



(RESEARCH ARTICLE)



Evaluation of the protective effect of melatonin on histological changes in the liver of adult male rats treated with gemcitabine

Hamideh Khakshour ¹, Behnam Ahmadnezhad ^{2, *}, Mostafa Shalimar ³, Pejman Mortazavi ⁴ and Mitra Aghazadeh ⁵

¹ Faculty of Pharmacy, Mazandaran University of Medical Sciences, Ramsar Campus.

² Department of Pharmacology, Faculty of Veterinary Sciences, Islamic Azad University, Research Sciences Branch, Tehran, Iran.

³ Department of basic sciences, Faculty of Veterinary Sciences, Islamic Azad University, Research Sciences Branch, Tehran, Iran.

⁴ Department of Pathology, Faculty of Veterinary Sciences, Islamic Azad University, Research Sciences Branch, Tehran, Iran.

⁵ Faculty of histological Veterinary Sciences, Islamic Azad University, Research Sciences Branch, Tehran, Iran.

GSC Biological and Pharmaceutical Sciences, 2022, 19(01), 297–303

Publication history: Received on 17 March 2022; revised on 23 April 2022; accepted on 25 April 2022

Article DOI: <https://doi.org/10.30574/gscbps.2022.19.1.0157>

Abstract

Melatonin is an endocrine hormone, produced by the pineal gland from the amino acid tryptophan. Melatonin level in the body according to a predetermined cycle and are affected by light received. Gemcitabine is an anti-cancer drug. The mechanism of action of gemcitabine affects the properties of the cellular phase and mainly destroys the cells that are making DNA (phase-s). Nephrotoxic side effects of gemcitabine have been reported in patients receiving this drug medication. Melatonin is a potent free radical scavenger and antioxidant and has protective effects against ischemic damage. The aim of the present study was to investigate the protective effect of melatonin on histological changes in the liver of adult male rats treated with gemcitabine and to evaluate the effects of different doses of melatonin on the protection of adult liver cells treated with gemcitabine. This experimental study was performed on male Wistar rats weighing approximately 250±20g and aged 8 weeks. Mice were randomly divided into 3 main groups. The first group (G1); the control group, received only normal saline. The second group (G2); the treated groups, divided into 2 subgroups T1 and T2, This group received melatonin at doses of 10 and 20 mg/kg and intraperitoneal (IP) with gemcitabine at a dose of 50 mg/kg, respectively. The third group (G3); negative control group, divided into 3 subgroups T3, T4 and T5. Subgroup T3, gemcitabine at 50 mg/kg, and T4 and T5, they received melatonin at doses of 10 and 20 mg/kg, intraperitoneally (IP) respectively. The number of mice in each group was 6 mice.

The results obtained from the injection of gemcitabine in groups clearly showed the side and cytotoxic effects in the liver tissue that the most lesions were observed in the subgroup (T3). In the groups receiving melatonin, the amount of these lesions was significantly reduced and the reduction of injuries was directly related to the dose of melatonin. The results of this study show that melatonin is effective in reducing the side effects of gemcitabine.

Keywords: Gemcitabine; Melatonin; Fat Degeneration; Chemotherapy; Inflammation

1. Introduction

Cancer has become very widespread in recent years. And the spread of this disease requires new therapies and new strategies to reduce the side effects of conventional therapies. Rutin drugs have side effects and are toxic to the body.

* Corresponding author: Behnam Ahmadnezhad

Pharmacology, Faculty of Veterinary Sciences, Islamic Azad University, Research Sciences Branch, Tehran, Iran.

One of the most effective anti-cancer drugs is gemcitabine, which is used to treat a variety of malignant tumors. In previous studies, the toxic effects and side effects on other tissues of the body has been proven during treatment [1]. The mechanism of action of gemcitabine is based on the effect on cell phase properties and mainly destroys the cells that are making DNA (phase-s) [2, 3]. Melatonin is secreted by the pineal gland in the brain, and also regulates immunity and temperature. In addition, melatonin affects cell proliferation and differentiation and can also be used as an antioxidant [4, 5]. Melatonin can detect and neutralize free radicals, including hydroxyl radicals, peroxy radicals, and peroxynitratrates [6].

The antioxidant properties of melatonin are important due to the use of chemotherapy drugs in the treatment of cancer patients. Therefore, the aim of this study was to investigate the protective effect of melatonin on histological changes in the liver of adult male rats treated with gemcitabine, as well as the effects of melatonin on the protection of liver cells.

2. Material and methods

The statistical society consisted of 48 male Wistar rats weighing approximately 230 ± 20 g, with the age of 8 weeks. Before starting the study, the mice underwent a week of adaptation with free access to food and water; then, they were randomly divided into 3 main groups and each group consisted of 8 mice.

- Control group (G1), which received only a specific dose of normal saline.
- Treatment group (G2), which was divided into 2 subgroups (T2, T1).
- T1 and T2 Subgroups, with melatonin at doses of 10 and 20 mg/kg, respectively, along with gemcitabine at a dose of 50 mg/kg through intraperitoneal (IP) and single dose. The duration of melatonin was 14 days (IP).
- Negative control group (G3), which was divided into 3 subgroups (T3, T4, T5).

Subgroup T3 received gemcitabine at a dose of 50 mg/kg intraperitoneally (IP) and as a single dose. Subgroup T4 and T5 received melatonin at doses of 10 and 20 mg/kg, respectively, intraperitoneally (IP) for 14 days.

2.1. Preparation of melatonin

Dissolve 500 mg of melatonin (Sigma) in propylene glycol and dilute to 2 times the volume with distilled water to a final concentration of 10% to increase the effective amount of melatonin to 10 mg / ml. In order to prepare the isotonic solution, sodium chloride was added and a 0.22 μ m filter was used for final filtration. The prepared melatonin was placed in containers away from light at a temperature of 4 °C [7].

2.2. Preparation of Gemcitabine

0.9% sodium chloride was used to dilute the injection form of gemcitabine. The maximum concentration for gemcitabine is 40 mg/kg. In this study, we used a concentration of 20 mg/kg [8].

2.3. Sampling

Two weeks after the first injection, the mice were anesthetized and after facilitation, the samples were separated from each group and placed in 10% formalin and prepared for tissue sections, and then examined using staining (H&E) and light microscopy [8].

2.4. Quantitative evaluation of parameters

Table 1 Quantitative evaluation

Visible damage rate / quality assessment	Value
No damage	0
Local damage 25% in hepatocytes	1
Local damage 25% -50% in hepatocytes	2
Extensive focal damage in hepatocytes	3
Extensive damage to hepatocytes	4

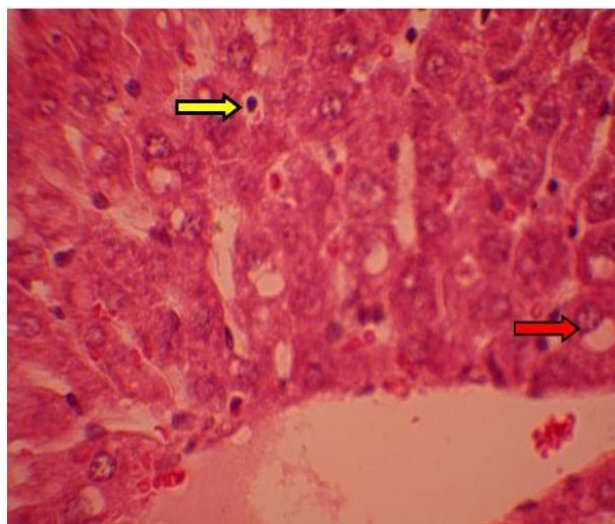
Injuries observed in different groups included cell swelling, fat degeneration, infiltration of mononuclear inflammatory cells and necrosis of hepatocytes. Pathological findings are qualitative. In order to compare between groups and the significance of differences between groups, these findings should be evaluated numerically and quantitatively. Table 1.

The overall results were significantly expressed \pm SEM (standard error), and the findings were considered as the mean percentage and standard deviation of expression, and $P < 0.05$ and $P < 0.01$ mean -Table 2.

Table 2 Histopathological lesions in experimental group (Mean \pm SD)

Group	sub Group	Fat degeneration	Cellular swelling	necrosis	inflammatory cells
G1	-	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
G2	T1	1.46 \pm 0.01	1.50 \pm 0.05	0.35 \pm 0.05	1.51 \pm 0.05
	T2	1.05 \pm 0.01	1.12 \pm 0.01	0.15 \pm 0.01	0.99 \pm 0.01
	T3	2.31 \pm 0.01	2.36 \pm 0.01	1.71 \pm 0.02	1.95 \pm 0.01
G3	T4	0.05 \pm 0.05	0.32 \pm 0.04	0.13 \pm 0.04	0.35 \pm 0.05
	T5	0 \pm 0	0 \pm 0	0 \pm 0	0.29 \pm 0.05

3. Results

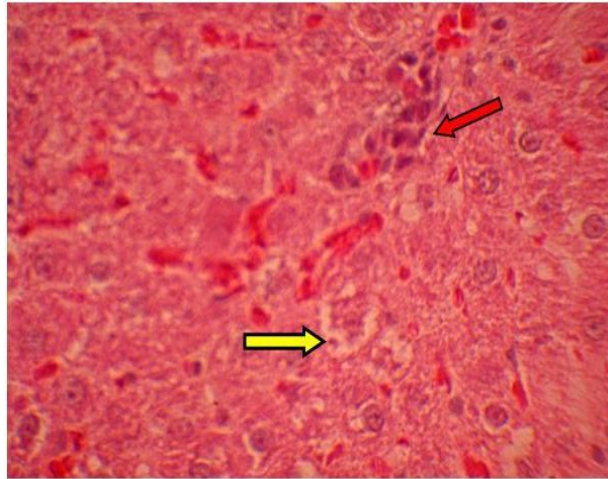


Yellow arrow: hepatocyte necrosis; Red arrow: fat degeneration

Figure 1 Histology micrograph of negative control group/subgroup T3 (Gancitabine), H&E \times 680

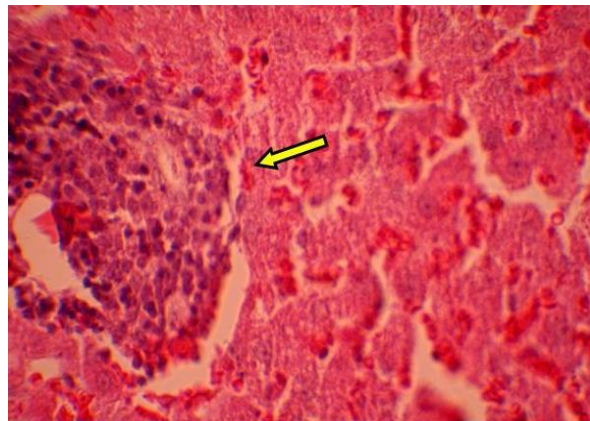
Examining control group samples did not show any significant changes, but histological examination of negative control group, subgroup (T3) had significant side effects, including effects of fat degeneration, penetration of inflammatory cells, and necrosis -Figure 1, Figure 2. Comparison of treatment groups, Concomitant use of gemcitabine and melatonin, showed a significant reduction in lesions in these groups (T1, T2) compared to group (T3).

The rate of injury was much lower in subgroup (T2) who received higher dose of melatonin than subgroup (T1) -Figure 3, Figure 4, Figure 5. Histology examination negative control group, subgroup (T4, T5), which received melatonin at doses of 10 and 20 mg/kg intraperitoneally, showed no specific and significant symptoms. Significant point in some histology slides of subgroup (T5) was observed. Very few lesions were seen in this area, however, they were not statistically significant. The incidence of these injuries was also inversely related to the dose of melatonin (dose comparison in T4 and T5 subgroups). It is possible that these injuries were caused by human and operational error - Figure 6.



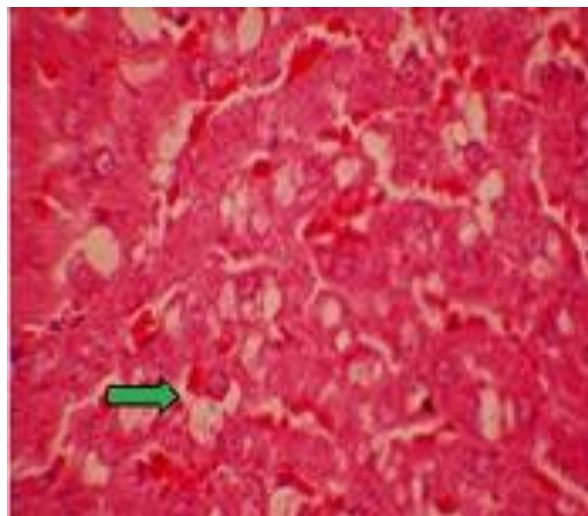
Yellow arrow: fat degeneration; Red arrow: infiltration of inflammatory cells

Figure 2 Histology micrograph of negative control group /subgroup T3 (Gamcitabine), H&E ×680



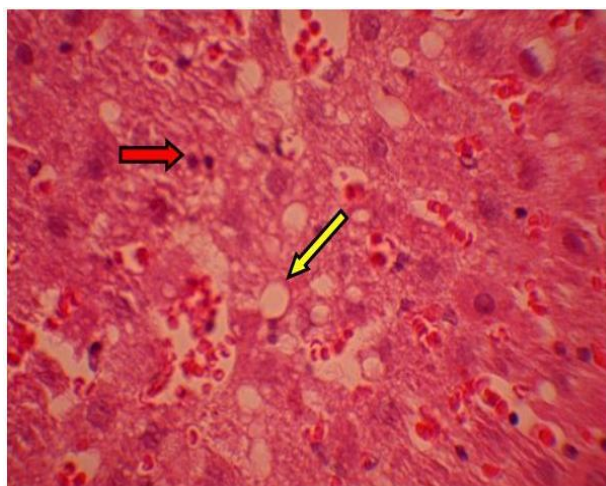
Yellow arrow: Infiltration of inflammatory cells

Figure 3 Histology micrograph of treatment groups/ subgroup T1 (Gemcitabine + Melatonin 10 mg/kg), H & E ×680



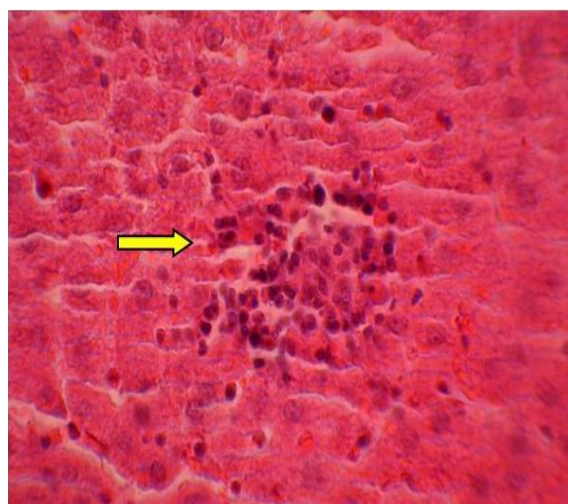
Green arrow: cell swelling and fat degeneration

Figure 4 Histological summary of treatment groups/ subgroup T1 (Gemcitabine + Melatonin 10 mg/kg), H & E ×680



Red arrow: hepatocyte necrosis; Yellow arrow: fat degeneration

Figure 5 Histology micrograph of treatment groups/ subgroup T2 (Gamcitabine+Melatonin 20 mg/kg), H & E ×680



Yellow arrow: Infiltration of inflammatory cells

Figure 6 Histological summary of negative control group /subgroup T5 (Melatonin 20 mg/kg), H & E ×680

4. Discussion

The incidence of side effects of anti-cancer drugs is very different in cancer patients which are being treated. Also, the dose of chemotherapy drugs, depending on the patient's weight and the patient's clinical parameters, as well as the length of treatment, vary according to the extent of the disease. Studies show that the risk level of gemcitabine increases when physicians mix gemcitabine with other drugs of chemotherapy to raise the efficiency of this treatment [9, 10]. The observed tissue and cellular changes completely indicate the side and toxic effects of this drug and indicate the alkylating property of gemcitabine [11].

Concluded that gemcitabine has Cytogenic, clastogenic and myotagenic effects. Past studies have shown that gemcitabine may cause apoptosis of lymphatic cancer cells due to DNA isolation [12]. In this study, these cases were observed in the treated group (subgroup T3) that was affected by gemcitabine.

Melatonin plays a role in regulating some physiological phenomena and other hormones in the body. The mechanism of melatonin is not fully understood, but its beneficial effects in treatment have been well observed and reported. Studies have shown the antioxidant and protective role of melatonin [13, 14]. The study on the protective effect of melatonin on the cytotoxic chemotherapy of busulfan referred to the fertility of testes in mice. The results of this study show that melatonin has a protective effect on busulfan-induced testicular lesions [15]. Also, in the study on the effects of melatonin (5-methoxy-N-acetyltryptamine) in cancer, its anti-cancer effect has been proven [16]. In this study, the

protective properties of this hormone in the treatment groups used in this study (T1, T2), which was associated with a reduction in toxic and side effects of gemcitabine on liver tissue were completely observed, which is the same as the results of previous studies.

5. Conclusion

Based on the results obtained in this study, the amount of injuries in subgroups (T1, T2), which received gemcitabine as well as melatonin in different doses, was significantly reduced compared to the subgroup (T3) that received only gemcitabine. This decrease indicates the protective effect of melatonin. Based on the results, the amount of injury reduction was directly related to the dose of melatonin.

Also in the negative control group, subgroup (T4, T5), which received only melatonin with different doses, scattered lesions were observed, but these lesions were not statistically significant. However, no evidence of melatonin toxicity has been found in high doses. The results of this study showed that these injuries were completely inversely related to the dose received and were not statistically significant.

This study suggests that melatonin may have a protective effect on liver tissue and function after gemcitabine administration, which was further observed at a dose of 20 mg/kg.

Compliance with ethical standards

Acknowledgments

We take this opportunity to thank all those who supported us, To share useful and educational comments. We sincerely thank them.

Disclosure of conflict of interest

All authors acknowledge that there is no conflict of interest / competing interests in this study.

Statement of ethical approval

The tested animals were kept in standard conditions, with free access to water and food. Under the supervision of a laboratory animal expert.

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