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Cytoprotective Role of Co-Enzyme Q10 on the Spleen of Immunosuppressed Female Albino Wistar Rats

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Author`s Contribution

[1,2,4]Substantial contributions to the conception or design of the work; or the acquisition, [3,4]Active participation in active methodology, [1,3]analysis, or interpretation of data for the work, [2]Drafting the work or revising it critically for important intellectual content

Funding Source: None
Conflict of Interest: None

Received: Feb 26, 2024
Accepted: Oct 7, 2024

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ABSTRACT

Objective: To assess the effects of Co-enzyme Q10 supplementation on the histology of the spleen in immunocompromised female Albino Wistar rats.

Methodology: A quasi-experimental study was conducted between July 2021 and December 2021 at the Anatomy Department of Isra University Hyderabad. Forty female adult Albino Wistar rats (n=10/group) were included in the present study, divided into 4 groups where A was kept as vehicle control, B as experimental group, C as pre-treatment group, and D was kept as post-treatment. All drugs were administered through the intraperitoneal route. On Day 09, animals were sacrificed through cervical dislocation and dissected. Spleen was carefully dissected from each animal and washed thoroughly with distilled water, and tissue was fixed in 10% formalin and processed for light microscopy.

Results: On the histopathological findings, the moderate and severe white pulp atrophy was significantly higher in group B (p-0.001). All the animals of control group A were without haemorrhagic findings, while mild haemorrhage was seen in 3 animals of group B, 3 animals in group C and 3 animals of group D. Moderate haemorrhage was seen in 5 animals of group B and 2 animals of group D, while severe haemorrhage was seen only in 2 animals of group B. The moderate and severe haemorrhage was significantly higher in group B (p-0.001). Furthermore, the moderate and severe necrosis was significantly higher in group B (p-0.001).

Conclusion: The histological changes, including white pulp atrophy, haemorrhage, and necrosis, were most frequently observed in the spleens of immunosuppressed female rats treated with cyclophosphamide. A significant cytoprotective effect of Coenzyme Q10 was noted in these rats, as spleen histology showed marked improvement in those treated with Coenzyme Q10 alongside cyclophosphamide, regardless of whether Coenzyme Q10 was administered before or after the start of cyclophosphamide treatment.

Keywords: Immunocompromised spleen, Co-enzyme Q10, Histology, Rats.

Cite this article as: Qureshi R, Memon ZA, Sangha SP, Khaskheli LB, Bano S, Naz K. Cytoprotective Role of Co-Enzyme Q 10 on the Spleen of Immunosuppressed Female Albino Wistar Rats. Ann Pak Inst Med Sci. 2024;20(Suppl. 2):834-838. doi: 10.48036/apims.v20iSuppl.2.1278.

Introduction

A life devoid of immune system is unimaginable. Immunological system responds to environmentally hazardous substances. Immune tissue establishes resistance towards infectious agents.[1-3] In recent decades, immunological system related disorders are spreading globally. Multiple international researches have suggested that rise in these types of disorders is due to compromised defense of human body leading to

mortality.[4,5] Spleen is the largest peripheral lymphoid organ which accomplishes multiple tasks such as purification of blood, elimination of debris and defense against pathogens.[6-8] Human splenic parenchyma consists of red and white pulp[9,10] and it shares similar anatomy to murines.[11] Splenic red pulp possesses splenic cords and sinuses whereas white pulp mainly has lymphocytes B and T. [11] Cyclophosphamide, a frequently prescribed medication was evolved in 1950s as an alkylating agent.[12]

Cyclophosphamide disrupts into active metabolite nitrogen mustard which later disintegrates the composition of DNA leading to cell death.[13] Production of free radicals through Cyclophosphamide results in oxidative stress and DNA damage at cellular level.[14] Therefore, cyclophosphamide is known as an immunosuppressant agent. [15]Cyclophosphamide further disrupts bone marrow cells which are non-malignant cells. Hence, it is essential to explore remedies that might be included in chemotherapy for the prevention of immunosuppressive diseases.

CoQ10 is considered as a frequently taken drug for a variety of factors. According to the National Health Industry Survey conducted in 2012, 1.3% of Americans used CoQ10 supplementations.[16] Coq10 a lipophilic antioxidant is naturally synthesized in the body.[17] Co-enzyme Q10 is also referred as ubiquinone due to the fact that it has quinone ring consisting of a benzoquinone ring, containing 10 subunits in humans moreover joining 6 and 10 subunits in variety of animal breed plus it is present in mammals.[19] Synthesis of reactive oxygen species from mitochondrial respiratory chain is age-related and these reactive oxygen species chiefly attacks the mitochondrial DNA.[18] Production of CoQ10 diminishes with age and its supplementation enhances its level and is beneficial for health.[20] Circumstances related to the health of the person concerned with supplementation of Co-Q10 are currently an essential requirement in health and sciences. Current study evidence on CoQ10 supplementation has primarily concentrated on cardiovascular health and the diseases of mitochondria, with few studies investigating its impact on immune-linked organs, particularly in immunocompromised patients. Although certain studies suggest that CoQ10 has immunomodulatory and protecting consequences, there is no histological evidence of its effect on spleen tissue in immunocompromised patients. This information gap underscores the importance of investigating the specific impact of CoQ10 on spleen histology in order to determine whether it may help retain or restore normal tissue structure and function in immune-challenged situations.

Methodology

A Quasi-experimental study was conducted at Isra University Hyderabad from March 2021 to Sept 2021. 40 female adult Albino Wistar rats (n=10/group) were included in the present study. Animals were divided into 04 groups where A was kept as Vehicle control, B as Experimental group, C as Pre-treatment group and D was kept as Post-treatment. The drug regime was introduced as follows. Group A (vehicle control) rats were given corn oil (100ul) for 7 days. Group B (experimental group) rats were given corn oil (100ul) for 7 days as a placebo and a single dose of cyclophosphamide (200mg/kg) was given on Day 08 of study. Group C (pre-treatment group) rats were given co-enzyme Q10 (300mg/kg) dissolved in corn oil (100ul) for 7 days and a single dose of cyclophosphamide (200mg/kg) was given on Day 08 of study. Group D (post-treatment group) rats were administered with a single dose of cyclophosphamide (200mg/kg) on Day 01 followed by Co-enzyme Q10 (300mg/kg) dissolved in corn oil (100ul) till Day 08. All drugs were administered through intra-peritoneal route.

On Day 09, animals were sacrificed through cervical dislocation and dissected. A careful dissection of spleen was done in each animal; surrounding tissues were cleaned with distilled water and immediately were fixed in the 10% neutral-buffered formalin for at least 24 hours to preserve tissue integrity. Gross morphometric parameters, i.e. length, weight and any gross abnormalities were recorded. Tissues were fixed in 10% formalin and processed for light microscopy. Tissues of the spleen were dehydrated in a graded series of ethanol, cleared in the xylene, and were embedded in the paraffin wax H&E staining is performed on the mounted sections observe general histological architecture and cellular integrity. A self-made proforma was used for data collection. Data was analyzed using SPSS version 22. For comparison between groups, one way- ANOVA followed by Post hoc Tukey's analysis was applied. P-value ≤ 0.05 was considered as significant.

Results

In this study, 40 female Albino Wistar rats were divided into four groups (n=10 per group): control group A, experimental group B, pre-treatment group C, and post-treatment group D. According to white pulp atrophy, in control group A, all animals (100%) showed normal

white pulp with no atrophy. In experimental group B, 30% of animals exhibited mild atrophy, 50% moderate atrophy, and 20% severe atrophy. In pre-treatment group C, 80% of animals had normal white pulp, 20% had mild atrophy, and none showed moderate or severe atrophy. In post-treatment group D, 50% displayed normal white pulp, 20% had mild atrophy, and 30% moderate atrophy, with none showing severe atrophy. These findings indicate a significant protective effect of Coenzyme Q10 in reducing white pulp atrophy (p = 0.0001). (Table I**)**

Table I: White pulp atrophy comparison among study groups (n=40)

Histology (White Pulp Atrophy)	Animal group				p-value
	A	B	C	D	
Normal	10	0	8	5	0.0001
	100.0%	0.0%	80.0%	50.0%	
Mild	0	3	2	2	
	0.0%	30.0%	20.0%	20.0%	
Moderate	0	5	0	3	
	0.0%	50.0%	0.0%	30.0%	
Severe	0	2	0	0	
	0.0%	20.0%	0.0%	0.0%	
Total	10	10	10	10	
	100.0%	100.0%	100.0%	100.0%	

Mild haemorrhagic change was seen in three animals in Group B, C and D while moderate haemorrhage was seen in five animals in Group B and two animals in Group D.

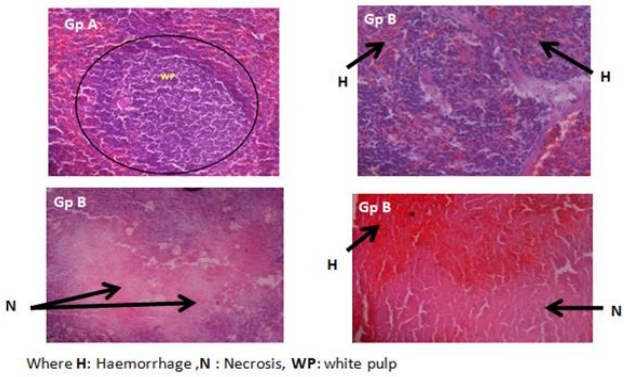
Severe haemorrhage was seen only in two animals in Group B. Moderate and severe haemorrhage was significantly higher in animals placed in Group B (p value 0.001). Furthermore, histological findings showed mild necrosis in three animals placed in Groups B and D whereas mild necrosis was seen in two rats in group C. Five rats in Group B and four in Group D presented with moderate necrosis in their splenic parenchyma. Additionally, severe necrosis in spleen was observed in two animals in Group B and D respectively. Upon comparison among groups, moderate and severe necrotic changes were highly significant in animals placed in Group B (p value 0.001). These findings indicate a significant protective effect of Coenzyme Q10 in reducing haemorrhagic and necrotic changes (p = 0.0001) as shown in table II & III.

Table II: Haemorrhagic findings comparison among study groups (n=40)

Histology (haemorrhage)	Animal group				p-value
	A	B	C	D	
Normal	10	0	7	5	0.0001
	100.0%	0.0%	70.0%	50.0%	
Mild	0	3	3	3	
	0.0%	30.0%	30.0%	30.0%	
Moderate	0	5	0	2	
	0.0%	50.0%	0.0%	20.0%	
Severe	0	2	0	0	
	0.0%	20.0%	0.0%	0.0%	
Total	10	10	10	10	
	100.0%	100.0%	100.0%	100.0%	

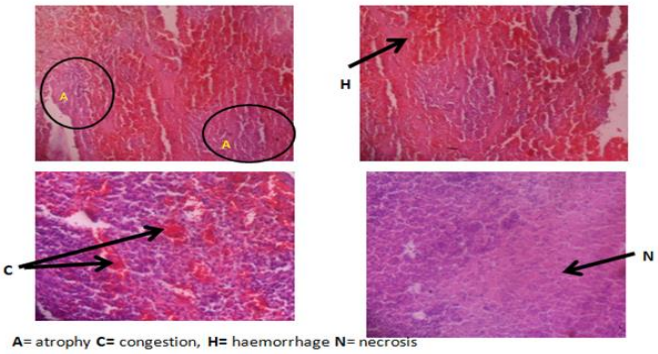
Table III: Necrosis findings comparison among study groups. (n=40)

Histology (necrosis)	Animal group				p-value
	A	B	C	D	
Normal	10	0	8	1	0.0001
	100.0%	0.0%	80.0%	10.0%	
Mild	0	3	2	3	
	0.0%	30.0%	20.0%	30.0%	
Moderate	0	5	0	4	
	0.0%	50.0%	0.0%	40.0%	
Severe	0	2	0	2	
	0.0%	20.0%	0.0%	20.0%	
Total	10	10	10	10	
	100.0%	100.0%	100.0%	100.0%	



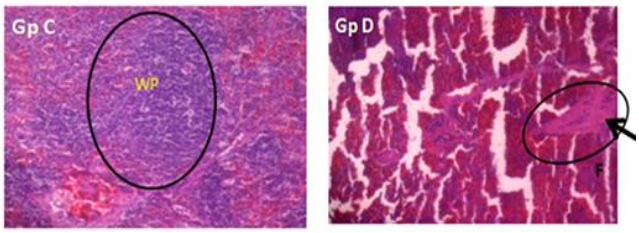
Where **H:** Haemorrhage ,**N** : Necrosis, **WP:** white pulp

Photomicrographs 01 showing histology of parenchyma of spleen of female albino Wistar rats of Gp A and B under H&E X 400



A= atrophy **C=** congestion, **H=** haemorrhage **N=** necrosis

Photomicrographs 02 showing parenchyma of Spleen of female albino Wistar rats Gp B under H&E X 200



Photomicrograph 3: Spleen of female Albino Wistar Rat showing necrosis areas replaced by fibrous tissue (Group D) Post- treatment group, under H&E X 200

Discussion

Spleen is essential for immune response because it filters blood, removes damaged cells, and facilitates the activation of immune cells, especially lymphocytes. For immunocompromised situations, the spleen's function can be severely affected. Immunosuppression can reduce the number and function of immune cells in the spleen, increasing vulnerability to infections and reducing the ability to remove pathogens and injured cells from the circulation. Furthermore, histological abnormalities including atrophy of lymphoid follicles, and changes in the red pulp might occur, all of which impair the efficacy of the immune system. Co-Q10, a powerful antioxidant and critical factor in mitochondrial production of energy, has been demonstrated to protect several organs, particularly the spleen, especially under conditions of oxidative stress and immunological impairment. This experimental study has been done to assess the effects of Co-enzyme Q10 supplementation on the histology of the spleen in immunocompromised female Albino Wistar rats and initially study found a significant reduction in spleen weight was observed in animals in Group B, which is consistent with the findings of Qi et al.[21] who also noted a loss of spleen weight due to cyclophosphamide exposure. The absence of mononuclear phagocytes in rodents' results in rapid weight loss and immunosuppression, as in a study suggested that cyclophosphamide contributes to the decrease in spleen weight. However, Voloshin et al[22] mentioned that spleen weight is a relatively insensitive indicator of immunosuppression.

According to histological analysis of the splenic pulp in this study revealed significantly higher levels of hemorrhage and necrosis in Group B, a finding consistent with previous studies by Araghi et al.,[23] and Khazaei et al.[24]. These studies also demonstrated that cyclophosphamide-induced oxidative stress leads to cellular necrosis and structural alterations within the spleen. The observed damage in Group B reinforces the susceptibility of splenic tissue to oxidative damage under immunosuppressive conditions, further highlighting the detrimental effects of cyclophosphamide on splenic histology.

In contrast, CoQ10 supplementation in the pre-treatment Group C showed well-preserved splenic parenchyma, indicating that CoQ10 effectively protected the spleen from oxidative damage. This protective effect aligns with findings from Mirmalek et al.,[25] and Rashid et al.[26] who reported CoQ10 as a potent antioxidant capable of neutralizing oxidative stress. The preservation of typical splenic architecture in Group C underscores CoQ10's role in mitigating tissue damage and supports its potential as a preventive measure against immunosuppressant-induced oxidative injury. However, in the post-treatment Group D, areas of necrosis were observed to be replaced by fibrous tissue, consistent with findings by Olama et al.,[27] who described fibrotic tissue formation following parenchymal destruction. This observation suggests that although Co-Q10 may offer some reparative effects post-exposure, its efficacy appears reduced compared to pre-treatment, possibly due to the extent of initial tissue damage and the limited regenerative capacity of splenic tissue once necrosis has progressed to fibrosis. The observations contribute to the field by providing evidence of CoQ10's protective effects on the spleen, particularly as a pre-treatment in immunocompromised conditions. Additionally, the study emphasizes the importance of timing in antioxidant supplementation, as the pre-treatment group showed markedly better outcomes than the post-treatment group. Furthermore, this study supports earlier research by Rashid et al.,[26] and Nyariki et al.,[27] which suggest CoQ10's benefits in oxidative damage prevention, immunomodulation, and neuroprotection. Our results contribute to a growing body of evidence supporting CoQ10 as a viable adjunct therapy for protecting immune organs in oxidative-stress-related immunosuppression. However, due to several limitations, further larger scale studies are recommended to validate the findings.

Conclusion

This study observed histological changes, such as white pulp atrophy, hemorrhage, and necrosis, were most prevalent in the spleens of immunosuppressed female rats treated with cyclophosphamide. Coenzyme Q10 demonstrated a significant cytoprotective role in these rats, as histological changes in the spleen were significantly improved in those receiving Coenzyme Q10,

whether administered before or after cyclophosphamide treatment. Further research is necessary to determine the optimal dosages and plasma levels of CoQ10 needed to enhance immune function and modulate inflammation. This knowledge will aid practitioners in designing effective treatment protocols for CoQ10 supplementation.

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