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Effect of gonadotropins on blood, bone marrow and spleen in cyclophosphamide exposed rats

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Cyclophosphamide (CTX) is an effective chemotherapeutic agent. Gonadotropins are molecules with various actions. Here, we investigated the effects of gonadotropins on the peripheral blood, bone marrow and spleen in rats administered with CTX. Three groups were formed: Control (C) group with no process; Sham (S) group: Physiological saline was applied; CTX group: A single dose of 200 mg/kg and 8 mg/kg CTX was administered for the next 14 days. All rats were superovulated with 150-300 IU/kg pregnant mare serum gonadotropin. Human chorionic gonadotropin @150-300 IU/kg was given, and complete blood counts, bone marrow smears, and spleen sections were examined; and also the expression of WNT-1, WNT-4, and β-catenin was analyzed. Although the hemoglobin and platelet value in the CTX group was lowest, it was still within the normal reference ranges. The C and S groups had significantly higher white blood cell values (p=0.017). In terms of number of megakaryocytes, Myeloid/ Erythroid ratio, lymphoid cell ratios, no significant differences were found in bone marrow aspiration smears. The CTX group had significantly higher β-catenin expression in the red pulp than the other groups (p=0.0001). The CTX group had the highest WNT-4 expression and very intense expression of WNT-1 in the white pulp. Our results indicate that the gonadotropins, promising in "treatment", have favourable effects on toxicity of CTX.

Keywords: Cytotoxic chemotherapy, Drug toxicity

Cyclophosphamide (CTX) is a frequently used, effective alkylating, cytotoxic drug. It is used not only as an antineoplastic but also in other fields of medicine such as rheumatology and bone marrow transplantation, sometimes alone or sometimes in combination with other drugs. It is a potent immunosuppressive agent. The toxic effects of CTX include bone marrow suppression, cardiotoxicity, hemorrhagic cystitis, gonadal toxicity, and carcinogenesis. The major risk factor for side effects is cumulative exposure[1-3]. CTX-induced bone marrow suppression can manifest as anemia, leukopenia, neutropenia, or thrombocytopenia. Neutropenia can increase the risk of infection. Antimicrobial prophylaxis can thus be given to appropriate patients. Neutropenic fever is a significant source of morbidity and mortality. If the infection is not treated, it will progress to sepsis and septic shock. Platelet and neutrophil counts typically decrease in the first 1-2 weeks, with an improvement expected after an average of 20 days[4,5].

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Chorionic gonadotropins are only found in primates (human chorionic gonadotropin (hCG) in humans) and equines. Luteinizing hormone (LH), which is released early by the embryo in mice, could have the same purpose as hCG[6-10]. It has recently been discovered that hCG, along with its hyperglycosylated analog (hCG-H), is a potent paracrine hormone that induces successful implantation and coordinated communication between the blastocyst and uterine tissues, despite the fact that it is traditionally known as a hormone that regulates progesterone production by corpus luteum cells[11-15].

Control of trophoblast to syncytiotrophoblast fusion, angiogenesis, immunity regulation, and endometrial susceptibility to implantation are all paracrine properties of hCG. hCG-H has been shown to act as a mitogen on NK cells, activating them[11-13]. Nongonadal tissues express luteinizing hormone-chorionic gonadotropin receptors, which are required for fetal growth. Adult mesenchymal stem/stromal cells have been shown to express these functional receptors outside pregnancy conditions, making them susceptible to hCG stimulation[14,15]. In recent years, studies have been conducted to show that hCG may not only have effects on immunity, but also in the

sense of 'treatment' like graft versus host disease (GVHD), and positive evidence has begun to be obtained in this direction[16,17]. There are few studies showing that hCG may have effects on hematopoietic tissues such as the spleen and bone marrow[18-21]. In addition, it has been shown that hCG can increase leukocyte cells such as neutrophils and monocytes in peripheral blood[18,19,22,23].

Recent studies show that gonadotropins can be a molecule with very different effects apart from their known properties. Our objective in this study was to determine whether gonadotropins, which are currently showing promise in "treatment," can mitigate the side effects of the commonly used cytotoxic chemotherapy drug CTX on the spleen and bone marrow. In this study, we have investigated the effect of gonadotropins on peripheral blood, bone marrow and spleen through ovulation induction in rats exposed to CTX.

Materials and Methods

Study design

Ethical approval was taken from the Pamukkale University Animal Experiments Ethics Committee Approval number: 2022/03). We used 24 adult female Wistar Albino rats. Rats were housed in separate cages in a quiet room with a temperature (21± 1°C) and humidity (65-70%) controlled 12-h light-dark cycle throughout the experimental protocol. Animals were given standard rat chow and tap water ad libitum. Eight week-old rats weighing 250-260 g were randomly divided into 3 groups: Group C: There was no process (n=8); Group S: The rats in this group were given physiological saline intraperitoneally for 14 days (n:8); and CTX group: A single dose of 200 mg/kg cyclophosphamide (Endoxan, EİP Eczacıbaşı, Turkey) was injected i.p. 8 mg/kg cyclophosphamide continued to be given i.p. for the next 14 days (n:8)[24] .

After 14 days, all rats were superovulated with an intraperitoneal injection of 150-300 IU/kg pregnant mare serum gonadotropin (PMSG; Adoog Bioscience, United States)[25]. After 48 h, 150-300 IU/kg human chorionic gonadotropin (hCG, Ovitrelle, Merck) was administered. 17-19 h after hCG administration, superovulated rats were anesthetized by intraperitoneal injection of ketamine/xylazine; blood samples were collected by cardiac puncture. Rats were sacrificed by cervical dislocation.

Complete blood counting (CBC)

Two milliliters of blood were drawn from the rats and placed in tubes containing ethylenediamine-tetraacetic acid (EDTA). A Mindray BC-2800Vet Auto Hematology Analyzer was used to perform complete blood counts (CBC) (Shenzhen Mindray Bio-Medical Electronics Co. Ltd, Shenzhen, China). Results of CBC included Red blood cells (RBC, reference interval 5.60-7.89× 10^{12}/L), hemoglobin (Hb, reference interval 12.0–15.0 g/dL), hematocrit (Hct, reference interval 36–46%), mean corpuscular volüme (MCV, reference interval 53.0-68.8 fL), mean corpuscular hemoglobin (MCH, reference interval 16-23.1 pg), mean corpuscular hemoglobin concentration (MCHC, reference interval 30.0–34.1 g/dL), red cell distribution width (RDW, reference interval 11.0-15,5%); mean platelet volüme (MPV, reference interval 3.8-6.2 fL), platelet distribution width (PDW), plateletcrit (PCT), white blood cells (WBC, reference interval 2.9–15.3 × 10^3/μL); platelet (plt, reference interval 100–1610 × 10^3/μL), percentage of neutrophil (Neu %, 7.3- 30.1), percentage of lymphocyte (Lym %, 63.7-90.1), percentage of monocyte (Mono %, 1.5-4.5), neutrophil count (Neu#, 0.4-3.2× 10^3/μL), lymphocyte count (Lym#, 2.6-13.5× 10^3/μL), monocyte count (Mono#, 0.0-0.5× 10^3/μL).

Preparation and evaluation of bone marrow aspiration smears

Both femoral bones were dissected and muscle and adipose tissue were removed. To expose the marrow stem, the femur was trimmed. The femur's upper and lower ends were severed. To release the cells into the petri dish, a 1 cc syringe with a 25 gauge needle was used to flush the bone marrow medium through the femur several times. It was pipetted into the Petri dish several times with 1 ml of PBS. The cell drop poured into the Petri dish was spread on a clean glass slide and dried. For three minutes, fixation was carried out in 100% methyl alcohol. It was dripped on May-Grünwald for five minutes. Giemsa was washed, dripped, and left for 25 min. After that, smear preparations that had undergone the washing procedure and been allowed to dry were double-blindly examined by one hematologist and one histologist under a light microscope. 200–500 cells were counted on a smear stained with May–Grünwald–Giemsa. At 100X magnification, myeloid, erythroid, lymphoid lineage cells, plasma, and blast cells were counted[26]. The leukocyte percentages (%) were as follows: Myeloid cells (including myelocytes,

promyelocytes, metamyelocytes, band neutrophils, segmented neutrophils, eosinophils, monocytes), erythroid cells, lymphocytes, plasma cells and blast cells.

Based on the Bolliger AP paper and with the help of the literature, the common and atypical features of cellular components in rats were assessed. Mega-karyocyte count was calculated to be per low power field-10X objective. With a 100X objective, the morphology of megakaryocytes was examined. This included the nuclei's segmentation, the existence of immature forms, dysplastic forms, micromega-karyocytes, platelet budding, cytoplasmic vacuolization, hypolobated forms, and hypogranular forms[27,28].

Histopathological examination of spleen

Spleen tissues were fixed in 10% neutral buffered formalin solution for at least 24 h. After dehydration and paraffin embedding serial sections of 5 µm thickness were taken. Spleen sections were examined under a light microscope after stained with hematoxylin and eosin (H&E).

Immunohistochemical staining of spleen

The avidin-biotin-peroxidase complex method was used for immunostaining. Citrate buffer (pH 6.0) was used for antigen retrieval by microwave after deparaffinization and rehydration of the sections. Endogenous peroxidase activity was then blocked for 15 min at room temperature (25°C) with 3% hydrogen peroxide in methanol. Sections were then incubated at 4°C overnight with primary antibodies (WNT-1: Biossantibodies, bs-1739R, diluted 1:300, USA, WNT-4: Biossantibodies, bs-6134R, diluted 1:300, USA, and Beta-catenin: Biossantibodies, bs-1165R, diluted 1:300, USA). After washing the primary antibodies, the sections were incubated for 60 min at room temperature with secondary antibody (1:1000), followed by 30 min with avidin-biotin complex. Under the light microscope, immunohistochemical reactions were visualized using diaminobenzidine chromogen. For counterstaining, Mayer's hematoxylin was applied for 15 min.

The following scale was used to assess immunohistochemical staining: (+++) indicates strong staining, (++) indicates moderate staining, (+) indicates weak staining, (/+/-) indicates very weak staining, (-) indicates negative, and (/) indicates no built-in model.

The expression of WNT-1, WNT-4 and β-catenin in the groups was assessed semi-quantitatively using the H score [H SCORE=Pi (I+1)]. It represents the intensity of the staining (0=no expression, 1=light, 2=medium, and 3=strong), and Pi represents the percentage of cells stained for each intensity[15].

Statistical analysis

All statistical analyses were performed using SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)). Continuous variables were defined by the mean ± standard deviation. Shapiro Wilk tests were used for the determination of normal distribution. For independent groups comparisons, we used One Way Analysis of Variance (post hoc: Tukey method) when parametric test assumptions were provided, Kruskal Wallis Variance Analysis (post hoc: Mann Whitney U test with Bonferroni Correction) was used when parametric test assumptions were not provided. The level of statistical significance was set at $P \leq 0.05$.

Results

Although the Hb value was lower in the group given CTX compared to the group C and S, it was still within the normal reference range of 12-15 g/dL. When comparing the groups, the Hb value of the group S was significantly higher than that of the CTX group (14.64±0.49, 12.9± 2.64, respectively) (p=0.027). In terms of the blood Hct levels, the Hct value of the CTX group was lower than that of the other two groups, and it was significantly lower than that of the group S (p=0.029). Similarly, RBC count was also lower in the group CTX, but it remained within the normal reference ranges of 5.6-7.89 × 10^{12}/L. An inter-group comparison revealed that the RBC level of the group S was significantly higher than that of the CTX group (7.88±0.18, 6.41±1.5 × 10^{12}/L, respectively) (p=0.001) (Table 1).

A significant difference of the MCV and MCH levels was detected between the groups (p= 0.001). In comparison to the other two groups, it was noticeably higher in the CTX group. When comparing the RDW values, CTX group presented a significantly higher level than in the other groups (p=0.0001).

Regarding WBC values, a significant difference was found between the groups. The blood WBC values of the groups C and S were significantly higher than those in the CTX group (p=0.017). When comparing the percentages of granulocytes and monocytes between the groups, the CTX group showed significantly higher levels compared to the other two

groups (p=0.0001). However, despite the significant difference in the percentages of granulocytes and monocytes, there was no significant difference in their absolute values. We also compared the groups in terms of lymphocyte percentages and absolute values, and the CTX group had lower levels compared tothe other groups (p=0.001) (Table 1).

A comparison of platelet levels in the complete blood count indicated that the CTX group had the lowest level (771,000±205,579.32/mm^3). When comparing the groups, the platelet level of the CTX group was found to be significantly lower than that of the Group C (p=0.027). Conversely, when comparing platelet distribution width (PDW) levels between the groups, it was found to be significantly higher in the CTX group (p=0.048) (Table 1).

Table 1 — Effects on peripheral blood cell counts

	Control Group (mean±S.D)	Sham Group (mean±S.D)	CTX Group (mean±S.D)	*P*
RBC	7.58±0.29	7.88±0.18	6.41±1.5	0.001[c]
Hb	13.84±0.68	14.64±0.49	12.9±2.64	0.027[c]
Hct	43.28±1.66	45.86±1.41	40.84±8.24	0.029[c]
MCV	57.22±0.54	58.24±1.01	64.6±4.67	0.001[bc]
MCH	18.24±0.36	18.53±0.37	20.3±1.38	0.001[bc]
MCHC	31.9±0.41	31.87±0.38	31.51±0.44	0.162
RDW	10.84±0.33	10.99±1.01	16.54±1.14	0.0001[bc]
WBC	4000±1426.53	3871.43±1070.38	2137.5±1193.96	0.017[bc]
NEU %	25.26±6.15	26.47±5.55	51.63±9.82	0.0001[bc]
NEU#	980±356.37	1000±244.95	1100±614.12	0.924
LYM%	72.24±6.81	71.21±5.84	44.15±10.1	0.0001[bc]
LYM#	2940±1182.37	2785.71±883.98	950±639.2	0.001[bc]
MONO%	2.5±0.76	2.31±0.49	4.23±0.4	0.0001[bc]
MONO#	80±44.72	85.71±37.8	87.5±35.36	0.936
Plt	1044600 ±151895.69	878285.71 ±87058.82	771000 ±205579.32	0.027[b]
MPV	5.98±0.16	5.83±0.22	6.04±0.21	0.167
PDW	16.72±0.27	16.57±0.1	17±0.51	0.048[c]
PCT	0.6±0.09	0.51±0.06	0.47±0.13	0.128

[**P* <0.05 statistically significant; SD: Standard Deviation. (A): Significant between Control and Sham; (B): Significant between Control and CTX; and (C): Significant between Sham and CTX. RBC, Red blood cells; Hb, Hemoglobin; HCT, Hematocrit; Mean corpuscular volume, MCV; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RDW, red cell distribution width; Neu, Neutrophil; Lym, Lymphocyte; Mono, monocyte; MPV, Mean platelet volüme; PDW, platelet distribution width; PCT, plateletcrit; WBC, white blood cell; and Plt, platelet]

Bone marrow aspiration smear results

A comprehensive examination of rat bone marrow included the determination of the myeloid: erythroid (M:E) ratio, with or without a 200- or 500-cell differential count. When comparing the number of megakaryocytes between the groups, although no significant difference was found between the groups, the CTX group had the highest count. There were no significant differences in the M/E ratio among the groups CTX, C and S (1.49±0.65, 1.48±0.72, 1.52±0.6, respectively). When comparing the lymphoid cell ratios of the groups, no significant difference was observed. However, the group S had the highest lymphoid cell ratio (54.29±16.97) (Table 2 and Fig. 1).

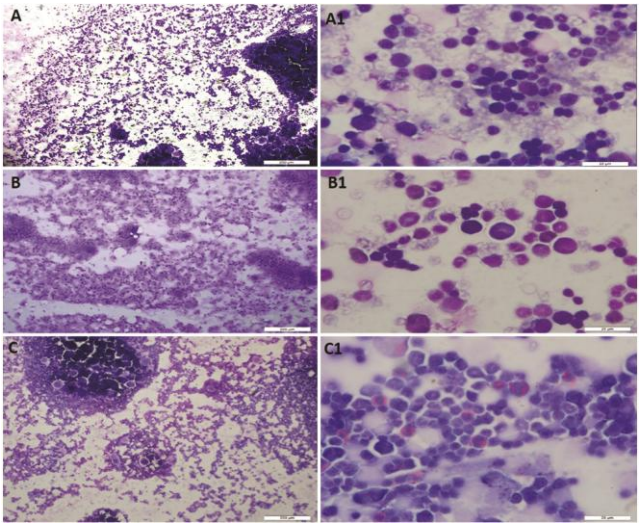


Fig. 1 — Photomicrograph of bone marrow smear Giemsa stain. (A & A1) Control; (B & B1) Sham; and (C & C1) CTX group. [A-C: X10 and A1-C1: X100]

Table 2 — Effect on manual differential cell count in the femoral bone marrow of rats

	Control group (mean±SD)	Sham group (mean±SD)	CTX group (mean±SD)	p
Myeloid cells (%)	30.68±9.27	26.06±12.36	26.65±11.16	0.755
Erythroid cells (%)	22.72±8.82	18.37±6.84	22.24±4.66	0.422
M: E ratio	1.49±0.65	1.48±0.72	1.52±0.6	0.88
No. of megakaryocytes (per low power field- 10X objective)	9.2±5.12	12.57±4.28	14.78±3.42	0.079
Lymphocytes (%)	45.8±14.48	54.29±16.97	50.44±12.45	0.596
Plasma cells (%)	0.8±0.84	0.71±0.49	0.44±0.73	0.475
Blast cells (%)	0±0	0.57±1.13	0.22±0.67	0.375

[In the control group, Some myeloid cells showed an increase in eosinophilic granulation. There were also micromegakaryocytes, In the sham group, there were numerous ring-shaped myeloid cells. Two segmented neutrophils were observed. There were also micromegakaryocytes and immature megakaryocytes. In the CTX group, In some areas, there was a decrease in cellularity and an increase in fat spaces. Eosinophilic granulation was increased in 50% of myeloid cells. There were numerous ring-form metamyelocytes. Two segmented neutrophils were observed. There were also micromegakaryocytes]

When comparing the plasma and blast cell ratios of the groups, no significant difference was found. In the bone marrow aspirations of the Group C, some myeloid cells exhibited increased eosinophilic granulation, along with the presence of micro-megakaryocytes. The bone marrow aspirations of the group S showed numerous ring-shaped myeloid cells and the presence of two segmented neutrophils. Additionally, micromegakaryocytes and immature megakaryocytes were observed. In some areas of the CTX group's bone marrow aspirations, a decrease in cellularity and an increase in fat spaces were noted. Eosinophilic granulation was increased in 50% of myeloid cells. Numerous ring-form metamyelocytes were also observed, along with two segmented neutrophils and micromegakaryocytes (Table 2 and Fig. 1).

Histological evaluation results

In sections stained with H&E, the spleen appeared normal in the groups C and S. However, in a few sections of the CTX-given group, it was observed that the white pulp lost its normal structure and showed signs of hemorrhage in that area. Additionally, although they were less in number, the presence of degenerative areas in the red pulp was noteworthy (Fig. 2).

The expression and localization of β-catenin were similar in the C and S groups. While expression was very weak in the white pulp, it was determined that the expression increased in the red pulp compared to the white pulp. It was noted that the expression in the CTX group was more intense compared to the other two groups. In this group, the expression in the white pulp was particularly weaker, especially in the germinal region and its surroundings. Intense expression was also observed in the red pulp. The expression of β-catenin in the white pulp was low in the C and S groups, while it was high in the CTX group. The expression of β-catenin in the red pulp was significantly higher in the CTX group compared to the other two groups (P=0.0001) (Fig. 3).

It is noteworthy that WNT-1 exhibited moderate/ intense expression in both the white and red pulp of the control and sham groups. However, the CTX group showed very intense expression specifically in the white pulp. The expression of WNT-1 in the red pulp of the CTX group was similar to that of the other two groups. High expression of WNT-1 was observed in the white pulp of the CTX group, and this was statistically significantly higher compared to the group S (p=0.001). Significant differences in WNT-1 expression were found in the red pulp between groups. The C and CTX groups showed significantly higher expression compared to the group S (p=0.004). WNT-4 expression was intensely observed in both the white and red pulp of all groups, with the highest expression seen in the white pulp of the CTX group (259.33±11.59). When comparing the groups, a significant increase was found in the CTX group compared to the group C in terms of white pulp (P=0.001). WNT-4 expression in the red pulp was also significantly higher in the CTX group (P=0.0001) (Fig. 3).

Discussion

We investigated the effects of ovulation induction on blood, bone marrow, and spleen in CTX-exposed rats in our study. Despite being lower than in the control C and sham S groups, the hemoglobin (Hb) value in the cyclophosphamide group was still within the typical reference. WBC value was found to be lower in the CTX-given group compared to the other groups. We found that the CTX group's lymphocyte percentages and absolute values were lower than those of the other groups. Similarly, the platelet value was found to be lower in the CTX group compared to the C and S groups. According to bone marrow aspiration smears, the CTX group had the most megakaryocytes, despite the fact that there was no statistically significant difference between the groups. The CTX group's bone marrow aspirations showed some regions with a reduction in cellularity and an increase in fat spaces. The white pulp in a few sections of the CTX-treated group lost its normal structure and showed signs of hemorrhage in that area. Furthermore, the presence of degenerative areas

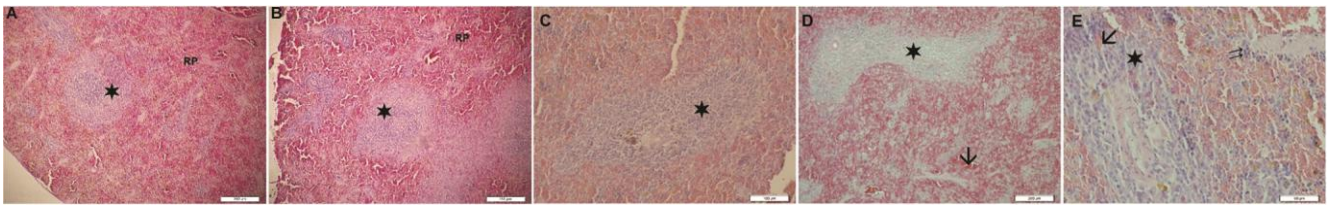
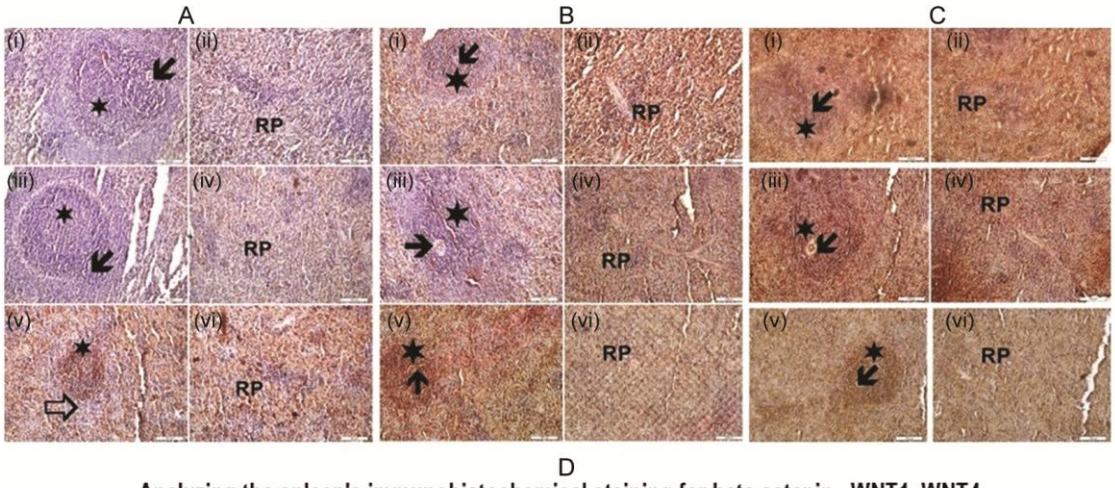


Fig. 2 — H&E staining sections of spleen from groups. (A) Control; (B) Sham; (C-E) CTX group. [RP, Red pulp; star, white pulp; arrow, hemorrhage; and double arrow, necrotic cells. [A, B and D: X10; C: X20; and E: X40]



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Analyzing the spleen's immunohistochemical staining for beta catenin , WNT1, WNT4

	Control Group (mean ± S.D)	Sham group (mean ± S.D)	CTX Group (mean ± S.D)	p
Beta-Catenin-White	10 ± 0	10 ± 0	221 ± 4.38	-
Beta-Catenin-Red	184 ± 17.12	182.67 ± 15.16	242.17 ± 13.86	0.0001[bc]
WNT1-White	211 ± 9.35	150.33 ± 19.14	281.33 ± 5.05	0.001[c]
WNT1-Red	287.2 ± 5.02	249.67 ± 14.96	286.17 ± 4.07	0.004[ac]
WNT4-White	13.4 ± 4.77	109.33 ± 10.89	259.33 ± 11.59	0.001[b]
WNT4-Red	167.4 ± 10.88	225.5 ± 13.55	284 ± 4.6	0.0001[abc]

*p<0.05 statistically significant; S.D: Standard Deviation; a: significant between Control and Sham; b: significant between Control and CTX; c: significant between Sham and CTX group

Fig. 3 — (A) Expression of β-catenin in [(i) & (ii)] Control group spleen tissue; [(iii) & iv)] Sham group spleen tissue; and [(v) & (vi)] CTX group spleen tissue. (B) Expression of WNT-1 in [(i) & (ii)] Control group spleen tissue; [(iii) & iv)] Sham group spleen tissue; and [(v) & (vi)] CTX group spleen tissue. (C) Expression of WNT-4 in [(i) & (ii)] Control group spleen tissue; [(iii) & iv)] Sham group spleen tissue; and [(v) & (vi)] CTX group spleen tissue. [RP, Red pulp; star, White pulp; and arrows, Central artery. Immunoperoksidase & Hematoxylin, X20, Bar= 200 µ. (D) Spleen's immunohistochemical staining for β-catenin, WNT1 and WNT4]

in red pulp was notable, despite its rarity. β-catenin expression in the white pulp was low in the C and S groups, while it was high in the CTX group. The expression of β-catenin in the red pulp was significantly higher in the CTX group than in the other two groups (P=0.0001). In the C and S groups, WNT-4 was found to be moderately/intensely expressed in the red and white pulps, but very intensely expressed in the white pulp of the CTX group.

CTX is inactive and must be activated by enzymes and chemicals. Nitrogen mustard forms cross-links between and within DNA strands, resulting in cytotoxicity. Aldehyde dehydrogenase detoxifies CTX. Cells with high proliferative potential may have high levels of aldehyde dehydrogenase, resulting in CTX resistance[1-3]. Common side effects of CTX include anemia, leukopenia, neutropenia, and thrombocytopenia. These effects are dose-dependent. The drug, as an alkylating agent, causes DNA damage in rapidly proliferating cells, including bone marrow cells. The lowest level of neutropenia and thrombocytopenia is expected one or two weeks after the drug is administered. In our study, after receiving CTX for 14 days, the rats were observed for a further 3 days. However, the development of anemia, leukopenia, neutropenia, and thrombocytopenia was not as evident as in the literature, indicating that pregnant mare serum gonadotropin and hCG therapy had a positive effect. In the study of Zhang *et al*.[29], CTX was administered intraperitoneally to rats for a total of seven days after the initial dose of 200 mg/kg. As a result, blood platelet values got decreased by approximately 49%, erythrocytes decreased by 28%, and leukocytes decreased by 25%[1-3,29,30]. Although the first dose we gave was the same, it would have been expected to cause much more hematological toxicity since the drug was given intraperitoneally for a total of 14 days. In rats given CTX, these rates were for platelets, erythrocytes, and leukocytes, respectively; 26.19, 15,43 and 46,56%. A positive effect of chorionic gonadotropin may be considered if platelets and erythrocytes decrease less than expected, but not leukocytes.

Although there was no significant difference in the number of megakaryocytes of bone marrow between the groups, it was highest in the CTX group. This may

be also a reaction of the bone marrow to protect the organism from thrombocytopenia. This supports the view that chorionic gonadotropins may have a protective effect on blood cells. In a study on the effect of hCG on cells, it was shown that this high hCG causes IL-8 production from PBMC (peripheral blood mononuclear cells) in the early stage of embryo implantation, a period when a high concentration of hCG can be obtained locally at the implantation site. IL-8 is a neutrophil chemoattractant/activating factor discovered in lipopolysaccharide-stimulated monocytes but is now known to be expressed in a variety of cells, including lymphocytes. Endothelial cell migration and proliferation have both been shown to be stimulated by IL-8[22,23]. Active chronic GVHD is a serious complication that can affect the skin, subcutaneous tissues, joints, or the gastrointestinal tract after allogeneic bone marrow transplantation. Low-dose hCG stimulated indoleamine-2,3-dioxygenase, IL 10, and regulatory T cells in patients with steroid-resistant GVHD, suppressing the clinical manifestations of chronic GVHD[14,15]. These findings imply the existence of an immune-endocrine network involving hCG and immune cells which is very important.

In the study of Xie RF, when mice were given a 0,12 mg/g body weight CTX injection, leukocytes were lowest on average on day 4, then increased from day 7 to day 14 compared to the control. Pluripotent hematopoietic stem cells-colony forming unit-spleen (CFU-S) nucleated cells in the bone marrow were minimal at 48 h and gradually returned to normal within two weeks. It was depleted abruptly within 24 h, then exponentially re-emerged on day 3, followed by a second decline, returning to normal on days 11 and 14. The granulocytic progenitor cell colony-forming unit-diffusion chamber (CFU-D) and colony-forming unit-culture (CFU-C) changes were quite like those of CFU-S, but their proliferation peak was at day 4. Peripheral leukocyte decrease was slower and the return to normal was earlier than hematopoietic cells. Therefore, recovery of the leukocyte count does not imply a true reconstruction of hematopoiesis. Bone marrow stroma and marrow microcirculation observed with colony-forming unit-fibroblastoid (CFU-F) assay was also damaged and did not return to normal during observation. Bone marrow stroma and microcirculation showed more severe damage[31]. In the CTX group's bone marrow aspirations in some areas, as if showing the damage here there was a decrease in cellularity and an increase in fat spaces. Eosinophilic granulation was increased in 50% of myeloid cells. There were numerous ring-form metamyelocytes. There were also dysplastic changes like two segmented neutrophils and micro-megakaryocytes. But no significant difference was found in the C, S, and CTX groups in terms of M/E ratio. The normal M: E ratio in rat bone marrow varies from 1.07 to 1.93[26,32,33]. The M: E ratio was found to be 1.49±0.65, 1.48±0.72, and 1.52±0.6 in the C, S and CTX groups. However, it was expected that the M: E ratio would be low, particularly in the CTX group; at this point, chorionic gonadotropins may have a positive effect.

In rats, the spleen plays an active role in hematopoiesis[34-36]. It is found that splenic dendritic cell precursors express β-catenin. β-catenin is the primary mediator of the WNT signaling pathway and is critical for numerous cellular functions, including hematopoietic cell fate determination and proliferation[37,38]. WNT signaling controls the specification of definitive and primitive hematopoiesis from human pluripotent stem cells[39,40]. WNT signals play a role in the regulation of many cellular functions, including embryo development, formation of adult tissues, and homeostasis of these tissues, as well as cell polarity, migration and proliferation, and disruptions in the pathway cause abnormal changes in tissue size, regulation and function[41-43]. β-catenin expression in the white pulp was low in the C and S groups, while it was high in the CTX group. The expression of β-catenin in the red pulp was significantly higher in the CTX group than in the other groups (p=0.0001).

WNT/ β-catenin pathways play a critical role in the maintenance of normal hematopoetic stem cells in early hematopoiesis[24,25]. Loss-of-function and gain-of-function experiments revealed that tight control of WNT signaling and β-catenin activity is required for hematopoietic cell function and cellularity control, including hematopoetic stem cells and mega-karyocytes[44,45]. When comparing the groups, the platelet and WBC levels of the CTX group was found to be significantly lower than that of the Group C. The Group CTX also had the most megakaryocytes in bone marrow aspiration smears, despite a statistically insignificant difference between the groups. WNT-1 and WNT-4 exhibited very intense expression specifically in the white pulp of the CTX group.

β-catenin expression in the white and red pulp was also high in the CTX group. This may be due to increased WNT expression in megakaryocytes in response to thrombocytopenia. In the study of Kuorelahti A, hCG was found to upregulate the WNT ligands in the mouse mammary gland regardless of ovarian steroidogenesis changes[45,46]. From this perspective, it is possible that hCG acted on WNT to increase the number of megakaryocytes.

Despite the prevailing notion that malignant transformation of hematopoetic stem cells is the main cause of leukemia, there is growing evidence that disruption of niche regulation by transformed hematopoietic cells capable of overexpressing WNT signaling or intrinsic stromal defects in gene expression is at least a collaborative factor. Therefore, insights into the normal and altered functions of niche components will aid a better understanding of normal and malignant hematopoiesis and how environmental factors influence these processes[39,40]. A study by Cismaru *et al*.[14] highlights the beneficial effects of hCG exposure on gene regulation in bone marrow-derived stem cells through the upregulation of pluripotent genes and selection of more primitive mesenchymal stem cells with better differentiation potential[14,15]. Although the CTX group's bone marrow aspirations revealed some areas with decreased cellularity and increased fat spaces, there was no significant difference in myeloid cell and erythroid cell percentages between the groups. This supports the role of chorionic gonadotropins in reducing CTX's bone marrow toxicity.

Conclusion

Gonadotropins have gained researchers attention recently for their potential in embryonic development. In this study, we observed the positive effects of the gonadotropins on the toxic effects of a frequently used cytotoxic chemotherapeutic like CTX on peripheral blood, bone marrow, and spleen. We have also found evidence for their beneficial effect via the WNT-β-catenin pathway.

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Conflict of Interest

Authors declare no competing interests.

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