Clinical and Experimental Health Sciences

Effects of Functional Pinealectomy on Immunity, Hematopoietic, Gastrointestinal and Urinary Systems in Experimentally Malnutritioned Rats

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ABSTRACT

Objective: The aim of this study was to demonstrate morphological changes in immunity, hematopoietic, gastrointestinal and urinary systems in different melatonin (MEL) release situations in a rat model of protein energy malnutrition (PEM).

Method: A total of 32 adult male Wistar rats were assigned into four equal groups: normal control; PEM light/dark; PEM light, called functional pinealectomy (Px); and PEM dark. PEM was produced with a 50% restricted diet, Px was produced by keeping rats in continuous light environment for 24 hours, and complete blood count and serum albumin level were analyzed at the end of the 6-week experimental period. Measurements of weights of body and some visceral organs were obtained, biochemical and morphological parameters were analyzed in addition to measurements of malondialdehyde (MDA), total glutatione (GSH), tumor necrosis factor (TNF)-α and interleukin-10 (IL-10) in tissue samples.

Results: A reduction in the weights of body and visceral organs of animals in the PEM groups was accompanied by hypoalbuminemia, anemia, leukopenia and lymphopenia, and higher MDA, GSH, TNF-α and IL-10 levels in visceral tissues. There was a significant decrease in parenchymal cells of the liver and spleen, duodenal villi, lymphoid structures and kidney glomeruli, but there was an increase in the spleen capsule thickness and renal Bowman's space, sinusoidal congestion and fat accumulation in the liver. Importantly, these findings were milder in the PEM dark group, while they were prominent in the PEM light group.

Conclusion: This study suggests that MEL has a protective role in reducing the negative effects of PEM, making it a potential therapeutic agent for further investigation.
Keywords: functional pinealectomy, malnutrition, melatonin, protein energy malnutrition, rat.

1. INTRODUCTION

Protein energy malnutrition (PEM) occurs due to decreased intake of macro and micro nutrients such as protein, carbohydrate and fat in less than their required amount (1,2,3,4). Even today, it is a serious health problem and an important cause of morbidity and mortality for all ages, especially in individuals who are hospitalized in intensive care units and had comorbid diseases, from developing countries (3,4,5).

Numerous studies revealed that PEM causes decreased numbers of hematopoietic cells, such as macrophages, lymphocytes and neutrophils, atrophic changes in the spleen, thymus and lymph nodes, blunting of intestinal microvilli and decreased IgA secretion; thus resulting in various visceral dysfunctions (6,7). Moreover, immunomodulatory cytokines released from macrophages, such as tumor necrosis factor (TNF)-α and interleukin (IL), play a regulatory role in inflammation (8), while the free radicals superoxide and hydrogen peroxide result in oxidative stress (1). It is well-known that some molecules which include glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) have vital roles in the antioxidative defense system, although malondialdehyde (MDA) is an indicator of lipid peroxidation. Theoretically, disruption of the critical balance between oxidative and antioxidative systems or accumulation of free radicals results in protein damage and DNA breaks (1).

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Many authors have reported that PEM causes an increased incidence of infections of the gastrointestinal, respiratory or urinary tract, due to oxidative stress, immunodeficiency and hypersensitivity to infectious agents (9). Clinically, it is important to reduce the economic losses associated with long hospitalizations, and the high morbidity and mortality rates related to infections, especially in people with PEM, who are hospitalized for various reasons in intensive care units. Otherwise, recent studies have demonstrated that melatonin (MEL) plays a role in the activation and regulation of T and B lymphocytes which are critical for immunity (10,11). Today, it is a well-known fact that MEL release was found to be in a distinct circadian rhythm that is 5-15 times higher at night than during the day (12). Thus, one could suggest that the antioxidants will become stronger with decreased free radicals due to increased MEL release in a dark environment.

The aim of the present work was to test the hypothesis that keeping adult individuals with PEM in a continuous light environment, as are found in the intensive care units of hospitals, will have negative effects on the health of patients. Therefore, the potential effects of different levels of MEL release provided by keeping malnourished adult rats in different light/dark cycle environments upon some biochemical parameters and histology of immune, hematopoietic, gastrointestinal and urinary systems was investigated.

2. METHODS

2.1. Ethical Statement

The animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals principles (13). The design of the experiment was approved by the ethics committee of Animal Experiments of Aydın Adnan Menderes University (Protocol No. 64583101/2020/087 of 17/09/2020).

2.2. Animals, Diets, and Experimental Design

A total of 32 male Wistar rats of 2-month-old, weighing between 240 and 310 g were used and they were kept in standard cages with 2 rats in each cage. During the experiment, standard laboratory conditions were provided (22°C room temperature, 40-60% humidity). As described by Leite et al. (14), the animals were acclimated for a 3-day adaptation period to determine the daily energy/calorie needs under laboratory conditions.

The rats were randomly divided into four groups for 6 weeks: normal control, i.e. intact or naive control, group (n=8) kept in 12-hour light/12-hour dark environment and fed standard diet; PEM light/dark group (n=8) kept in 12-hour light/12-hour dark environment and fed PEM diet; PEM light, functional pinealectomy (Px), group (n=8) kept in 24 hours of light and fed PEM diet; and PEM dark group (n=8) kept in 24 hour dark environment and fed PEM diet. At the end of the experiment, blood and tissue samples were taken from the rats for biochemical and morphological analyses.

2.3. Functional Pinealectomy and Malnutrition Diet

Functional Px was produced by keeping rats in a continuous light environment for 24 hours (15). A standard rat diet was given without restriction according to the requirements for laboratory animals by the National Research Council (16). For the control group, the daily calorie requirements of the rats were calculated as 25-35 kcal/kg. The PEM diet was provided with a diet that was 50% restricted compared to control rats, as previously described (14,17,18). The water consumption of the rats was unrestricted in all groups.

2.4. Sample Collection

At the end of the experiment before sacrificing the animals by cervical dislocation under anesthesia by intramuscular administration of drug combination consisting of 40 mg/kg ketamine (Ketalar, Eczacıbaşı, Turkey) and 5 mg/kg xylazine (Rompum, Bayer, Germany), blood samples from all rats were taken by the intracardiac route using a 5 cc syringe and they were placed in tubes with ethylenediamine tetraacetic acid (EDTA) and without anticoagulant to be studied simultaneously. The samples were centrifuged at 1000 g for 10 minutes. The complete blood count (CBC) tests, analysing hemoglobin, erythrocyte, leukocyte and lymphocyte counts, were performed from the whole blood samples with EDTA, while serum albumin levels were obtained from the samples. The serum albumin level was studied spectrophotometrically with a routine biochemistry autoanalyzer (Architect C8000, Abbott, IL, USA). The CBC was studied with Mindray BC 6800 (Nanshan, Shenzhen, China) analyzer. Visceral organs were removed from each of the animals for macroscopic evaluation, and then frozen at – 85 degrees without adding any additive for biochemical analysis. After the tissues were removed from the deep freezer and thawed, they were mixed with phosphate buffer at pH: 7.4 at 1/10 volume ratio and homogenized with a homogenizer (PRO 250 Scientology Inc., Monreo, CT USA). The hemogenate was centrifuged at 10000 g for 15 minutes. MDA, total GSH, TNF-α and IL-10 measurements were taken from the supernatant on the same day, as described below.

2.5. Tissue Analysis

MDA production was evaluated by the method of Ohkawa et al. (19). MDA formed a pink complex at high temperature in the presence of thiobarbituric acid and this color was read spectrophotometrically at a wavelength of 532 nm. Tetraethoxypropane was used as a standard and the results are given in nmol/mg protein.

GSH content in tissue supernatants was measured according to the method of Beutler et al. (20). Absorbance was measured at 412 nm using the Shimadzu UV-160 spectrophotometer (Shimadzu UV-160). The GSH concentration was determined using the standards of GSH. Results are expressed as nmol/mg protein.

TNF-α and IL-10 levels in tissue were determined by the immunosorbent assay (ELISA) kit (SunRed Biological Technology, Jufengyuan Road Baoshan, District, Shanghai,

China). Measurements were made with an ELISA microplate reader (DAR 800, Diagnostic Automation, CA 91302, USA). According to the TNF-α kit content, the sensitivity of the test is 4.752 pg/mL, the working range is 5-15000 pg/mL, the CV within and between experiments is < 9 and 11%. The sensitivity of the test according to the IL-10 kit contents is given as 3.002 pg/mL, working range 5-9000 pg/mL, intra-experimental and inter-experiment CV < 9 and 11%.

The levels of protein in the tissue were determined by the Lowry method, which is based on thSSe principle that copper ion (Cu+2) forms a complex with the peptide bonds in proteins and is reduced to Cu+1 in alkaline medium (21).

2.6. Macroscopic Evaluation

Body weight (BW) of each of the rats in the study was measured and recorded once a week from the beginning of the experiment and macroscopic photographs were taken. At the end of the experiment, the abdominal and thoracic cavities of each animal sacrificed were exposed and the visceral organs including liver, spleen, kidney (right and left) and first part of small intestine (duodenum) were removed separately from each animal for weight measurement.

2.7. Microscopic Evaluation

Visceral organ samples from the animals in the control and PEM groups were fixed in neutral buffered formaldehyde and a routine tissue preparation process was performed. 5 µm histological sections of tissues embedded in paraffin blocks were placed on polylysine slides and then they were stained with hematoxylin & eosin (HE) and Mallory's trichrome. These sections were visualized with an Olympus BX-51 light microscope and Olympus DP72 digital camera after staining. Morphometric analyses were performed on the photographs taken by two blinded histologists (Y.U. and C.T.) using CellSensEntry 3.1 (Japan). A total of five different measurements were taken from the structures analyzed in 25 different areas in 25 different sections randomly selected from all animals.

2.8. Statistical Analysis

The assumption of normal distribution of the data was checked with the Kolmogorov-Smirnov test. Descriptive statistics are given as means ± standart error of the mean (SEM). One-way ANOVA was used for group comparisons, while repeated ANOVA measures were used to compare repeated measures. Tukey test was applied as a post hoc method and paired t-test was used to compare dependent measures. Statistical significance was considered as $p<0.05$.

3. RESULTS

Measurement results of mean weekly BWs in control and PEM groups are shown in Figure 1. There was no significant difference in BWs of rats between the groups at the start of the study, but the change in weekly measurement results during the experiment was statistically significant ($p<0.001$). Rats in the control group showed a 25% BW gain, whereas rats from PEM groups given a restricted diet showed a progressive reduction in BW, especially in those from the PEM light group, where loss of BW was close to 30%, resulting in a clear difference between their macroscopic appearances (Fig. 2). Furthermore, hair thinning and shedding, broken nails, muscle atrophy, apathy and agitation were observed in the animals in the PEM groups. The differences between the weights of the visceral organs of the animals in PEM groups and those of the control were found to be significant (Table 1). Amongst the PEM groups, it was noted that visceral atrophy was more prominent in animals from the PEM light group, while atrophy was milder in those from the PEM dark group.

Table 1. *Results of visceral organ weight measurement*

	Liver (g)	Spleen (g)	Duodenum) (g)	Kidney (g)	
				Right†	Left†
Normal control (intact or naive control)	14.07±0.97	1.05±0.15	1.25±0.16	2.14±0.35	2.04±0.24
PEM light/ dark	6.47±0.33*	0.45±0.02*	0.70±0.05*	1.10±0.11*	1.11±0.13#
PEM light	6.29±0.52*	0.43±0.06*	0.56±0.16*	0.99±0.11*	1.01±0.10*
PEM dark	7.50±0.76	0.53±0.06*	0.87±0.11*,§	1.14±0.06*	1.17±0.09

*Differences between study groups for liver, spleen, duodenum, and right and left kidney weights (mean ± SEM) are significant (p<0.001); n=8/ group. *Significant difference with normal control (p<0.001). §Significant difference with PEM light (p<0.001). #Significant difference with control (p<0.05). †Differences between right kidney weight measurements and left kidney weight measurements are significant (p<0.05 for control and PEM light groups, p<0.005 for PEM dark group)*

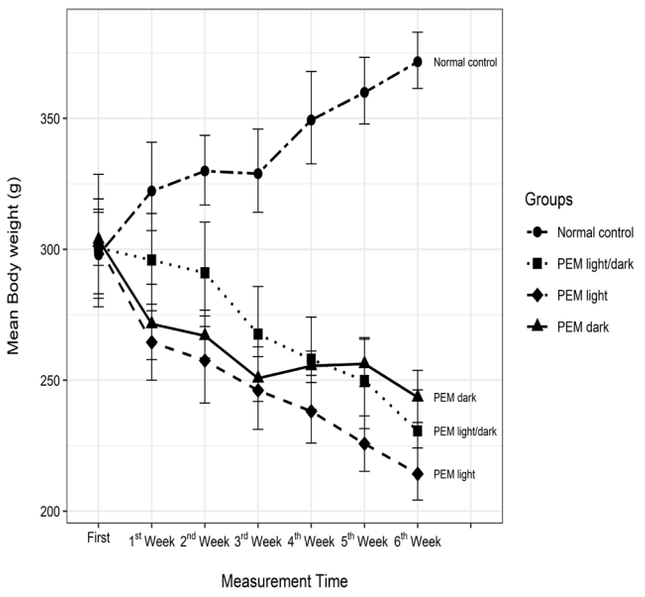

Figure 1. *Weekly change in body weight in rats of the study groups over the course of the experiment (mean ± standard error of the mean). The differences between the normal control (A) and experimental PEM groups (B, C and D) are significant (p<0.001). PEM, protein energy malnutrition.*

Serum albumin levels of PEM groups were found to be lower than those of the control group (p<0.001) and CBC results from animals with PEM clearly demonstrated the presence of anemia, leukopenia, and lymphopenia (Table 2). Hematological findings related to PEM, especially the leukocyte count, were milder in the PEM dark group. MDA, GSH, TNF-α and IL-10 levels in the liver, spleen, duodenum and kidney were also higher in PEM groups compared to the control group (Figs. 3, 4, 5, 6). On the other hand, there was a significant difference between the PEM light group and the PEM dark group in terms of MDA levels of liver and kidney, GSH levels of liver and spleen, TNF-α levels of spleen and duodenum, and the IL-10 level of duodenum (Figs. 3, 4, 5, 6).

Table 2. *Results of serum albumin level and complete blood count (CBC)*

	Albumin (g/L)	Hemoglobin (g/dL)	Erythrocyte count (106)	Leukocyte count (103)	Lymphocyte (%)
Normal control (intact or naive control)	36.65±0.97	16.46±0.59	10.32±0.87	5.95±1.29	82.51±6.25
PEM light/ dark	31.60±0.88*	15.83±0.48	8.91±0.64‡	2.76±0.30*	76.36±9.31
PEM light	30.60±1.00*	15.36±1.15#	8.08±1.05*	2.25±0.33*	70.81±4.84#
PEM dark	31.99±1.17*,§	15.96±0.52	9.05±0.29#	3.82±1.06*	77.98±6.56

*The data are presented as mean ± SEM; n=8/group. Differences between study groups for albumin levels as well as erythrocyte and leukocyte counts are significant (p<0.001), while those between both hemoglobin levels and lymphocyte counts are significant (p<0.05). *Significant difference with normal control (p<0.001). §Significant difference with PEM light (p<0.05). #Significant difference with the control (p<0.05). ‡Significant difference with the control (p<0.01)*

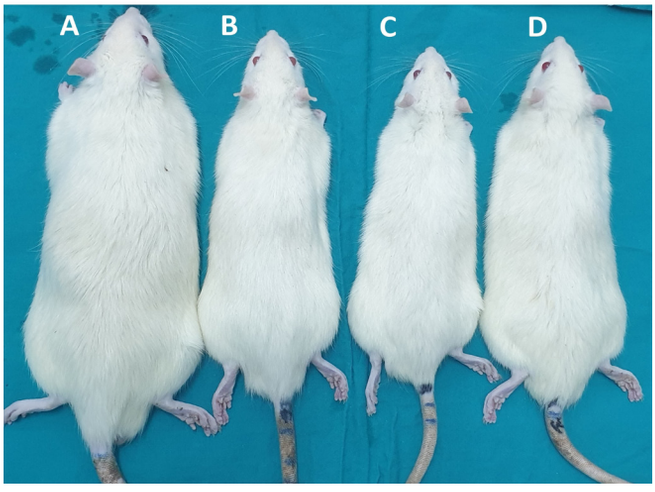

Figure 2. *Photographs of the macroscopic appearances of the animals in the normal control (A) and PEM groups (B, C and D) taken at the end of the experimental study period are shown. Note that among animals in experimental PEM groups, the animal in the PEM dark group (D) is more macroscopically similar to the animal of the control (A). PEM, protein energy malnutrition.*

Table 3. *Histological changes in liver, duodenum and kidney tissue*

Tissues Groups		Normal control (intact or naive control) (n=8)	PEM light/ dark (n=8)	PEM light (n=8)	PEM dark (n=8)
Histological changes in liver					
Parenchyma	Intracytoplasmic edema	None	++	+++	+
	Nuclear hypertrophy	Normal	++	+++	+
	Vena centralis dilatation	Normal	++	+++	+
Stroma	Sinusoidal dilation	Normal	++	+++	+
	Portal triad dilatation	Normal	++	+++	+
Histological changes in duodenum					
Change in epithelial type		N	++	+++	+
Villus atrophy		N	++	+++	+
Reduction in villus size		N	++	+++	+
Reduction in intestinal (duodenum) wall thickness		N	++	+++	+
PMNL infiltration in lamina propria		N	++	+++	+
Degeneration of Lieberkühn and Brünner glands		N	++	+++	+
Histological changes in kidney					
Degeneration of renal corpuscles		N	++	+++	+
Degeneration in podocytes and edema		N	++	+++	+
Cystic degeneration in renal corpuscles		N	++	+++	+
Dilation and congestion in peritubular vessels		N	++	+++	+
Vacuolization in proximal tubules		N	++	+++	+
Epithelial changes in the distal tubules		N	++	+++	+

Abbreviations: N: normal histological structure; +: low level of change; ++: moderate level of change; +++: high level of change

Both number and size of the hepatocyte cells in the liver of the individuals in the PEM groups were found to be decreased in contrast to those of the control animals (Figs. 7, 8). In PEM groups, vacuolization, mononuclear cell infiltration, fat accumulation and sinusoidal congestion and dilatation of the periportal hepatocytes were prominent, whereas they were at a minimal level in PEM dark group (Figs. 7, 8) (Table 3). There was also a statistical difference between the morphometric results of the liver sinusoid and v. centralis diameters of the groups (p<0.001) (Figs. 9, 10, 11).

There was a decrease in the splenic capsule thickness in samples belonging to the PEM light/dark and PEM light groups compared to those of controls (p<0.001) (Figs. 7, 8, 9, 12). Furthermore, narrowing in the a. centralis, disappearance of the lymph node-like structure, and the fibrotic foci in the red pulp were also noted in those of PEM groups (Figs. 7, 7, 9, 12). In the PEM dark group, however, the capsule was similar to that in the PEM light/dark group and there was also a decrease in cord cells, as well as narrowing in the a. centralis structure and the fibrotic foci in the red pulp (Figs. 7, 8, 9, 12).

Group	Liver	Spleen	Intestine (duodenum)	Kidney
Normal control	109.84 ± 8.36*	153.58 ± 12.51	167.04 ± 79.36	145.7 ± 4.46*
PEM light/dark	122.61 ± 7.33	177.15 ± 16.88	219.35 ± 133.33	151.55 ± 8.77
PEM light	145.01 ± 32.19	215.64 ± 113.21	266.52 ± 88.46	160.87 ± 10.66
PEM dark	114.99 ± 7.05*	165.49 ± 43.28	195.57 ± 64.31	147.77 ± 8.00*

* Represent significant difference with PEM light

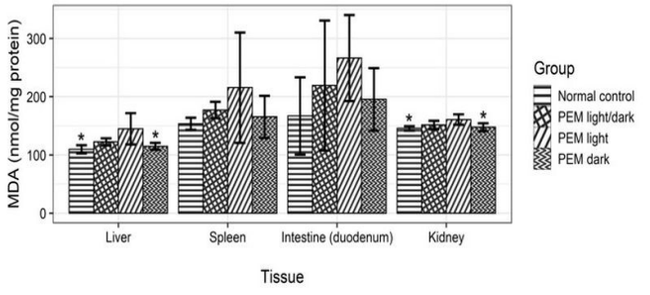

Figure 3. *Results of MDA levels in tissue homogenates taken from the animals in the control and experimental PEM groups taken at the end of the study period are shown. The data are presented as mean ± SEM; n=8/group. *p<0.05between the indicated groups in each tissue sample analysis. MDA, malondialdehyde; PEM, protein energy malnutrition.*

Group	Liver	Spleen	Intestine (duodenum)	Kidney
Normal control	15.34 ± 5.39*	8.01 ± 4.74*	18.1 ± 9.51	13.73 ± 5.37
PEM light/dark	20.04 ± 6.84*	16.09 ± 3.88*#	33.84 ± 47.77	16.02 ± 4.24
PEM light	31.25 ± 12.54	25.63 ± 6.67	46.91 ± 39.94	20.44 ± 6.94
PEM dark	17.93 ± 4.89*	12.24 ± 3.66*	21.28 ± 15.21	14.73 ± 6.02

* Represent significant difference with PEM light; # Represent significant difference with Normal control

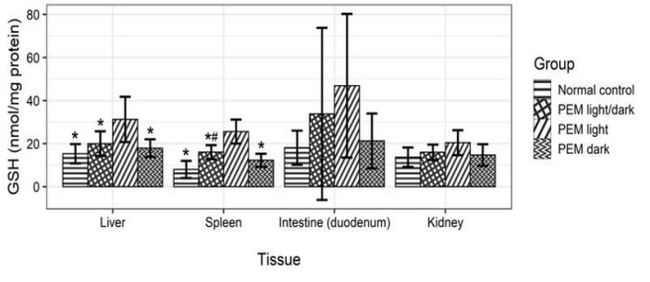

Figure 4. *Results of GSH levels in tissue homogenates taken from the animals in the control and experimental PEM groups taken at the end of the study period are shown. The data are presented as mean ± SEM; n=8/group. *p<0.05 between the indicated groups in each tissue sample analysis. GSH, glutathione; PEM, protein energy malnutrition.*

Group	Liver	Spleen	Intestine (duodenum)	Kidney
Normal control	225.88 ± 55.07	283.94 ± 55.05*	295.77 ± 13.51*	238.72 ± 40.03
PEM light/dark	254.27 ± 38.37	310.49 ± 43.2*	379.56 ± 98.75	257.8 ± 34.79
PEM light	273.6 ± 73.4	401.52 ± 88.03	442.09 ± 132.37	301.3 ± 75.34
PEM dark	243.29 ± 32.51	291.4 ± 67.98*	298.04 ± 55.25*	248.41 ± 37.68

* Represent significant difference with PEM light

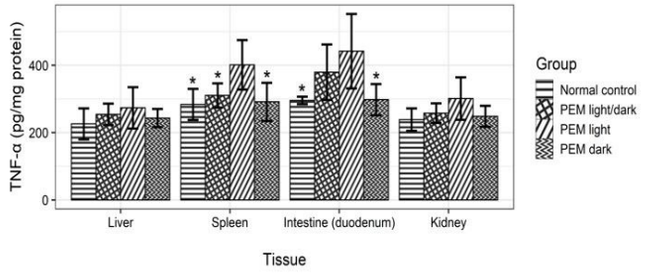

Figure 5. *Results of TNF-α levels in tissue homogenates taken from the animals in the control and experimental PEM groups taken at the end of the study period are shown. The data are presented as mean ± SEM; n=8/group. *p<0.05 between the indicated groups in each tissue sample analysis. PEM, protein energy malnutrition; TNF-α, tumor necrosis factor-α.*

Group	Liver	Spleen	Intestine (duodenum)	Kidney
Normal control	204.06 ± 14.66	246.01 ± 39.54*	230.54 ± 42.31*	252.64 ± 16.47*
PEM light/dark	227.5 ± 32.23	275.63 ± 27.48	264.23 ± 49.87*	259.95 ± 41.71
PEM light	238.65 ± 30.95	297.00 ± 40.84	349.15 ± 97.38	293.78 ± 36.17
PEM dark	226.47 ± 42.65	264.88 ± 16.8	240.08 ± 13.41*	254.94 ± 17.9

* Represent significant difference with PEM light

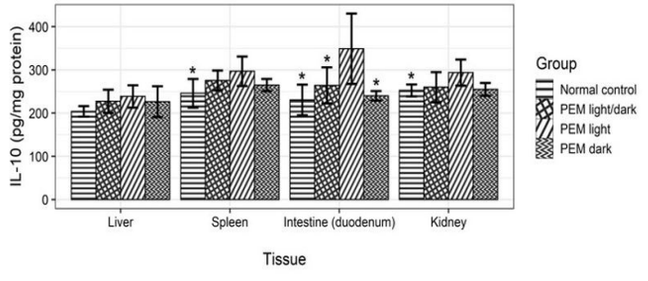

Figure 6. *Results of IL-10 levels in tissue homogenates taken from the animals in the control and experimental PEM groups taken at the end of the study period are shown. The data are presented as mean ± SEM; n=8/group. *p<0.05 between the indicated groups in each tissue sample analysis. IL-10, interleukin-10; PEM, protein energy malnutrition.*

In PEM groups, both number and length of the duodenal villi were decreased, and atrophy was identified in Lieberkuhn and Brunner's glands (Figs. 7, 8, 9, 13) (Table 3). Thinning and single-layered cuboidal epithelium, indistinguishable epithelial-connective tissue boundary, and increased collagen fibers in the lamina propria and duodenal PMNL infiltration

were observed in the PEM light/dark group, although the PEM dark group was similar to that of the control group (Figs. 7, 8, 9, 13) (Table 3).

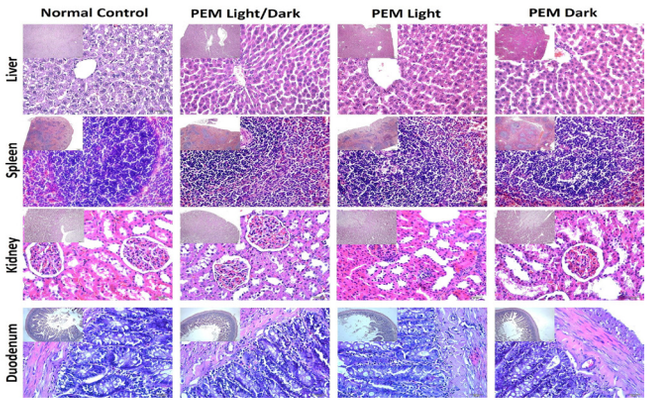

Figure 7. *Representative microscope images of H&E-stained visceral organs (liver, spleen, duodenum, and kidney) in the study groups. Small photos 4x magnification (magnification bar: 20 µm); large photos 40x magnification (magnification bar: 50 µm).*

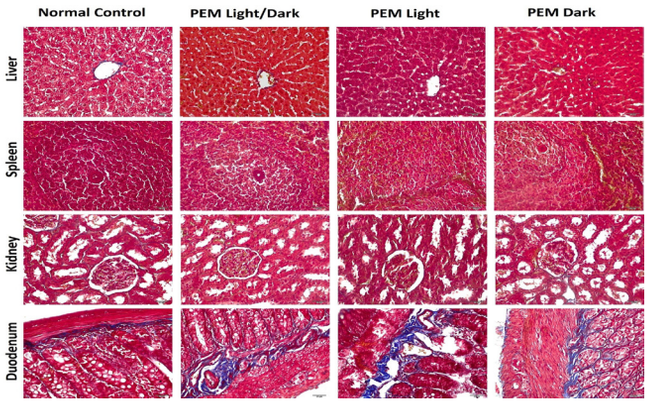

Figure 8. *Representative microscope images of Mallory's trichrome-stained visceral organs (liver, spleen, duodenum, and kidney) in the study groups. Small photos 4x magnification (magnification bar: 20 µm); large photos 40x magnification (magnification bar: 50 µm).*

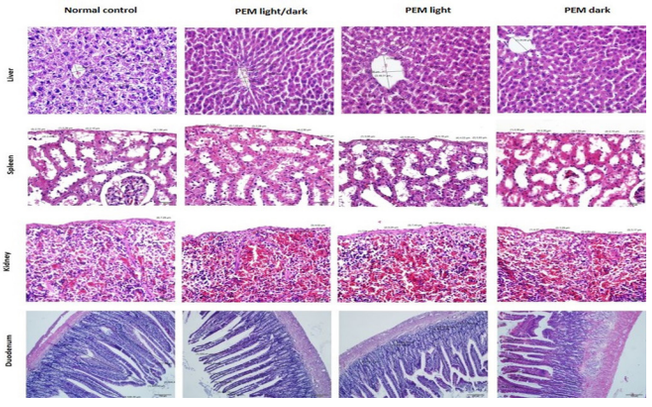

Figure 9. *Histopathology of the tissues of visceral organs (liver, spleen, duodenum, and kidney) in the study groups. 40x magnification (magnification bar: 50 µm). Images of morphometric analyzes made with CellSensEntry 3.1 digital analysis program. Five different measurements were taken from the structures analyzed in 25 different areas and in 25 different sections randomly selected from all animals in each group.*

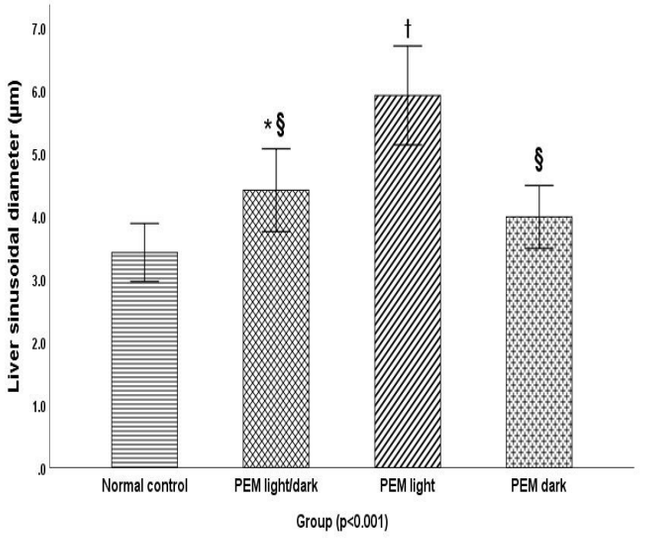

Figure 10. *Results of morphometric measurement of liver tissue sinusoid diameter in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group. *Significant difference with control (p<0.05). †Significant difference with control (p<0.01). §Significant difference with PEM light (p<0.01).*

In the PEM groups, there was decreased size, volume, and glomeruli number of the kidney, while there was increased collagen in the kidney, resulting in glomerulosclerosis and interstitial fibrosis (Figs. 7, 8, 9, 14, 15) (Table 3). Likewise, vacuolization, cystic dilatation, vasodilatation and tubular congestion, and edematous glomerular mesangial cells were also observed (Table 3). However, the findings in the PEM dark group were similar to those of control animals (Figs. 7, 8, 9, 14, 15). Morphometrically, the group having the greatest capsule thickness was the PEM light/dark group (Fig. 14), while the group with the largest space in Bowman's capsule was the PEM light group (Fig. 15).

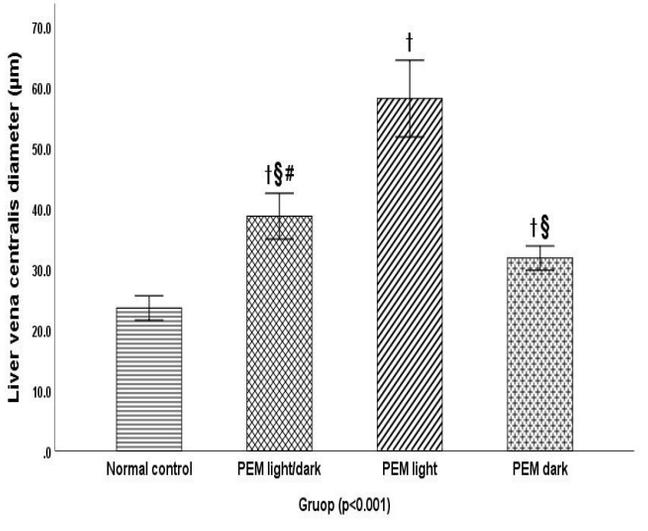

Figure 11. *Results of morphometric measurement of liver tissue vena centralis diameter in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group. †Significant difference with control (p<0.01). §Significant difference with PEM light (p<0.01). #Significant difference with PEM dark (p<0.05).*

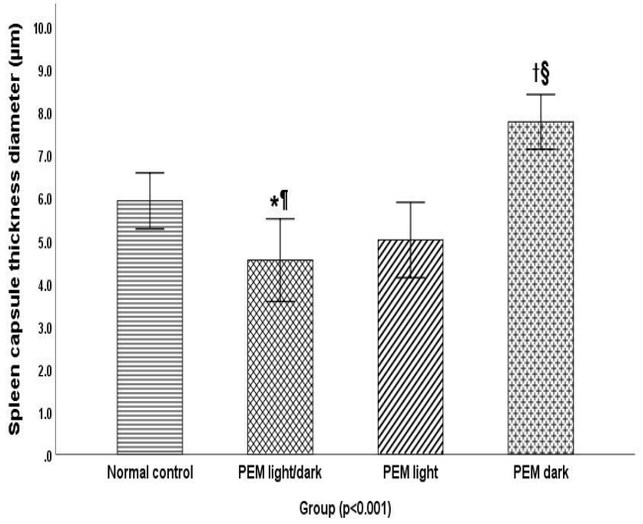

Figure 12. *Results of morphometric measurement of the spleen tissue capsule thickness in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group. *Significant difference with control (p<0.05). †Significant difference with control (p<0.01). §Significant difference with PEM light (p<0.01). ¶Significant difference with PEM dark (p<0.01).*

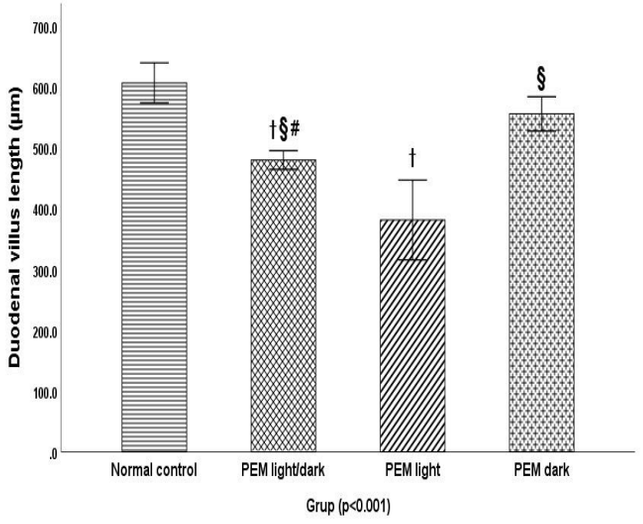

Figure 13. *Results of morphometric measurement of duodenal villus length in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group. †Significant difference with control (p<0.01). §Significant difference with PEM light (p<0.01). #Significant difference with PEM dark (p<0.05).*

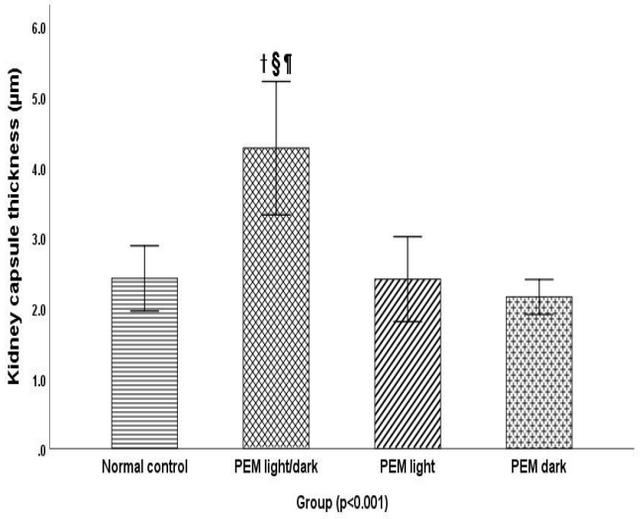

Figure 14. *Results of morphometric measurement of kidney capsule thickness in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group. †Significant difference with control (p<0.01). §Significant difference with PEM light (p<0.01). ¶Significant difference with PEM dark (p<0.01).*

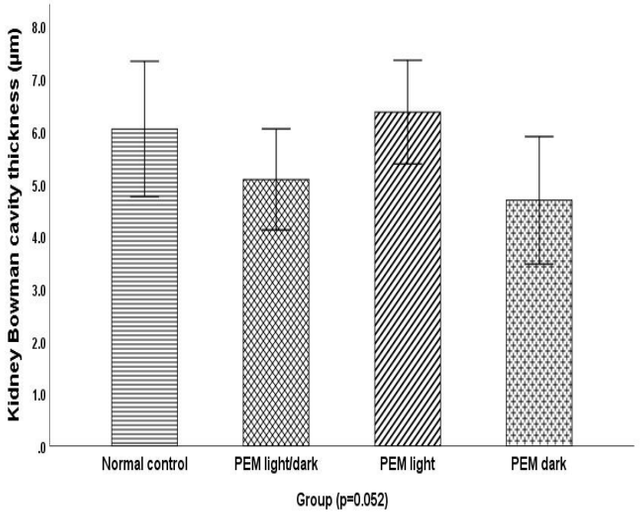

Figure 15. *Results of morphometric measurement of kidney tissue Bowman capsule cavity in the study groups are shown comparatively. The data are presented as mean ± SEM; n=8/group.*

4. DISCUSSION

In this study, we found that the biochemical and morphological findings were milder in the PEM dark group, while they were most prominent in the functional Px group, suggesting that there are potential effects of different light/dark cycles on immunity, hematopoietic, gastrointestinal and urinary systems.

Amongst the factors affecting the development of the human body, the most important one is nutrition and PEM can lead to various infectious diseases by affecting the immunity and

inflammatory response (22,23). In the case of PEM, various morphological and functional changes related to the visceral organs like the liver, spleen, intestine and kidney in addition to hematopoietic and immune systems may occur (24). There is an increase in lipid peroxidation and weakening of the antioxidant defense system, resulting in morphological and functional changes in the visceral organs of individuals with PEM (25). It has also been reported that macrophage activation, cytokine production, dysfunction of T-lymphocytes and cell death occur as a result of apoptosis and cell necrosis in immunity-related organs (4,6,26,27,28,29). Cortes-Barberena et al. (27) reported reduced spleen/body weight ratio, T-lymphocyte count, and cell number and proliferation in different phases of the cell cycle in the spleen of rats with PEM. We found severe atrophic changes in the splenic and intestinal lymphoid structures and a prominent lymphocytopenia in the blood of PEM groups, although the changes were milder in the animals from the PEM dark group, possibly due to endogenous MEL secretion.

Further studies have revealed reduced cell proliferation in the bone marrow and increased DNA damage in lymphoid tissues, including the spleen and circulating lymphocytes (27,30). Ortiz et al. (4) suggested that splenic atrophy occurs due to increase in spontaneous apoptosis in the rat PEM model. Likewise, Santos et al. (31) reported hematopoietic alterations associated with bone marrow atrophy, and impaired homeostasis and immune response. Cortés-Barberena et al. (27) claimed that PEM increased the time for DNA synthesis and the total cycle of the splenic cell. We determined an increased thickness in the splenic capsule but decreased cords in the red pulp in all PEM groups, consistent with previous studies (5,28,31). More importantly, the changes were milder in the animals in the PEM dark group, suggesting a mitigating effect on the aforementioned effects.

In acute and chronic PEM, the lymphopoiesis is impaired due to apoptosis (4,28). Mello et al. (32) found an increase in both the percentage of T-cells and the production of anti-inflammatory IL-10. Similarly, an elevated IL-10 level was found in serum of newly weaned mice fed a low-protein diet (33). Our results confirm a defense mechanism acts to control inflammation in rats with PEM, especially in the functional Px group, although it is difficult to say whether the increase is due to protein or energy/calorie deficiency. Furthermore, lower serum albumin levels related with protein metabolism and an antioxidant defense system in PEM groups are consistent with reports in the literature (3,14,34). We think that the partial increase in the albumin level of the animals in the PEM dark group may be associated with the reparative effects of endogenous MEL on the liver.

In individuals with PEM, normochromic normocytic anemia occurs due to various reasons (5,26) and in addition to energy and/or protein deficiency, deficiency of iron or micronutrients such as vitamins and trace elements cause certain negative effects on erythropoiesis (35). In PEM, erythrocyte and reticulocyte counts are low due to decreased oxygen consumption and erythropoietin production as well as impaired erythroblast maturation (34,36,37). It is widely accepted that PEM affects the hematopoietic system, resulting with hypoplasia/atrophy and decreased myeloid/erythroid cell proliferation in the bone marrow (5,26,31). The hemopoietic tissues have high rates of self-renewal and proliferationand the coexistence of anemia and leukopenia in individuals with PEM confirms the high protein requirement for hematopoiesis (5,26). We think that low values of the erythrocyte count and hemoglobin in PEM groups is associated with suppression of erythropoiesis and the short life span of erythrocytes. However, animals in the PEM dark group had higher CBC values compared to other PEM groups, suggesting a stimulatory effect of endogenous MEL on erythropoiesis. Similarly, splenic hypocellularity and decreased erythrocyte count in the animals from PEM groups, in addition to an increased splenic capsule thickness, suggest a negative effect on the hematopoietic system.

Even today, the morphological and functional changes in the digestive tract, including the liver and duodenum, are still debated (34,38). Previously, Miguel Parra et al. (39) reported decreased weights of visceral organs such as the liver, spleen, and kidney in acute PEM, in addition to a decreased size and number of their parenchymal cells. Comparably, the intestinal mucosa is markedly thinned, with a decrease in the height/number of villi with structural blunting and lymphocyte infiltration (34,38). In contrast, some authors found a partial increase in liver cell ballooning and steatosis, edema, hypoalbuminemia, and anemia in PEM animal models (40,41). It has been reported that mice exposed to PEM in the neonatal period have increased inflammation and oxidative stress in liver (41). In an acute PEM piglet model, Lykke et al. (34) found vacuolization and lipid droplets in hepatocytes, in addition to decreased BW, intestinal atrophy and anemia.

Recently, chronic inflammation and immune dysfunction in the intestines of animals with PEM have been reported (42). Some authors reported an increased level of IL-10 in the liver and jejunum as a response to systemic inflammation to reduce the effects of IL-1β and TNF-α secreted by monocytes and macrophages (43,44). Similarly, Dewan et al. (45) reported an increase in serum TNF-α and IL-10 levels in children with PEM. In our study, MDA, TNF-α, GSH and IL-10 activities were found to be high in the liver and duodenum in all PEM groups, but there was a significant improvement in those of the PEM dark group, suggesting anti-inflammatory effects of MEL upon the gastrointestinal system. However, it is difficult to make a comment regarding the functional features of the duodenums of rats exposed to PEM, such as the wall permeability, motility and enzyme activities. Moreover, the effects of severe PEM on the intestinal tract are well-known, but those related with mild or moderate PEM are not clear (46). Therefore, our experimental model is invaluable, because it demonstrates the changes in the small intestine consistent with mild to moderate PEM. Our morphological findings were most prominent in the functional Px group, while they were minimal in the PEM dark group, suggesting a hepatoprotective effect of MEL. Additionally, an increase in Bowman's capsule space due to the presence of interstitial fibrosis and glomerular degeneration, but a decrease in both weight and volume of the kidney occur in individuals with PEM, resulting in clinical

hypertension and chronic renal failure (24). Santoso et al. (24) demonstrated a close relationship between PEM and the immunological status of the kidney by assessing macrophages producing proinflammatory cytokines such as TNF-α, IL-6, and TGF-β cytokines in mice, as also seen in our study. Based on our findings, we expect an improvement in the renal function of animals in the PEM dark group, although confirmation of this idea will only be possible with additional studies in future.

This experimental study is the first in the literature to evaluate the effects of functional Px on immunity, hematopoietic, gastrointestinal, and urinary systems in malnourished rats. Furthermore, it is worth emphasizing that the rat PEM model is easy, applicable, and reliable, but there are some limitations regarding the applicability of the results obtained in this model to humans, and the measurement of the serum level of MEL and levels of pro-inflammatory/anti-inflammatory cytokines. In this study, we found that animals with PEM with reduced BWs and weights of visceral organs had hypoalbuminemia, anemia, leukopenia, and lymphopenia, and higher MDA, GSH, TNF-α and IL-10 levels in tissue samples. Our morphological findings also revealed a reduction in parenchymal cell count of the visceral organs, lymphoid structures of the spleen and intestine, number and height of intestinal villi, kidney glomeruli count, but an increase in the spleen capsule thickness and kidney Bowman's capsule cavity, and sinusoidal congestion and fat accumulation in the liver. Importantly, these findings were mild in the PEM dark group, in contrast to those of the functional Px group. In summary, we speculate that keeping adult patients hospitalized, especially in intensive care units, with PEM in a continuous light environment will have negative effects on their health.

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Acquisition of data for the study: YBT, CT, MY

Analysis of data for the study: YBT, YU, CT, MY, ÖÇ

Interpretation of data for the study: YBT, YU, CT, MY, ÖÇ

Drafting the manuscript: YBT, CŞ, YU

Revising it critically for important intellectual content: YBT, CŞ, YU, ÖÇ

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