EFFECT OF SPIRULINA PROTEIN HYDROLYSATE ON MALE REPRODUCTIVE SYSTEM IN CYCLOPHOSPHAMIDE-INDUCED STRESS IN RATS

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Abstract

Cyclophosphamide (CP) is an alkylating agent commonly used in the treatment of various types of cancer. However, despite its effectiveness in treating cancer, it also induces toxicity on non-target cells by generating oxidative stress, particularly in reproductive tissues, leading to fertility. Recently, the consumption of algae-based protein has gained popularity due to its rich of amino acid content and pharmacological properties. Thus, the objective of this study was to determine the protective effects of protein hydrolysate (PS) derived from *Arthrospira platensis* on the male reproductive system of CS-induced stress in rats. Thirty-six male Wistar rats were randomly divided into four groups. Groups 1 and 2 served as the normal control and negative control groups and received distilled water orally, whereas groups 3 and 4 were treatment groups that received PS orally at doses of 3 and 6 mg/kg, respectively. Oral treatments were administered for 14 consecutive days. On day 7 and 14 of the experiment, rats in groups 2-4 were intraperitoneally injected with CP at a dose of 200 mg/kg. After the treatment period, reproductive organ weights, sperm quality, and reproductive organ histopathology were evaluated. The results showed that CP has negatively effects on the structure and function of male reproductive organs of rats. Significant increases ($p<0.05$) in testicular and epididymal weights, slight increases in prostatic and seminal weights, decreases in sperm concentration, motility, and viability, along with a significant increase in sperm morphological abnormalities and histopathological alterations in the reproductive organs, were observed in the negative control group compared to the normal control group. The treatment of PS at both doses could restored these damages. In conclusion, protein hydrolysate from A. *platensis* at doses of 3 and 6 mg/kg could protect against damage to male reproductive organs caused by CP-induced stress in rats.

Introduction

Nowadays, daily activities such as smoking, drinking alcohol, eating fast food, breathing in air pollution, and taking certain drugs can increase the risk of health problems. Cyclophosphamide (CP) is an alkylating agent widely used as an immunosuppressive drug for treating various types of cancer. In target cells, CP is converted into acrolein and phosphoramide. These metabolites inhibit cancer cell division and induce apoptosis by forming crosslinks in DNA strands and generating reactive oxygen species. Although CP effectively inhibits cancer cells, it also has severe cytotoxic effects on various normal cells, particularly those in the male reproductive system, leading to infertility. Previous research has reported that CP induces testicular dysfunction by altering the hypothalamic-pituitary-gonadal axis and disrupting testicular steroidogenesis. Male rats treated with CP showed reduced testicular weight, as well as lower levels of testosterone, follicle-stimulating hormone, and luteinizing hormone, compared to normal rats [1]. Furthermore, CP disrupts the normal structure of testicular tissues. Rats treated with CP exhibited detached germinal epithelium, irregular outlines of the germinal epithelium, and empty seminiferous tubule lumens. Moreover, CP significantly reduced sperm concentration, viability, and normal morphology in mice [2].

Recently, there has been growing interest in using microalgae, particularly *Arthrospira platensis* (spirulina), as a nutritious food source and health-promoting supplement. This blue-green alga is rich in proteins, vitamins, minerals, carotenoids, and Omega-3 fatty acids. The bioactive properties of spirulina protein hydrolysates include antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. However, the efficacy of protein hydrolysates varies depending on the culture conditions and the hydrolysis technique used. Therefore, the aim of this research was to study the effects of protein hydrolysate (PH) from *Arthrospira platensis*, developed by the Algal and Cyanobacterial Research Laboratory at Chiang Mai University, on the reproductive system of male rats subjected to cyclophosphamide-induced stress.

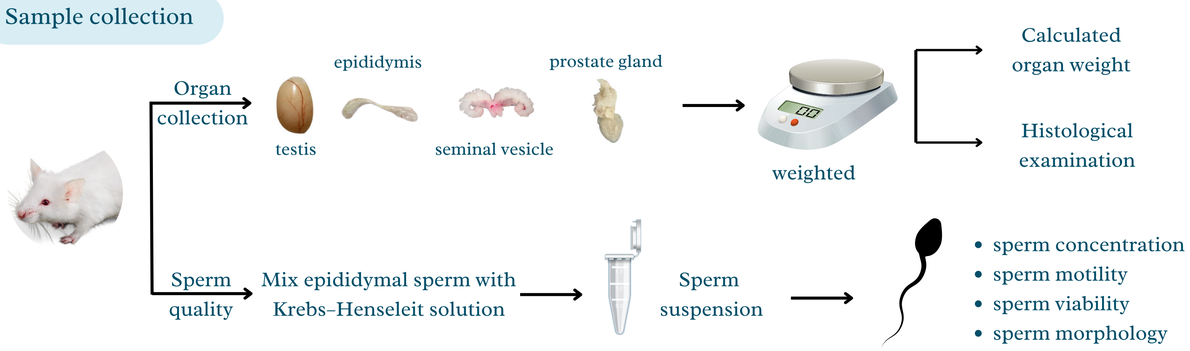
Material and Methods

- Male Wistar rats

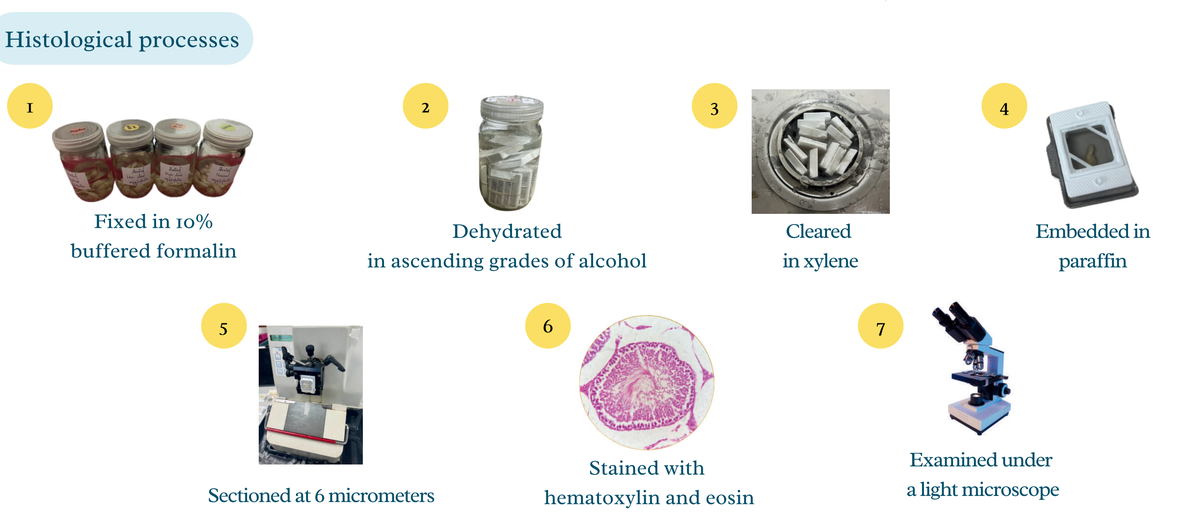
CP : cyclophosphamide
PH : protein hydrolysate

Groups		intraperitoneal injection	oral treatment	volume
Group 1	Normal control (NC)	85 % normal saline	distilled water	1 ml
Group 2	Negative control (CP)	CP 200 mg/kg	distilled water	1 ml
Group 3	CP+PH 3 mg/kg BW	CP 200 mg/kg	PH 3 mg/kg BW	1 ml
Group 4	CP+PH 6 mg/kg BW	CP 200 mg/kg	PH 6 mg/kg BW	1 ml

Sample collection



Histological processes



Results

Table 1 Relative weight of reproductive organ and sperm parameter.

Parameters		Normal control	Negative control	Low dose	High dose
Weight index	Testis (g/100g BW)	0.43 ± 0.02^{c}	0.57 ± 0.05^{a}	0.46 ± 0.05^{bc}	0.49 ± 0.03^{b}
	Epididymis (mg/100g BW)	0.13 ± 0.01^{c}	0.18 ± 0.02^{a}	0.16 ± 0.02^{b}	0.15 ± 0.01^{b}
	Prostate gland (g/100g BW)	0.14 ± 0.02^{a}	0.16 ± 0.03^{a}	0.21 ± 0.07^{a}	0.15 ± 0.01^{a}
	Seminal vesicle (g/100g BW)	0.28 ± 0.05^{a}	0.37 ± 0.20^{a}	0.23 ± 0.04^{a}	0.33 ± 0.09^{a}
Sperm concentration		47.35 ± 3.75^{a}	45.83 ± 6.49^{a}	49.50 ± 2.25^{a}	51.31 ± 3.27^{a}
Sperm motility	Progressive	26.81 ± 3.77^{a}	13.88 ± 6.27^{c}	13.91 ± 4.64^{c}	20.88 ± 3.12^{b}
	Non-progressive	11.59 ± 1.75^{a}	12.04 ± 5.43^{a}	7.59 ± 2.55^{b}	6.97 ± 1.53^{b}
	Immotile	11.28 ± 4.12^{c}	24.08 ± 7.70^{ab}	28.44 ± 4.87^{a}	22.16 ± 3.86^{b}
	Circular	0.31 ± 0.32^{a}	0.00 ± 0.00^{b}	0.06 ± 0.12^{b}	0.00 ± 0.00^{b}
Sperm viability	Alive	74.03 ± 14.39^{a}	39.55 ± 14.42^{b}	70.31 ± 6.47^{a}	79.34 ± 7.64^{a}
Sperm morphology	Normal	64.13 ± 9.97^{a}	56.83 ± 1.04^{a}	57.50 ± 4.95^{a}	53.75 ± 1.55^{a}
	Head abnormality	0.63 ± 0.63^{ab}	1.83 ± 0.76^{a}	0.38 ± 0.48^{ab}	0.63 ± 0.95^{b}
	Tail abnormality	35.25 ± 9.56^{a}	41.33 ± 1.26^{a}	41.88 ± 4.84^{a}	39.38 ± 1.18^{a}
	Head and tail abnormality	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.25 ± 0.29^{a}	0.25 ± 0.29^{a}

Values are expressed as mean ± SD. The sapeascript letters indicate statistically significant differences ($p<0.05$) between the experimental groups. (Duncan test)

Testes

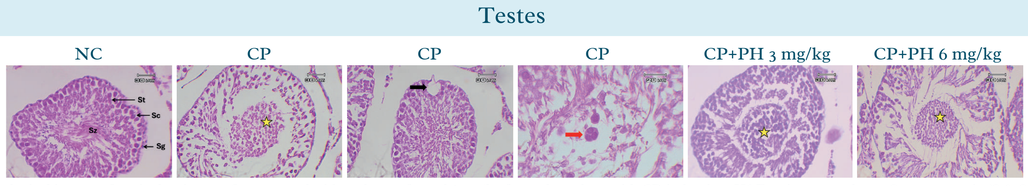


Figure 1. Histological images of testicular tissues of rats treated with at doses of 3 and 6 mg/kg for 14 days along with CP-induction. H&E stain, 20x and 40x. Spermatogonia (Sg), Spermatocyte (Sc), Spermatid (St), Spermatozoa (Sz), Exfoliation germ cell (yellow star), vacuolization (black thick arrow), Multinucleated giant cell (red thick arrow).

Epididymis

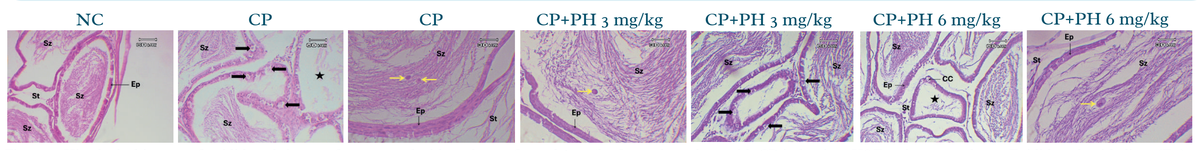


Figure 2. Histological images of epididymal tissues of rats treated with at doses of 3 and 6 mg/kg for 14 days along with CP-induction. H&E stain, 10x and 20x. Epididymal epithelium (Ep), Stroma (St), Spermatozoa (Sz), Clear cell (cc), Clear cell hyperplasia (black thick arrow), Decrease spermatozoa (black star), Germ cell sloughing (yellow thin arrow).

Prostate gland

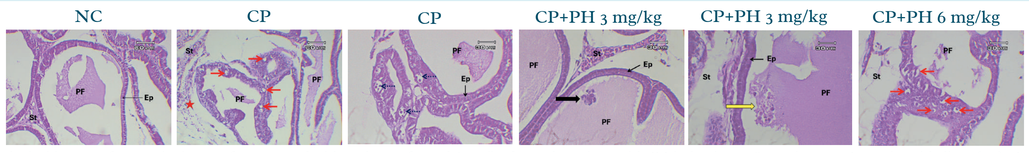


Figure 3. Histological images of prostatic tissues of rats treated with at doses of 3 and 6 mg/kg for 14 days along with CP-induction. H&E stain, 20x. Prostatic epithelium (Ep), Prostatic fluid (PF), Stroma (St), Vacuolization (red thin arrow), Leucocyte infiltration (red star), Apoptosis (blue dotted arrow), White blood cell (yellow thick arrow), Germ cell sloughing (black thin arrow)

Seminal vesicle

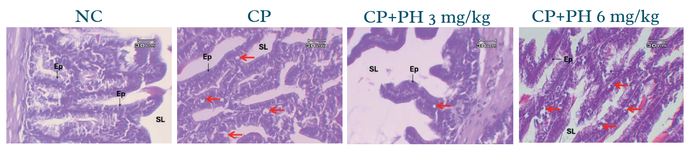


Figure 4. Histological images of seminal tissues of rats treated with at doses of 3 and 6 mg/kg for 14 days along with CP-induction. H&E stain, 20x. Seminal epithelium (Ep), Seminal fluid (SL), Vacuolization (red thin arrow).

Conclusion

The protein hydrolysate from Spirulina, at doses of 3 and 6 mg/kg, administered to rats for 14 days, was able to reduce the weight of the testes and epididymis induced by CP. It also increased sperm concentration, motility, and viability, while reducing morphological abnormalities, with a more significant effect observed in the high-dose group. However, it did not affect the weight of the seminal vesicles and prostate gland. Additionally, it helped restore structural changes of the testes, epididymis, seminal vesicles, and prostate gland of CP-induced rats.

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References

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