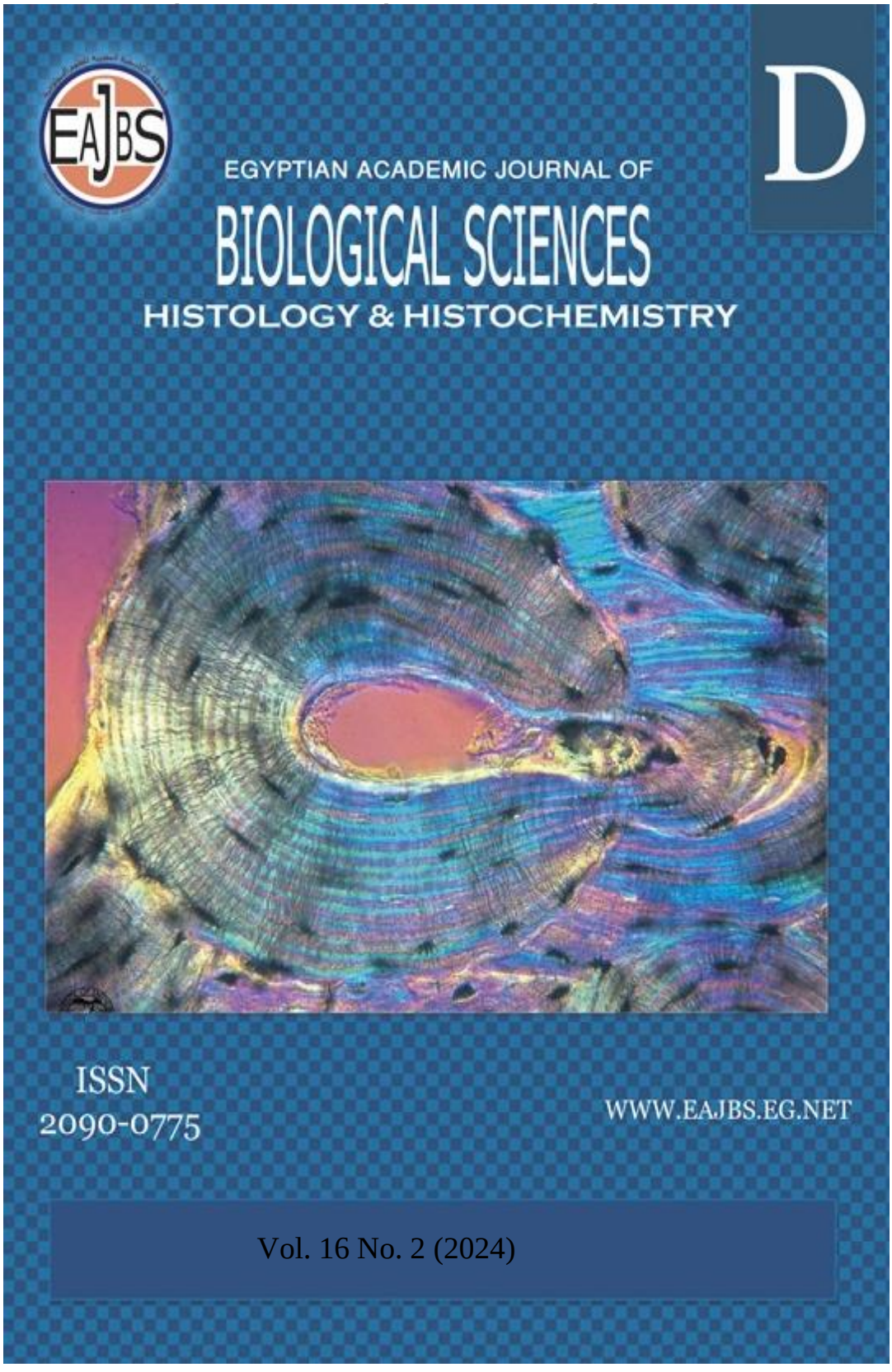

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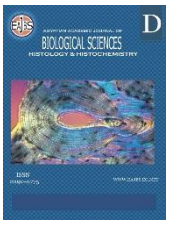

Histological Analysis of White Pulp of Spleen Due to Supplementation of Coenzyme Q10 in Immunocompromised Adult Female Albino Wistar Rats

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ABSTRACT

Aim: The present study was conducted to determine the immune-enhancing effects from supplementation of Coenzyme Q10 on a cyclophosphamide-induced immunosuppressed rat model. Study Design & Setting: Quasi experimental study design was selected. The Study was placed at Animal House, Husbandry and Veterinary Sciences, Sindh Agricultural University (SAU) Tandojam and Post Graduate lab, Isra University from November 2020-April 2021.**Materials & Methods:** 40 adult healthy female albino Wistar Rats were selected and placed in 4 groups (n=10/group). Food and water were provided ad libitum. Control group A rats were given corn oil (100ul) intraperitoneally from Day 1-7. Experimental group B rats were given corn oil (100ul) intraperitoneally from Day 1-7 as a placebo followed by single dose of cyclophosphamide (200mg/kg) intraperitoneally on Day 8. Pre-treatment group C animals were given Co-enzyme Q10 (300mg/kg) dissolved in corn oil (100ul) intraperitoneally from Day 1- 7 followed by a single dose of cyclophosphamide (200mg/kg) intraperitoneally on Day 8. A Single dose of 200mg/kg of cyclophosphamide was initially given intraperitoneally to Post-treatment group D animals followed by Co-enzyme Q10 (300mg/kg) dissolved in corn oil (100ul) intraperitoneally from Day 2-8. On Day 9, animals were weighed and sacrificed. Spleen was preserved and prepared for light microscopy. **Results:** Graded white pulp atrophy was observed in animals in Groups B, C & D. Marked effectiveness of Coenzyme Q 10 was observed in animals of the pretreatment group. **Conclusion:** It can be concluded that Coenzyme Q10 Supplementation in the Pre-treatment Group displayed marked effectiveness in immunocompromised adult female Wistar albino rats.

INTRODUCTION

Immunology plays a critical role in defense against organisms from harmful microbes. Malignancies and infections have been one of the leading causes of immunosuppression (Dunkelberger & Song WC, 2010) (Haddad, Azar, Groom, & Boivin, 2005). Thus, it is essential for us to search for therapies that boost our immunological system in immunodeficient diseases.

Cyclophosphamide (CP), an immunosuppressive agent was introduced in a rodent model for immunosuppression (Ahmed & Hombal, 1984) (Noh, Kim, Lee, Song, Joung, & Yang, 2019). In 1995, cyclophosphamide was licensed as a chemotherapeutic agent for organ transplantation, nephrotic syndrome, multiple myeloma, leukemia, and lymphoma along with several malignancies related to ovaries, breasts and lungs.

These health conditions are treated by cyclophosphamide through its immunosuppressive action (Brayfield, 2017). Its mechanism of action reveals inhibition of the replication of DNA through the addition of alkyl group thus interrupting its cell cycle from dividing and replicating (Huyan, Lin, & Gao, 2011). Adverse effects related to cyclophosphamide are leucopenia, lymphocytopenia, nausea, vomiting, alopecia, hemorrhagic cystitis, infertility and pulmonary fibrosis (Cengiz, 2018).These adverse consequences are because reactive oxygen species (ROS) enable the destruction through oxidative stress (Elshater, Haridy, & Salman, 2018).Coenzyme Q10, a component of the electron transport chain, is present abundantly in mitochondria, particularly in the brain, heart and muscles (Tóth, *et al.,* 2017) (Kumar, *et al.,* 2010). As cells are particularly susceptible to the harmful consequences of oxygen free radicals, Coenzyme Q10 has a significant preventive antioxidant effect. The Quantity of Coenzyme Q10 diminishes as a result of aging, heredity, and the use of statins (Littaru & Langsjoen, 2007). Coenzyme Q10 is lipophilic in characteristic and is produced from the polymerization of a benzoquinone ring with a hydrophobic chain of isoprenoids, all in trans configuration with a double bond (Littaru & Langsjoen, 2007).The third most widely utilized dietary supplement Coenzyme Q10 can also cure non-communicable illness (Marta, Suñé-Negre, & García-Montoya, 2020). Diminished levels of Coenzyme Q10 cause fibromyalgia, cancer, diabetes and neurodegenerative diseases (Garrido-Maraver, Cordero, & Oropesa-Avila, 2014). Coenzyme Q10 levels in blood are normally between 0.7 to 1.0 μg/mL (Villalba, Parrado, Santos-Gonzalez, & Alcain, 2010). The rationale of the present study was to determine the immune-enhancing outcomes from supplementation of Coenzyme Q10 on a cyclophosphamide- induced immunosuppressed rat model.

MATERIALS AND METHODS

Experimental Design:

Quasi-experimental design was selected for this study. The study was conducted in Animal House, Husbandry and Veterinary Sciences, Sindh Agricultural University (SAU) Tandojam and Post Graduate lab, Isra University. The Sample size was calculated by using formula E= total number of animals / number of groups.40 adult female Albino Wistar Rats of 8-12 weeks old weighing around 180-250 g were taken and placed into 4 Groups (n=10/group). Non- Probability Purposive sampling technique was used. Adult female rats weighing 180-250g without any gross abnormality were included in this study. Morbid, underweight and pregnant rats were excluded. The Group A (Vehicle control group) (n=10) animals were given corn oil (100ul) (Xu, Lu, Dong, Shapoval, Soriano, & Liu, 2017) intraperitoneally till day 7. Group B (Experimental group) (n=10/group) animals were administered corn oil (100ul) intraperitoneally from Day 1-7 as a placebo followed by a single dose of cyclophosphamide (200mg/kg) intraperitoneally on Day 8 of study. Group C (Pre-treatment group): 10 Rats were administered Coenzyme Q10 (300mg/kg) dissolved in corn oil (100ul) intraperitoneal for Days 1- 7 followed by a single dose of cyclophosphamide (200mg/kg) (Olama, Taha, & Rady, 2018) intraperitoneally on Day 8 of study. Group D (Post-treatment group) 10 rats were administered a single dose of 200mg/kg of cyclophosphamide intraperitoneally on Day 1 followed by Coenzyme Q10 (300mg/kg) (Olama, Taha, & Rady, 2018) dissolved in corn oil (100ul) intraperitoneal from Day 2- 8. On Day 9, the final body weight of rats was calculated and noted. Animals were sacrificed by cervical dislocation.

Light Microscopy:

Spleen were removed, weighed and fixed in 10% formalin solution. Tissue was stained by haematoxylin and

eosin for any observable histological changes under light microscope.

Statistical Analysis:

Collected data was analyzed using SPSS version 21. Mean ± standard deviation was calculated for frequency distribution. One-Way ANOVA was applied for comparison of variables among groups.

RESULTS

General Observation:

In the study, all rats survived the duration of the experiment. Results of body weight, weight of spleen and histological features of white pulp of spleen of animals in different groups were compared.

Body Weight and Weight of Spleen:

Difference in initial body weight of animals in all study groups was found to be statistically insignificant (p value 0.702), while final body weight of animals placed in Group B, C & D was found to be decreased in comparison to animals placed in control group A ($p<0.05$) as being shown in Table 1. Average weight of spleen was 373.3 mg in Group A, 289.09 mg in Group B, 301.00 mg in Group C and 359.00 mg in Group D. The findings were statistically insignificant according to the study groups' p value 0.113. Post hoc analysis of weight of spleen also displayed insignificant results among groups ($p>0.05$) as shown in Table 2.

Table 1: Comparison of initial and final body weight of rats placed in different groups**.**

Groups	Initial body weight (g)		F-value	p-value
A vs B	197.9±3.14	197.30±7.18	0.474	0.991
A vs C	197.9±3.14	199.20±1.31		0.893
A vs D	197.9±3.14	198.90±1.52		0.847
B vs C	197.30±7.18	199.20±1.31		0.726
B vs D	197.30±7.18	198.90±1.52		0.658
C vs D	199.20±1.31	198.90±1.52		1
	Final body weight (g)			
A vs B	193.70±5.43	128.09±34.38	12.452	0.0001
A vs C	193.70±5.43	131.00±27.83		0.0001
A vs D	193.70±5.43	130.20±29.88		0.0001
B vs C	128.09±34.38	131.00±27.83		0.995
B vs D	128.09±34.38	130.20±29.88		0.998
C vs D	131.00±27.83	130.20±29.88		1

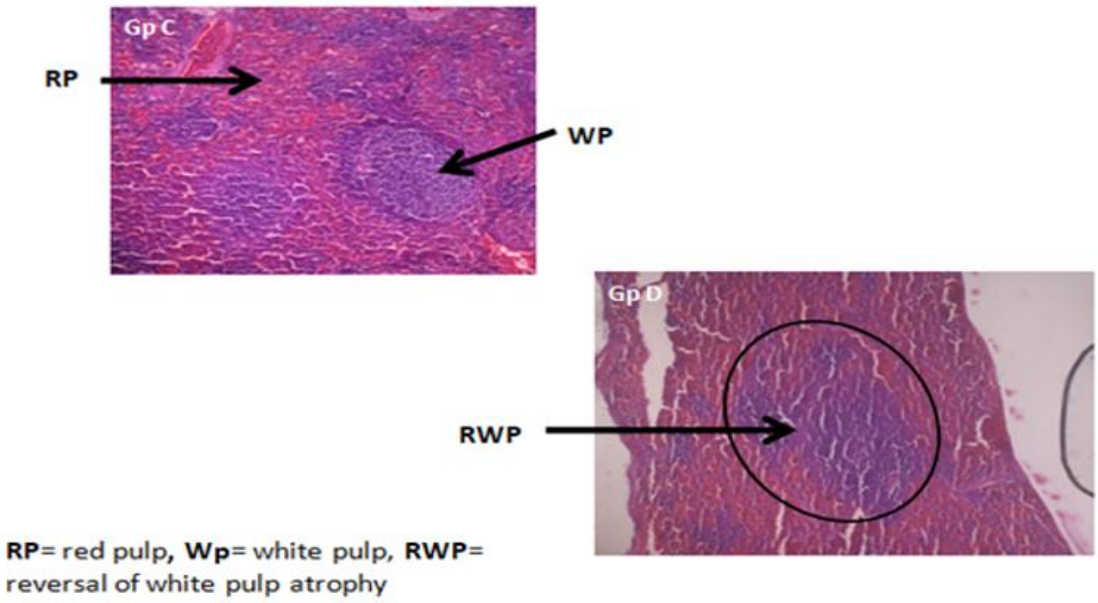
Table 2: Comparison of weight (mg) of spleen in different groups (n=40) where p value < 0.05**.**

Groups	Spleen weight (mg)		F-value	p-value
A vs B	373.33±86.31	289.09±69.49	2.135	0.179
A vs C	373.33±86.31	301.00±106.92		0.315
A vs D	373.33±86.31	359.00±95.27		0.986
B vs C	289.09±69.49	301.00±106.92		0.99
B vs D	289.09±69.49	359.00±95.27		0.302
C vs D	301.00±106.92	359.00±95.27		0.484

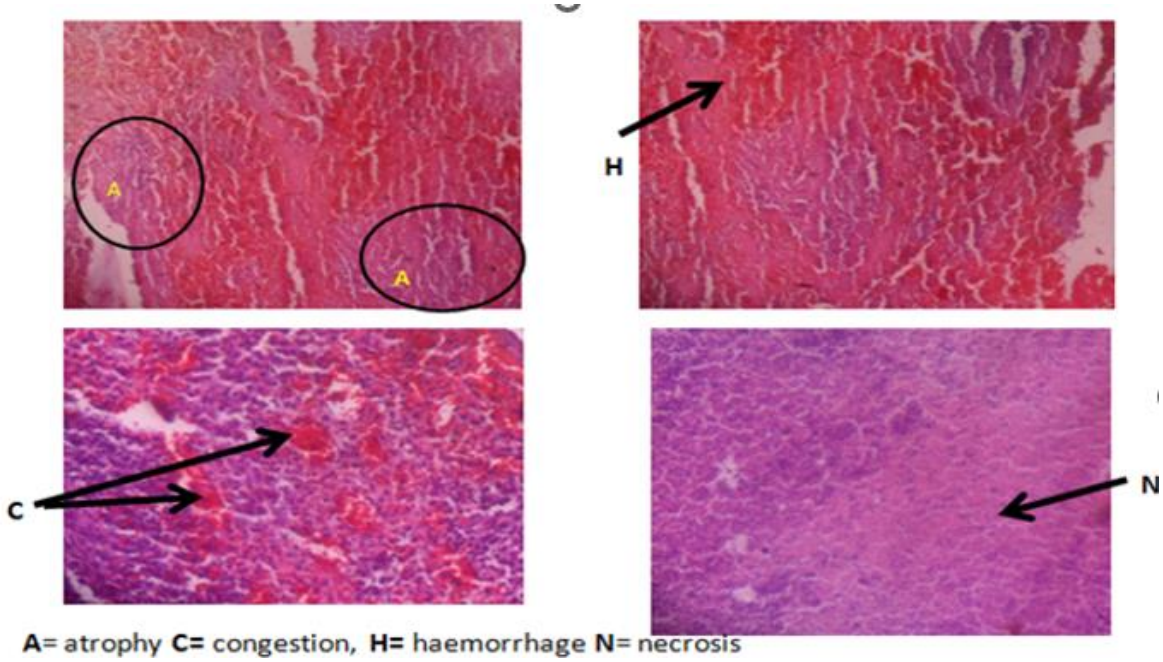
Light Microscopy:

Under a light microscope, the spleen tissue of animals in control group A was found to be normal. Mild white pulp atrophy was observed in 3 animals placed in group B, 2 animals of Group C and Group D respectively as seen in photomicrograph 1.

The white pulp of the spleen of 05 animals in group B displayed moderate atrophy whereas 3 animals of group D had white pulp atrophy. The Spleen of two animals placed in Group B displayed severe atrophy. Moderate to severe white pulp atrophy was significantly higher in animals in Group B (p value 0.001) as displayed in Table 3 and seen in photomicrograph 2.



Photomicrograph 1. Showingparenchyma of spleen of female albino Wister rats Gp C&D under H&E x200.



Photomicrograph 2.Photomicrograph 1. Showing parenchyma of spleen of female albino Wister rats Group B under H&E x200.

Table 3 shows histological changes in white pulp of spleen among different groups (n=10/group).

		Animal Groups					
		A	**B**	**C**	**D**	**Chi- value**	**p- value**
White Pulp atrophy	Normal	10	0	8	5	27.584	0.0001
		100%	0%	80%	50%		
	Mild	0	3	2	2		
		0%	30%	20%	20%		
	Moderate	0	5	0	3		
		0%	50%	0%	30%		
	Severe	0	2	0	0		
		0%	20%	0%	0%		
Total		10	10	10	10		
		100%	100%	100%	100%		

DISCUSSION

In the present study, histological analysis revealed highly significant white pulp atrophy in animals of Group B which was found to be consistent with the study conducted by Qi Q, *et al.,* 2018 and Park Hr *et al.,* 2023 showing Cyclophosphamide induced atrophy of immune organs (Qi *et. al.,* 2018) (Yoo, Lee, Ku, & Lee, 2020).However, these atrophic changes might be associated with the reduction of lymphoid cells specifically T cells (Park, *et al.,* 2023).

Cyclophosphamide is known for causing atrophy, and weight loss of lymphoid organs along with an imbalance of leukocytes in mice blood which ultimately impairs immunity (Chen, *et al.,* 2006) (Tanahashi, *et al.,* 2017). In the present study supplementation of CoQ10 in Pre-treatment Group C showed typical splenic parenchyma which might be a result of activated natural killer cells moreover, natural killer cells have been recommended as a therapeutic approach for cancer therapy and immunosuppressive disorders (Tanahashi, *et al.,* 2017) (E, S, & K, 2004).

Farsi *et al.,*2019 also co-relates with the present study that CoQ10 decreases inflammatory mediator levels (Farsi, *et al.,* 2019) by decreasing MDA and MPO levels thus minimizing free radicals and lipid peroxidation (Sakat, *et al.,* 2018). Studies also reveal that supplementation with Coenzyme Q10 reduces plasma levels of C-reactive protein, IL-6 and TNF which supports CO-Q10 as anti-apoptotic, and anti-inflammatory effects (Heidari, *et al.,* 2018).

Coenzyme Q10 as an antioxidant decreases NADPH oxidase retards NO generation and defends against DNA damage and protein oxidation (Ratliff, *et al.,* 2016). Treatments with Coenzyme Q10 raise mitochondrial number decrease oxidative stress efficiently and reduce mitochondrial dysfunction. Study conducted by Sherif IO *et al.,* 2018 also concluded that the use of antioxidant supplementation protects CP-induced toxicity (Sherif, 2018)

CONCLUSION

In the current study, it can be concluded that histological comparison of normal splenic parenchyma exhibited noticeable outcomes with Pre-treatment over Post-treatment Group with Coenzyme Q10. Hence it can be stated that supplementation of Coenzyme Q10 in the Pre-treatment Group displayed marked effectiveness. These findings help us understand the role of Co enzyme Q 10 supplementation prior to the start of any chemotherapeutic treatment.

Declarations:

Ethics Approval: Ethical approval was obtained from the Ethical Committee of Isra University Hyderabad. Consent from participants is not applicable as it is an animal study.

Conflict of Interest: There is no conflict of interest among authors.

Author Contribution: Syna P. Singha, conceived the study and

designed the experiment and revised the manuscript for intellectual content. Rida Qureshi, collected the data and wrote the initial draft of the manuscript. Amir D. Isaac, performed statistical analysis, interpreted the results. Abroo F. Qazi , provided critical revisions and editing.

All authors reviewed and approved the final manuscript.

Data Availability Statement: The collection of data developed and/or assessed throughout the present work is available through the corresponding author upon reasonable request.

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