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STUDY THE PROPHYLACTIC EFFECTS OF MELATONIN AND CYANOCOBALAMIN AGAINST CYCLOPHOSPHAMIDE-INDUCED CARDIOTOXICITY

Waleed Khalid Ghanim* , Muhsin Sagheer Ghalib and Karamallah Al-Yousof

Department of Pharmacology and Toxicology, College of Pharmacy, University of Basra, Basra, Iraq

English Cyclophosphamide chemotherapeutic agent, it's used associated with multiorgan toxicity including the heart. Melatonin and cyanocobalamin possess cardiac-protective properties. This study was designed to evaluate the prophylactic effects of melatonin and cyanocobalamin. Fifty adult male rats have used in the study, divided into five groups each containing ten rats, melatonin, and cyanocobalamin were given orally for seven consecutive days before injection of (150 mg/kg) of cyclophosphamide on day seven. ***Group I:*** *Control group, in which rats were intraperitoneally injected with (1 ml/kg/day) of normal saline.* ***Group II:*** *Rats were intraperitoneally injected with a single dose of cyclophosphamide.* ***Group III:*** *Rats were given orally melatonin at a dose (1 mg/kg/day) and cyclophosphamide.* ***Group IV:*** *Rats were given orally cyanocobalamin at a dose (0.1 mg/kg/day) and cyclophosphamide.* ***Group V:*** *Rats were administered orally a mixture of melatonin at a dose (1 mg/kg/day) and cyanocobalamin at a dose (0.1 mg/kg/day) and cyclophosphamide. On day eight rats were sacrificed, serum and heart extracted for malondialdehyde, interleukin-1 beta, and troponin I measurement, furthermore cardiac immunohistochemical study was performed. It was found that both melatonin and cyanocobalamin significantly ($P<0.05$) decrease serum levels of malondialdehyde, interleukin-1 beta, and troponin I in comparison with group II and improve immunohistochemical changes in cardiac tissue when compared with the group treated by cyclophosphamide only (group II).*

Keywords: *cyclophosphamide, melatonin, cyanocobalamin, cardiac toxicity*

INTRODUCTION

Cyclophosphamide (CPD) is a cytotoxic and immunosuppressive agent belonging to the nitrogen mustard alkylating agent which is used to treat different malignant diseases and organ transplantation [1]; previous animal studies have been showing that it's used associated with multi-organ toxicity including kidney, bone marrow, and lung[1&2]. Although the exact mechanism of CPD-induced cardiac toxicity is not fully elucidated, however, these toxicities may be attributed to the metabolic biotransformation of CPD that occurs in the liver to phosphoramide mustard and acrolein[3]. These metabolites will induce oxidative stress and damage to endothelial capillaries[3]. Furthermore, CPD promotes inflammatory cytokines and enhances the phosphorylation of tumor necrosis factor-alpha (TNF-α), nuclear factor- kappa B (NF-*k*B), and interleukin- 1 beta (IL-1β)[4]. All these events may be responsible for cardiotoxicity induced by CPD.

Melatonin is an endogenous neurohormone produced by the pineal gland, released in dark, and regulates the sleep cycle, immune responses, and appetite[5]. Melatonin possesses pleiotropic effects including antioxidant, anti-inflammatory, and free radicals scavenger activity[6] thus play important role in neuroprotection, neurogenesis, maintenance of oxidative

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*Corresponding author: Waleed Khalid Ghanim, E-mail: waleed.ghanim@uobasrah.edu.iq

balance, modulation of the immune system, and cardiovascular system[7]. It was found that melatonin possesses potent antioxidant properties by being a direct scavenger of hydroxyl radicals[8], stimulating the biosynthesis of antioxidant enzymes, and blocking the transcription of proinflammatory cytokines[8& 9].

Cyanocobalamin (vitamin B12) is a water-soluble vitamin[2], which is found in fish, red meat, and dairy products[2]. Human beings are unable to synthesize B12, so they depend on dilatory intake[10]. Vitamin B12 functions as a cofactor for two important reactions involved in the biosynthesis of succinyl-CoA and methionine thereby decreasing the level of homocysteine, in which accumulation of later compounds may responsible for free radicals generation[11]. The elevated plasma level of homocysteine concentration is considered a clinical biomarker for higher risk of cardiovascular disease, myocardial infarction, and ischemic stroke; furthermore increased levels of homocysteine may disturb mitochondrial membrane integrity causing a release of cytochrome c also disturb nitric oxide synthase activity leading to more tissue damage[12].

The present study aims to evaluate the prophylactic effects of melatonin and cyanocobalamin against cyclophosphamide-induced cardiac toxicity

MATERIALS AND METHODS

Fifty healthy adult male rats weighing from 200-230 gm were used in the present study they were bought from the College of the Science University of Dhi Qar and kept under temperature control in the Animal House of Basrah University's College of Pharmacy. The animals were fed commercial pellets and had free access to the water supply throughout the trial.

Drugs

Five hundred milligrams of CPD vial were provided by Baxter in the United States. Melatonin syrup (3 mg/ml) was provided by NOW company, USA. Cyanocobalamin tablet (1 mg) was provided by TQ pharma, Japan.

Study design

The healthy experimental male rats were divided into five groups each one contains ten rats as follows:

- **Group I:** Represents the control group, in which rats were intraperitoneally (IP) injected with (1ml/kg/day) of normal saline (NS) for seven consecutive days.
- **Group II:** Rats were intraperitoneally injected with one dose of CPD (150 mg/kg)[2].
- **Group III:** Rats were given orally melatonin at a dose (1 mg/kg/day)[13] for seven consecutive days and one dose of CPD at a dose (150 mg/kg) on day seven.
- **Group IV:** Rats were given orally cyanocobalamin at a dose (0.1 mg/kg/day)[2] for seven consecutive days and one IP injection of CPD at a dose (150 mg/kg), which is administered on day seven.
- **Group V:** Rats were administered orally a combination of melatonin at a dose (1 mg/kg/day) and cyanocobalamin at a dose (0.1 mg/kg/day) for seven consecutive days and one IP injection of CPD at a dose (150 mg/kg), which is administered on day seven.

At the end of the experiment (on day eight), rats were anesthetized by using diethyl ether. Intracardiac puncture to obtain 6 ±1 mL of blood, which was collected in gel-activated tubes for collection of serum to measure malondialdehyde (MDA), interleukin-1beta (IL-1β), troponin I, and heart extracted for immunohistochemical study.

Immunohistochemical Study

The terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay is used to determine apoptosis in heart tissue[14].

Statistical Analysis

Statistical analysis was done by using Statistical Package for Social Sciences (SPSS) version 25 for windows software. Data were expressed as Mean ± SD. A one-way analysis of variance (ANOVA) was used to assess the statistical significance of the differences

between the experimental groups. P-values less than 0.05 were considered statistically significant differences.

RESULTS AND DISCUSSION

Results

Table 1 showed that rats IP injected with CPD at a dose of (150 mg/kg) (**Group II**) caused a significant elevation ($P< 0.05$) in the level of MDA in the serum when compared with the relevant level in the control group (**Group I**). Moreover, (table 1) showed that there was a significant reduction ($P< 0.05$) in serum MDA level in groups treated with **(**melatonin (1 mg/kg/day) for seven days (**group III**), vitamin B12 (0.1 mg/kg/day) for seven days (**Group IV**), and a combination of melatonin (1 mg/kg/day) plus vitamin B12 (0.1mg/kg/day) for seven days (**Group V)**) before IP injection of (150 mg/kg) of CPD compared to the relevant serum level to (**Group II**).

Table 2 showed that rats IP injected with CPD at a dose of (150mg/kg) (**Group II**) cause a significant elevation ($P<0.05$) in the serum level of IL-1β in comparison with the corresponding level in the control group (**Group I**). Moreover, (table 2) showed that there was a significant reduction ($P< 0.05$) in serum level of IL-1β in groups treated with **(**melatonin (1 mg/kg/day) for seven days (**group III**), cyanocobalamin (0.1mg/kg/day) for seven days (**Group IV**), and melatonin (1mg/kg/day) with cyanocobalamin (0.1 mg/kg/day) (**Group V**) for seven days**)** before IP injection of (150 mg/kg) of CPD at day seven compared to the relevant serum level to (**Group II)**.

Table 3 showed that rats IP injected with CPD at a dose of (150 mg/kg) (**Group II**) produce a significant elevation ($P< 0.05$) in the serum level of troponin I in comparison with the corresponding level in the control group (**Group I**).

Table 1: Effects of Melatonin and Cyanocobalamin on Serum MDA level

Groups/Treatment	Mean MDA level ng/ml ±SD
Group I (IP NS 1 ml/kg/day) for seven days	67 ± 0.632^{a}
Group II CPD single IP 150mg/kg	949 ± 0.894^{b}
Group III Melatonin (1mg/kg/day) for seven days prior to CPD (IP 150mg/kg)	449 ± 0.894^{c}
Group IV Cyanocobalamin (0.1mg/kg/day) for seven days prior to CPD (IP 150mg/kg)	749.1 ± 0.752^{d}
Group V Melatonin (1 mg/kg/day) plus Cyanocobalamin (0.1mg/kg/day) for seven days prior to CPD (IP 150mg/kg)	239.1 ± 0.752^{e}

Each value represents mean ± standard deviation (SD). Values expressed in small letters (a, b, c, d, and e) are significantly different (P<0.05). Number of animals in each group=10

Table 2: Effects of Melatonin and Cyanocobalamin on Serum IL-1β level

Group/Treatment	Mean IL-1β level pg/ml ±SD
Group I (IP 1 ml/kg/day) for seven days	44.1 ± 0.752[a]
Group II /CPD single IP 150mg/kg	418.8 ± 0.983[b]
Group III Melatonin (1mg/kg/day) for seven days prior to CPD (IP 150mg/kg)	249 ± 0.894[c]
Group IV Cyanocobalamin (0.1mg/kg/day) for seven days prior to CPD (IP 150mg/kg)	309.1 ± 0.983[d]
Group V Melatonin (1 mg/kg/day) plus Cyanocobalamin (0.1mg/kg/day) for seven days prior to CPD (IP 150mg/kg)	159.3 ± 0.816[e]

Each value represents mean ± standard deviation (SD). Values expressed in small letters (a, b, c, d, and e) are significantly different ($P<0.05$). Number of animals in each group=10

Table 3: Effects of Melatonin and Cyanocobalamin on Serum Troponin I level

Group/Treatment	Mean troponin I level pg/ml ±SD
Group I (IP 1 ml/kg/day) for seven days	40.6 ± 0.516[a]
Group II /CPD single IP 150mg/kg	829.6 ± 0.516[b]
Group III Melatonin (1mg/kg/day) for seven days prior to CPD (IP 150mg/kg)	539.3 ± 0.816[c]
Group IV Cyanocobalamin (0.1mg/kg/day) for seven days prior to CPD (IP 150mg/kg)	619 ± 0.894[d]
Group V Melatonin (1 mg/kg/day) plus Cyanocobalamin (0.1mg/kg/day) for seven days prior to CPD (IP 150mg/kg)	311.1 ± 0.752[e]

Each value represents mean ± standard deviation (SD). Values expressed in small letters (a, b, c, d, and e) are significantly different ($P< 0.05$). Number of animals in each group=10

Immunohistochemistry (TUNEL assay) of rats' heart tissue

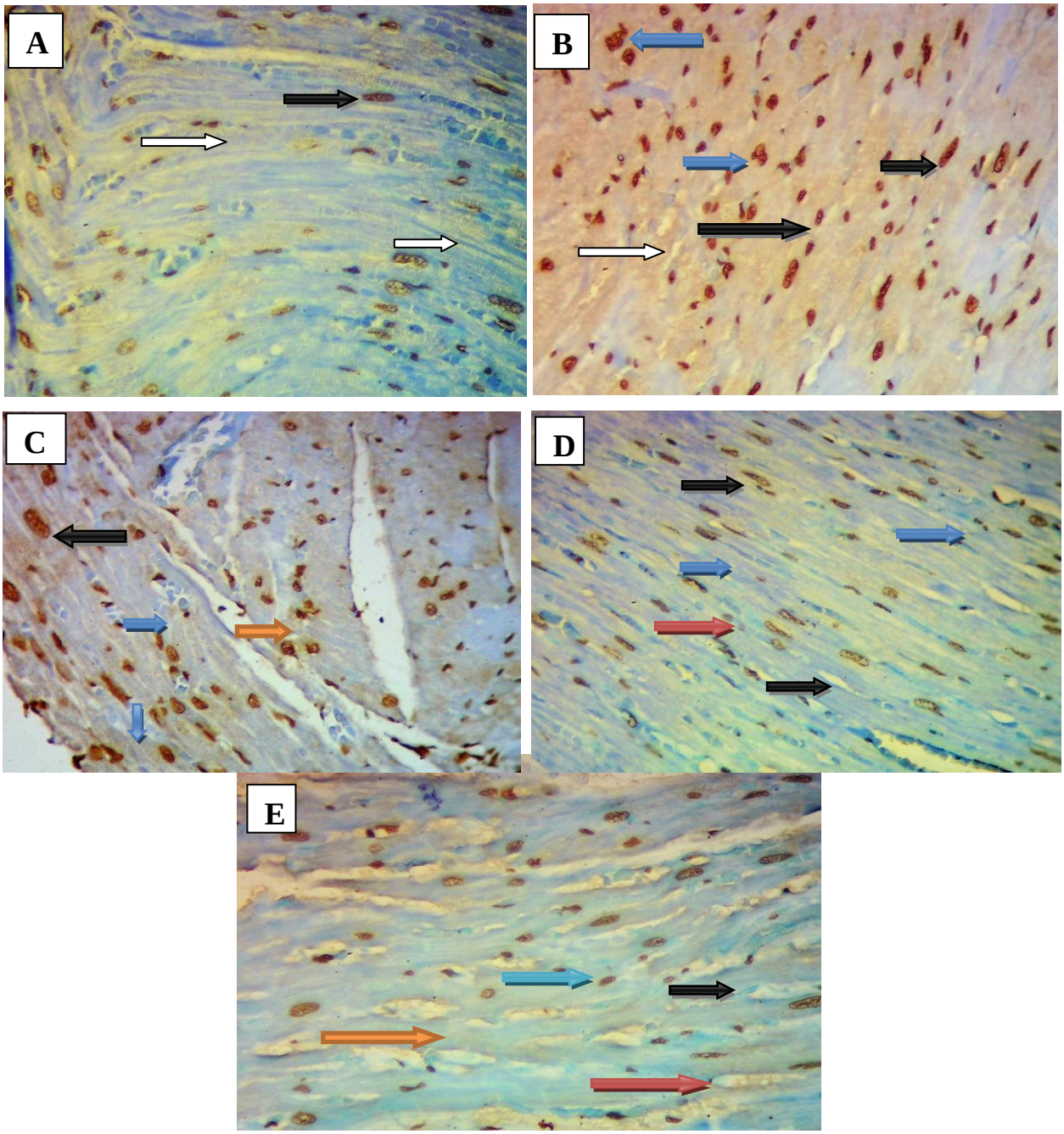

Fig.1: Light micrograph of immunohistopathological changes in heart tissue with Tunel assay of male rats are presented on the plate. **A)** Normal cardiac muscle in the longitudinal section of the control rat shows a syncytium of myocardial fibers with centrally located nuclei (black arrow)., striated muscle (white arrow), and sarcomeres (red arrow). **B)** In Cardiac tissue of rats treated with 150mg /kg B.W of CPD, the myocardial fibers are losing cross-striations (white arrow), a different form of the nucleus, undergoing karyolysis (black arrow) as well as apoptotic bodies (blue arrow).

C) this section of the heart treated with 1 mg/kg B.W of melatonin shows the appearance of cardiac fibers, less than B plate with normal sarcomeres (blue arrow), nucleus fewer effects have spindle shape (black arrow), and other under karyolysis (green arrow). **D)** the myocardial was treated with 0.1 mg/B. W of Vit. B12 shows are beginning to cross striation (red arrow), the nucleus is normal spindle ship respectively (black arrow), finally normal sarcomeres (blue arrow). **E)** cardiac muscle of rat treated with a combination of 1 mg/kg of melatonin plus 0.1 mg/kg Vit. B12 shows normal striated muscle (red arrow), with centrally located nuclei (blue arrow) normal cardiac muscle fibers branches (green arrow), and normal sarcomeres (black arrow). Tunel assay, 20X.

Moreover, (table 2) showed that there was a significant reduction (P<0.05) in serum level of troponin I in groups treated with **(**melatonin (1mg/kg/day) for seven days (**group III**), cyanocobalamin (0.1mg/kg/day) for seven days (**Group IV**), and melatonin (1mg/kg/day) with cyanocobalamin (0.1mg/kg/day) (**Group V**) for seven days before IP injection of (150mg/kg) of CPD compared to the corresponding serum level of troponin I in (**Group II)**.

Discussion

In the current study, rats treated with CPD (150 mg/kg) cause a significant elevation in the serum levels of MDA, IL-1β, and troponin I (P< 0.05) compared to the control group this indicated that cardiac toxicity induced by CPD, although the precise mechanism of toxicity not fully elucidated, however, it could be attributed to the active metabolite of CPD which include phosphoramide mustard and acrolein [15]. It is believed that these metabolites could induce oxidative stress and free radicals generation which may cause damage to capillary endothelial lining with resultant extravasation and leakage of erythrocytes, proteins, and free radicals which may cause further damage to myocardium and blood vessels[16]. Furthermore, it was found that treatment with CPD may cause inhibition of carnitine palmitoyl transferase-I gene expression and heart-type fatty acid-binding proteins in the heart[17], such inhibition may lead to decrease energy production and result in fatty acid oxidation and free radicals generation with subsequent cardiac damage[17]. Moreover, CPD promotes the expression of proinflammatory cytokine[18], enhances the phosphorylation of nuclear factor-kappa B, TNF-α, IL-1β, and increases serum level of cyclooxygenase-2 which may cause multi-organ toxicity including heart, kidney, lung, and bone marrow[18].

The results of the present study demonstrated that oral administration of melatonin (1 mg/kg/day), cyanocobalamin (0.1 mg/kg/day), and their combination for seven consecutive days produce a significant reduction in serum levels of MDA, IL-1β, and troponin I compared to rats treated with 150 mg/kg of CPD, these effects could be explained as follows.

Melatonin has potent antioxidant properties, it protects organs and tissues from damage caused by free radicals[19], melatonin act as a direct scavenger for the most powerful free radicals which is hydroxyl radical which can attack DNA, lipids, and proteins and cause pathogenesis and also for other reactive oxygen and nitrogen species[19], unlike biological antioxidant molecules like vitamin C and vitamin E, melatonin doesn't undergo redox cycling, thus it will not promote further oxidation [20]. it was found that melatonin can promote DNA repair and biosynthesis of antioxidant enzymes, furthermore, melatonin attenuates the expression of inflammatory cytokines like IL-1β, transcription of TNF-α, and COX-2 expression which may reduce cardiac damage induced by CPD[21].

Moreover, cyanocobalamin can act also as a direct scavenger for superoxide anion[22], and also being as a cofactor for enzymatic conversion of homocysteine to methionine in which accumulation of homocysteine may cause cardiac damage and induce oxidative stress conditions furthermore cyanocobalamin modulate inflammatory cytokine release[23], all these events may explain the effects of cyanocobalamin on serum levels of MDA, IL-1β, and troponin I these results agree with the work of *Mauro S, et al* [24] and *Farah M, et al*[25].

In the present study rats treated with 150 mg/kg of CPD shows, that the myocardial fibers are losing cross-striations, a different form of the nucleus, undergone karyolysis as well apoptotic bodies when compared with the control group. whereas rats treated with 1 mg/kg of melatonin before IP injection of CPD shows the appearance of cardiac fibers, less than B plate with normal sarcomeres (blue arrow), a nucleus with fewer effects has spindle shape (black arrow), and other under karyolysis when compared with rats treated with 150 mg/kg of CPD. furthermore, rats treated with 0.1 mg/kg of cyanocobalamin before IP injection of CPD show are beginning to cross striation, the nucleus is normal spindle ship respectively when compared with rats treated with 150 mg/kg of CPD, finally normal sarcomeres cardiac muscle of rat treated with a combination of 1 mg/kg of melatonin plus 0.1 mg/kg Vit. B12 shows normal striated muscle,

with centrally located nuclei normal cardiac muscle fibers branches, and normal sarcomeres when compared with rats treated with 150 mg/kg all these events may be attributed to the effects of melatonin and cyanocobalamin as an antioxidant and/or their effects on the expression inflammatory cytokines transcription of TNF-α, and COX-2[21, 22, 24].

Conclusions

Cyclophosphamide has serious cardiac toxic side effects, so to reduce such effects, pre-treatment with melatonin and/or cyanocobalamin will reduce this toxic effect.

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نشرة العلوم الصيدلية
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دراسة الاثار الوقائية للميلاتونين والسيانوكوبالامين ضد السمية القلبية التي يسببها سيكلوفوسفامايد

وليد خالد غانم – محسن صغير غالب – كرم الله اليوسف

قسم الأدوية و السموم ، كلية الصيدلة ، جامعة البصرة ، العراق

عامل العلاج الكيميائي سيكلوفوسفاميد ، يستخدم مرتبطًا بتسمم العديد من الأعضاء بما في ذلك القلب. يمتلك الميلاتونين والسيانوكوبالامين خصائص واقية للقلب. تم تصميم هذه الدراسة لتقييم الآثار الوقائية للميلاتونين والسيانوكوبالامين. استخدم خمسون فأرًا بالغًا للدراسة ، مقسمة إلى خمس مجموعات تحتوي كل منها على عشرة جرذان ، الميلاتونين ، وسيانوكوبالامين ، تعطى عن طريق الفم لمدة سبعة أيام متتالية قبل حقن (١٥٠ مجم / كجم) من سيكلوفوسفاميد في اليوم السابع. المجموعة الأولى: المجموعة الضابطة ، حيث تم حقن الجرذان داخل الصفاق بـــ (١ مل / كجم / يوم) من محلول ملحي عادي. المجموعة الثانية: تم حقن الجرذان داخل الصفاق بجرعة وحيدة من سيكلوفوسفاميد. المجموعة الثالثة: أعطيت الجرذان الميلاتونين عن طريق الفم بجرعة (١ مغ / كغ / يوم) وسيكلوفوسفاميد. المجموعة الرابعة: تم إعطاء الجرذان سيانوكوبالامين عن طريق الفم بجرعة (٠.١ مجم / كجم / يوم) وسيكلوفوسفاميد. المجموعة الخامسة: تم إعطاء الجرذان عن طريق الفم خليط من الميلاتونين بجرعة (١ مجم / كجم / يوم) وسيانوكوبالامين بجرعة (٠.١ مجم / كجم / يوم) وسيكلوفوسفاميد. في اليوم الثامن تم ذبح الجرذان ، واستخراج المصل والقلب من أجل قياس malondialdehyde ، و interleukin-١ beta ، و Troponin I ، علاوة على إجراء دراسة الكيمياء المناعية لانسجة القلب. وجد أن كلا من الميلاتونين والسيانوكوبالامين يقللان بشكل كبير ($P<0.05$) من مستوى المصل من malondialdehyde و interleukin-1 beta و Troponin I بالمقارنة مع المجموعة الثانية ويحسن التغيرات المناعية في الأنسجة القلبية عند مقارنتها بالمجموعة المعالجة بسيكلوفوسفاميد فقط (المجموعة الثانية).