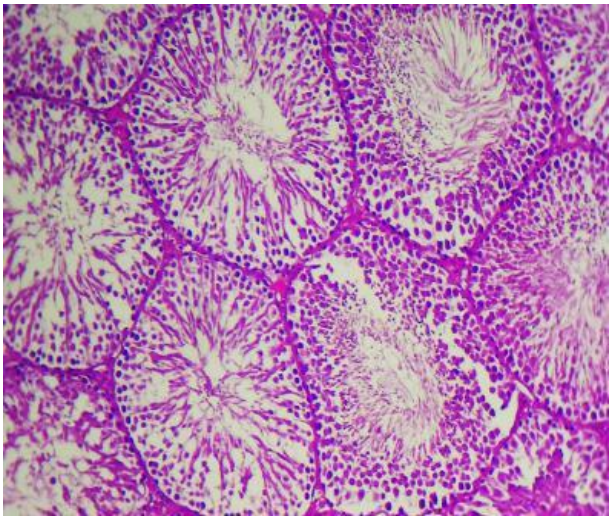

Fig (2): Histopathological partition of testes for control group showing normal histological structures (H&E, stains 200X).

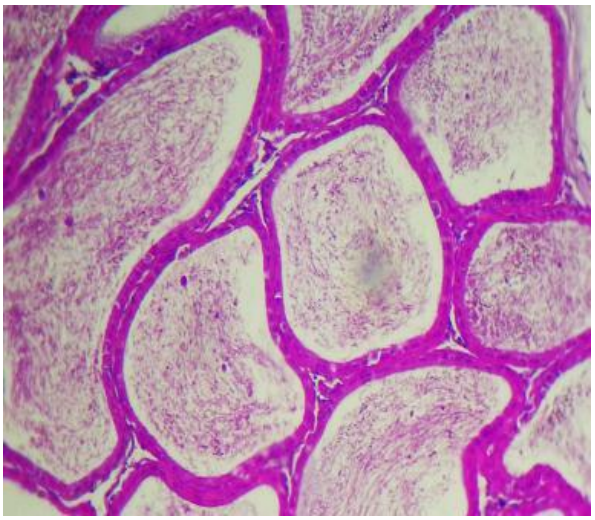

Fig (3): Histopathological partition of epididymis for control group showing normal histological structures (H&E, stains 200X).

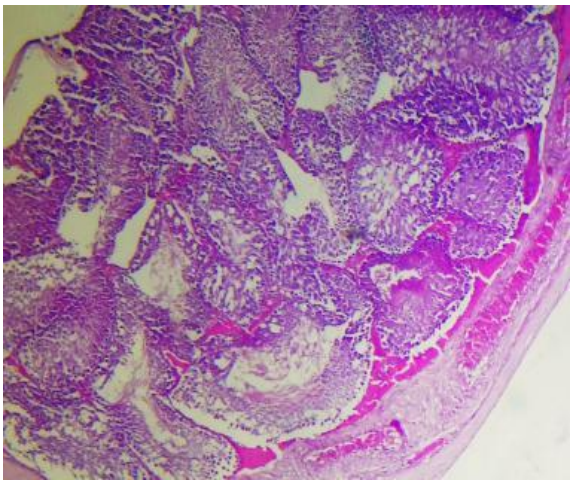

Fig (4): Histopathological partition of testes for control positive group showing thickening od tunica albuginea due to hemorrhage, edam and inflammatory cells infiltration, also necrosis in the seminiferous tubules (H&E, stains 200X).

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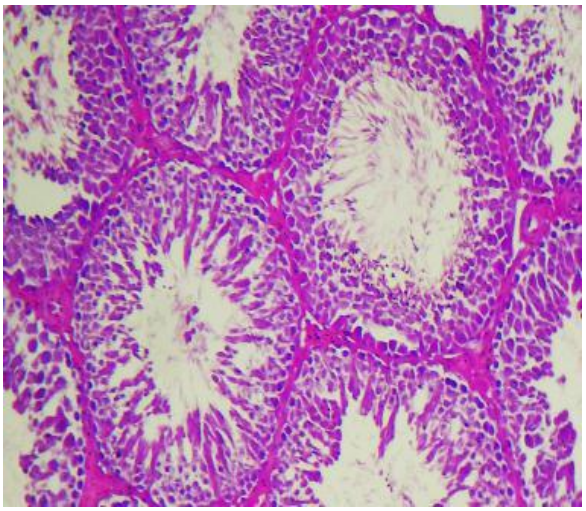

Fig (5): Histopathological partition of testes for TD-AP extract group showing dilation and congestion of blood vessels, with regeneration in some tubules and sloughing in some spermatogenesis cells in other (H&E, stains 200X). inflammatory cells infiltration, also necrosis in the seminiferous tubules (H&E, stains 200X).

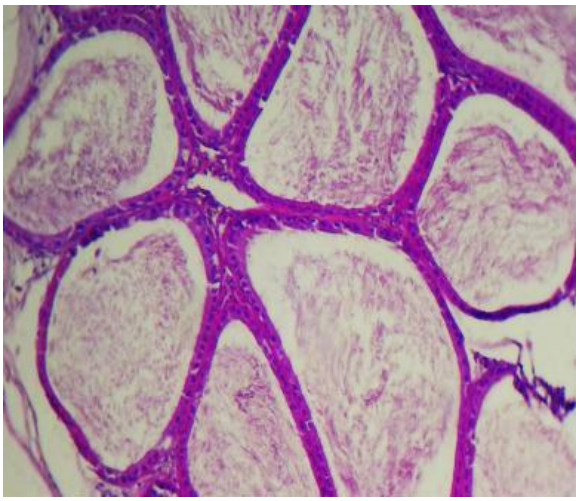

Fig (6): Histopathological partition of epididymis for TD-AP extract group showing irregular distribution of seminal fluid (H&E, stains 200X).

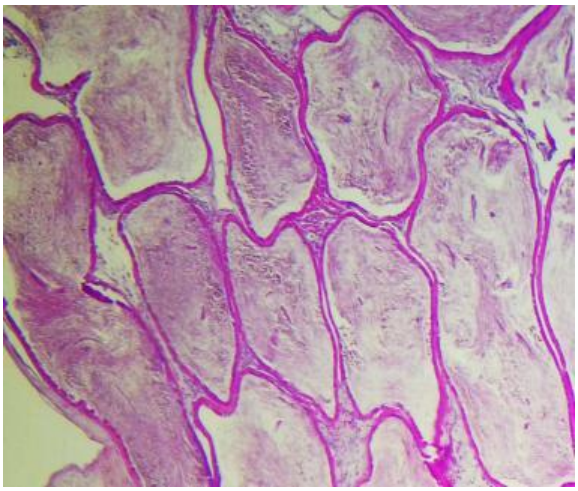

Fig (7:) Histopathological partition of epididymis for nano group showing increased sperm production with regular distribution of seminal fluid (H&E, stains 100X).

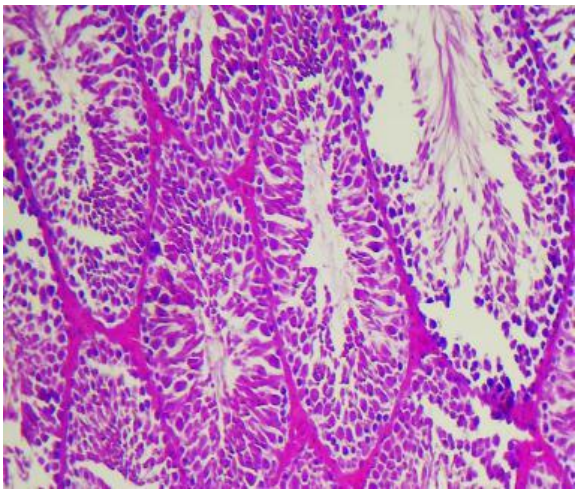

Fig (8): Histopathological partition of testes for nano group showing regeneration of seminaries tubule with increase in spermatogenesis cells (H&E, stains 200X).

Discussion

The current study in table (2) revealed significantly reduced total antioxidant capacity (TAOC) and elevation of TAOC in the ROS groups especially untreated, while *D-AP extract* a *TD-AP loading chitosan* effectively restored TAOC levels. These findings agreement with (14), who reported TAOC enhancement with AP supplementation. The observed antioxidant effects are mediated through different pathways : direct ROS scavenging in renal tissues (15) (16), and hydroxyl radical neutralization ROS and

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depend on number bonds (17). This oxidative stress manifestation was accompanied by a marked decrease in glutathione (GSH) levels was confirmed by PHZ's dual action in simultaneously promoting reactive oxygen species (ROS) generation while depleting endogenous antioxidant defenses.

AP, when loading with chitosan and its components, like phycocyanin, chlorophyll, and various vitamins, scavenge ROS. This result was disagreement with the findings of (18) who noted that the high phenolic compounds in hydro alcoholic extracts of AP might have potent antioxidant activity. On the other hand, antioxidant activity when using DPPH appear a perfect association with quantity of phenol in the different extracts of AP that was also recorded recently by (19).

Herbal medicine traditionally utilized for infertility , herbal preparations are believed to alleviate various reproductive disorders, including sexual weakness, ejaculatory problems, reduced libido, erectile dysfunction, and abnormalities in production of sperm such as azoospermia or and oligospermia (20).

Their exact mechanisms of action in the body remain unclear; therefore, researchers have increasingly turned to the ocean as an abundant source of natural therapeutic agents. The marine environment serves as a vast reservoir of bioactive compounds with significant pharmacological potential. Among these, C-phycocyanin, a biologically active pigment-protein complex extracted and purified from Spirulina platensis, has gained considerable attention (21). Recent study reported that C-phycocyanin has multiple health-promoting characteristics and may play a beneficial role in increase functions of reproductive systems. Recent study by (22) noted Mice pretreated with C-phycocyanin exhibited notable improvements in sperm count and concentration, along with a decrease in sperm malformation rates and enhanced fertility. These findings suggest that C-phycocyanin can protect the male reproductive system and help maintain sperm quality and overall fertility in mice. Low testosterone levels and/or a reduced sperm count are characteristic features of hypogonadism, which can be classified into primary, secondary, or mixed types depending on whether the defect originates within testes, hypothalamic, or pituitary axis (23, 24). Measurement of serum testosterone is considered the most critical diagnostic indicator for male hypogonadism. Patients with primary hypogonadism typically exhibit decreased testosterone levels accompanied by elevated luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, whereas those with secondary hypogonadism present with low testosterone and abnormally low or normal LH and FSH levels (18). In this research, to evaluate the tests function of rats, serum concentrations of androgen, LH were determined. The main active compounds C-Phycocyanin, β-Carotene, γ-Linolenic Acid,Vitamin E and Polyphenols act synergistically to improved Protects germ cells, improves sperm quality, enhances membrane integrity, motility Prevents oxidative membrane damage, Scavenge ROS, and protect Sertoli/Leydig cells (25).

The study concluded that AP and AP-loaded chitosan nanoparticles affect positively as a therapeutic value to treat the induced testicular damage in rats which is done by different pathways via lowering oxidative stress, by Nano-formulation further than improved AP therapeutic efficacy to prescribed in many health problems with higher safety as compared with synthetic drugs.

Conflict of interest: Authors declare no conflict of interest.

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