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Histological Effect of Gemcitabine on the Liver and Kidney of Male Rat with and without Melatonin

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Abstract

AIM: The goal of this work is to identify the effect of gemcitabine and the role of melatonin on the liver and kidney tissues of rat and whether melatonin has any protective effect on these tissues.

MATERIALS AND METHODS: Thirty-two adults male Wistar rats were divided into four groups. The control group is Group A which administered only normal saline. Group B received gemcitabine alone (25 mg/kg) body weight intraperitoneally once weekly for 4 successive weeks. Group C received gemcitabine intraperitoneally (25 mg/kg) and melatonin orally in a dose of 10 mg/kg once per week for 4 successive weeks. Group D received only melatonin 10 mg/kg once per week for 4 weeks.

RESULTS: The histological study of liver tissue of Group B showed disorganization of hepatic tissue with infiltration by chronic inflammatory cells. In addition to congestion around blood vessels and periportal area. Nuclei of some hepatocytes were vesicular with steatosis. In Group C, liver sections showed inflammatory cell infiltration with mild pyknosis of some hepatocytes. Liver sections of Group D were limited to degeneration of some hepatocytes. Renal sections of Group B revealed that degenerative and necrotic changes of the epithelial cells lining the tubules with blood vessel wall thickening, congestion, and thrombus formation with cystic appearance in the interstitial tissue were detected. While in group C, the histological sections showed epithelial cells swelling with vascular congestion. Renal sections of Group D were more or less normal.

CONCLUSION: This study concluded that gemcitabine-induced toxic effect on liver and kidney of male rats and melatonin may play a protective effect on the tissues of these organs.

Introduction

The numbers of cancer patients are increasing all over the world, thus medical researchers afford and present new antineoplastic drugs to the market [1]. Gemcitabine is one of the most advised worldwide cytotoxic medicine (pyrimidine analog 2′, 2′-difluorodeoxycytidine), classified as an antimetabolite with many brand names including gemzar [2]. It acts by inhibiting DNA synthesis and begins intracellular activation through deoxycytidine kinase [3].

Gemcitabine is detoxified in the liver to the active diphosphate and triphosphate nucleosides by the aid of nucleoside kinase enzyme [4]. This drug cannot differentiate between normal cell and malignant cell, so it produces its toxic effect on both cells but the normal cells can repair the injury and retain their normal function while the malignant cell will be destructed and all the adverse reactions of gemcitabine are due to this process [5]. As all other anticancer agents, it may cause gastrointestinal upset including nausea, vomiting, and constipation, in addition to other side effects such as hair loss, bone marrow suppression, and fertility complications [6]. It is metabolized in liver and excreted by kidney so it is recommended to be given with attention in patients with renal and hepatic insufficiency [7], [8].

Melatonin (*N*-acetyl-5-methoxytryptamine) hormone is a night-time released substance throughout dark hours being formed by the pineal body [9]. It has a significant effect by regulating the biological rhythm, enhancing healthy sleep with an immune regulating effect [10]. It enhances deep sleep through reducing the wake enhancing signals [11].

It is proven as a scavenger which destructs the free radicles such as hydrogen peroxides to promote its antioxidant effect on tissues [12].

Melatonin has anti-inflammatory action as it is an important free radical hunter and scavenger and enhance the proinflammatory factors in early stages of inflammation [13]. It is believed that melatonin can reduce liver injuries caused by some drugs through reducing oxidative destruction, mitochondrial and microsomal peroxidation, and inflammatory cell infiltration [14]. In addition, it has a protective effect against renal tissue injury induced by drugs due to its antioxidant property [15].

The goal of this work is to identify the effect of gemcitabine and the role of melatonin on the liver and

kidney tissues of rat and whether melatonin has any protective effect on these tissues.

Materials and Methods

Drugs

Gemcitabine was supplied by manufacturer Onko ilac San. Ve. Tic. A.S. Gebze/Kocaeli/Turkey under traditional name (Gemtu) 1000 mg/10 ml. Melatonin was supplied by manufacturer Shlaf Gut, Istanbul, Turkey.

Ethical approval

The ethical approval was obtained by the ethical committee/college of medicine/university of Mosul.

Animals

Thirty-two adults male Wistar rats were supplied by the animal house section of veterinary college at the University of Mosul. They are weighing 200–300 g at the beginning of the study. The animals were kept in typical conditions (temperature 22 ± 2°C, light-dark cycle of 12:12 h). All rats were fed by ad libitum and supplied with drinking water. They were weighed every week throughout the experiment.

Experimental design

The treated animals were separated into four groups with eight rats in each group. The control group was Group A which administered only normal saline. Group B administered gemcitabine alone (25 mg/kg) body weight intraperitoneally once weekly for 4 successive weeks. Group C received gemcitabine intraperitoneally in a dose of 25 mg/kg and melatonin orally in a dose of 10mg/kg once per week for 4 successive weeks. Group D received only melatonin 10mg/kg once per week for 4 weeks [16], [17].

One week later after the end of the experiment, the treated animals were sacrificed. Anatomical dissection of the abdomen to dissect both liver and kidney was done and prepared for histological examination.

Histological examination

Paraffin sections were obtained using Reichters microtom to get 5 mm thickness sections. Hematoxyline and eosin stain were used to examine the pathological changes [18].

Observations and Results

Group A (control)

Physical observations

Animals of this group looked normal, with very good activity and appetite.

Histological examination

Normal histological appearance of liver tissue with normal histological features of the hepatocytes is shown in Figure 1. Normal histological picture of renal tissue, with normal looking glomeruli and tubules, is shown in Figure 2.

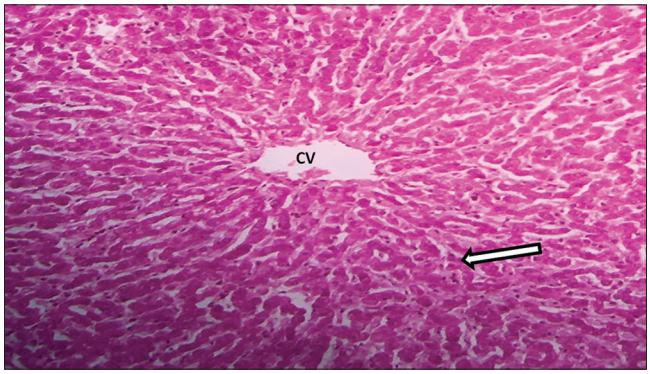

Figure 1: Normal hepatocytes (white arrow) with normal central venule. (HE stain 400×)

Group B

Physical observations

Animals of this group looked ill, with decreased activity and reduced appetite compared to control group.

Histological examination

The histological study of liver tissue showed disorganization of hepatic tissue with infiltration by chronic inflammatory cells. In addition to congestion,

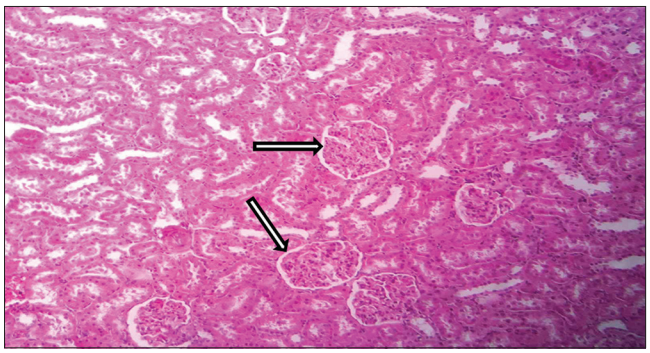

Figure 2: Normal glomeruli and tubules. (white arrows). (HE stain 400×)

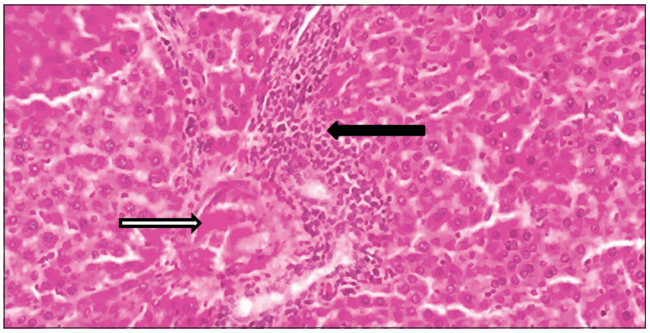

Figure 3: Congestion of portal area (white arrow) with infiltration by chronic inflammatory cells (black arrow). (HE stain 400×)

around blood vessels and periportal area are shown in Figure 3. Some nuclei of hepatocytes were vesicular and there were steatosis (Figure 4), congestion of sinusoids, and central vein, with increase in the number of Kupffer cells (Figure 5). Binucleated hepatocytes were also observed occasionally (Figure 6).

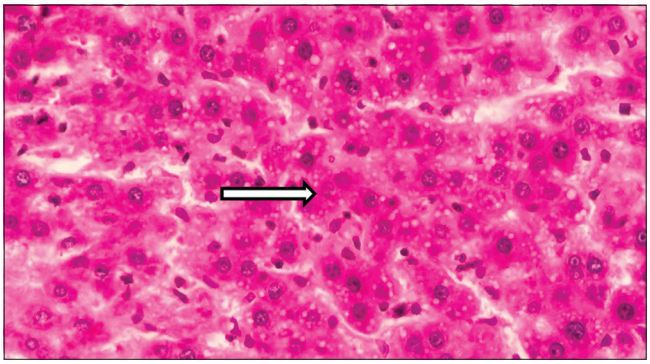

Figure 4: Disorganization of hepatic parenchyma. The nuclei are vesicular, and steatosis is detected (white arrow). (HE stain 600×)

The kidney sections showed various severe histological changes characterized by degenerative and necrotic changes of epithelial lining cells (Figure 7). Other sections showed thickening of blood vessel wall, congestion, and thrombus formation (Figure 8). Cystic appearance in the interstitial tissue was also observed (Figure 9). The glomeruli showed lobulation and shrinkage with expansion of renal space with inflammatory cells infiltration around blood vessels and interstitial tissue (Figure 10). Other areas showed

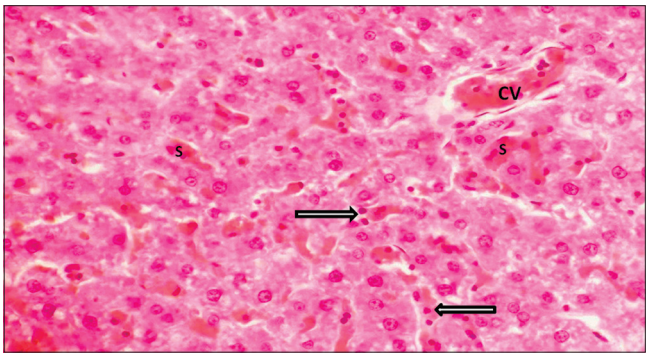

Figure 5: Congestion and dilatation of sinusoids (s), central vein (cv), with increase in the number of Kupffer cells (white arrows). (HE stain 400×).

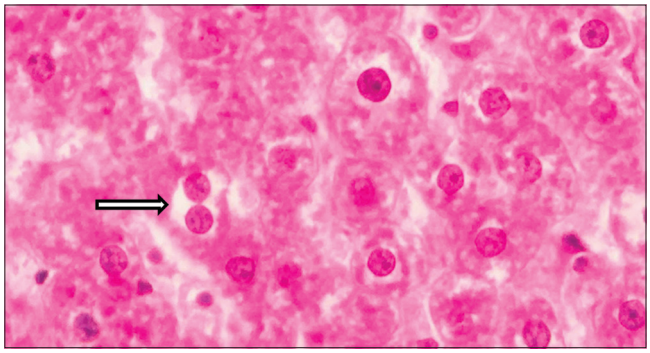

Figure 6: Binucleated hepatocytes (white arrow). (HE stain 1000×)

accumulation of proteinous substance [hyaline cast] in the lumen of renal tubules (Figure 11).

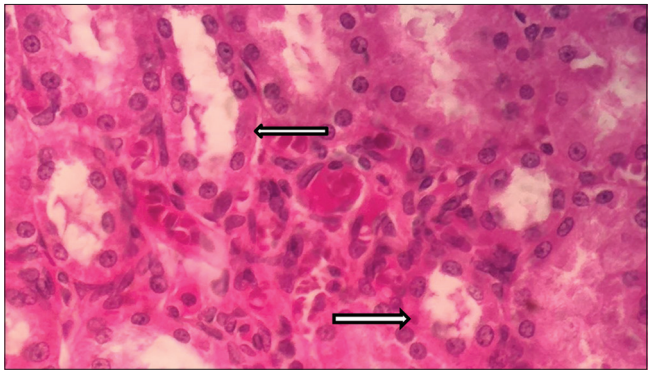

Figure 7: Degenerative and necrotic changes of epithelial cells (white arrows). (HE stain 600×).

Group C

Physical observations

Animals of this group looked ill, with decreased activity and reduced appetite compared to control group.

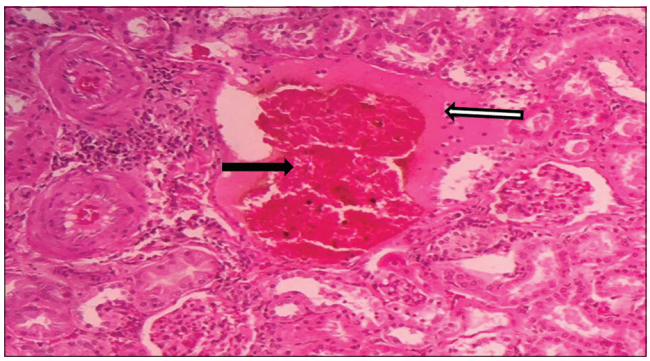

Figure 8: Blood vessel wall thickening (white arrow), congestion and thrombus formation (black arrow). (HE stain 400×)

Histological examination

The treatment of animals with melatonin before cytotoxic drug produced comparatively less pathological changes as compared to group of animals treated with cytotoxic drug only (Group B). Inflammatory cell infiltration with mild pyknosis and necrosis of other hepatocytes is shown in (Figure 12). The kidney

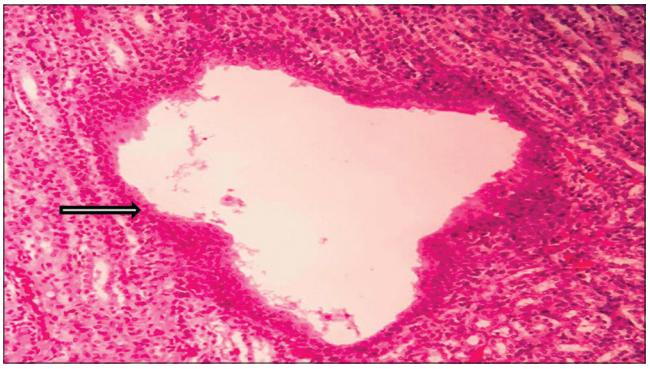

Figure 9: B shows cyst formation in the interstitial tissue of renal section (white arrow). (HE stain 400×)

shows epithelial cells swelling with vascular congestion (Figure 13).

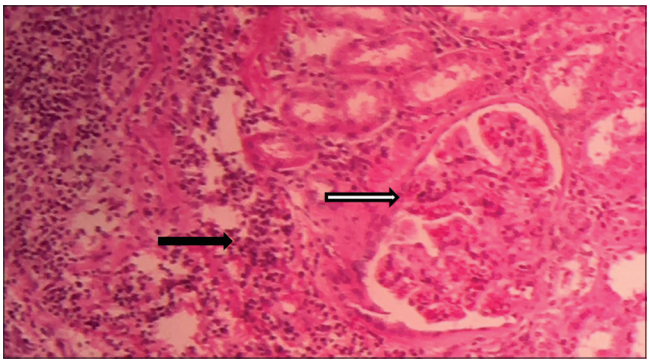

Figure 10: Lobulation and shrinkage of glomerular tuft widening of bowman space (white arrow) with infiltration of inflammatory cells in the interstitial tissue (black arrow). (HE stain 400×)

Group D

Physical observations

Animals of this group looked normal with good activity and appetite compared to other groups.

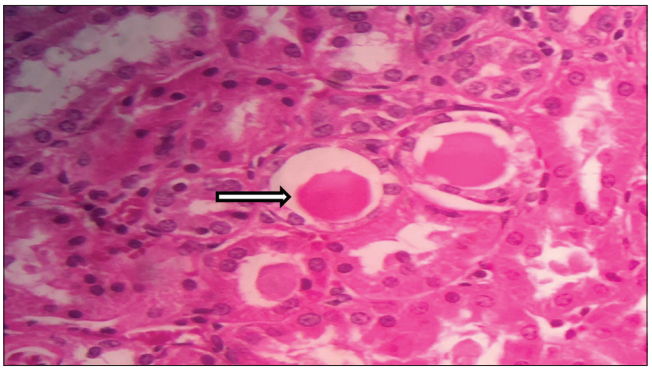

Figure 11: Cast formation inside the lumen of renal tubules (white arrow). (H and E stain 600×)

Histological examination

The histological changes were limited to degeneration of some hepatocytes (Figure 14), while histological examination of renal sections showed no tubular degeneration, necrosis, or inflammation more or less as control (Figure 15).

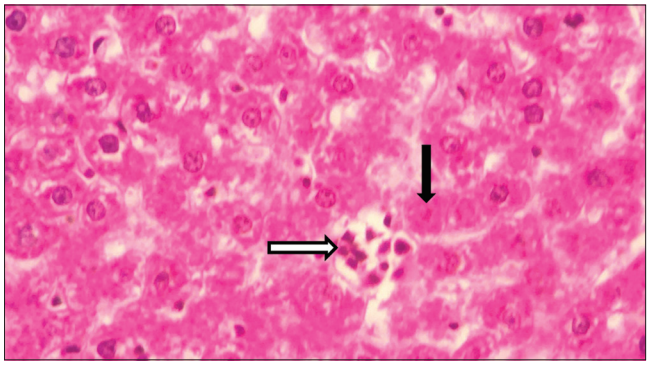

Figure 12: Infiltration by chronic inflammatory cell. (white arrow) with mild pyknosis and necrosis of other hepatocytes (black arrow) (H and E stain, ×600).

Discussion

Gemcitabine is undergone the process of phosphorylation to the active metabolite di or triphosphate which are very toxic especially when the level of intracellular adenosine triphosphate is low. There will be inhibition in polymerases enzymes of DNA with cessation of its synthesis, so this leads to inhibition of duplication of DNA in the cellular cycle at S-phase [19].

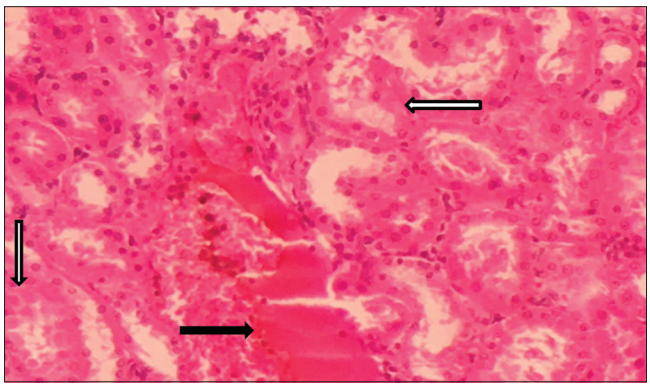

Figure 13: Epithelial cells swelling (white arrows) with vascular congestion in the interstitial tissue (black arrow). (H and E stain 400×)

Liver sections of Group B (received only gemcitabine) showed more severe pathological

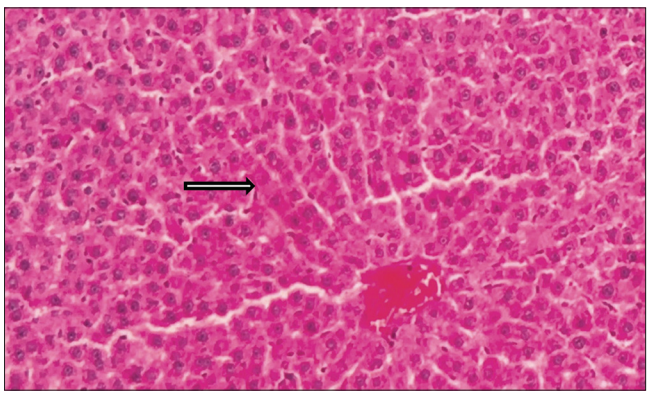

Figure 14: No hepatocytes necrosis or inflammatory reaction. Degeneration of some hepatocyte is noticed (white arrow). (HE stain 400×)

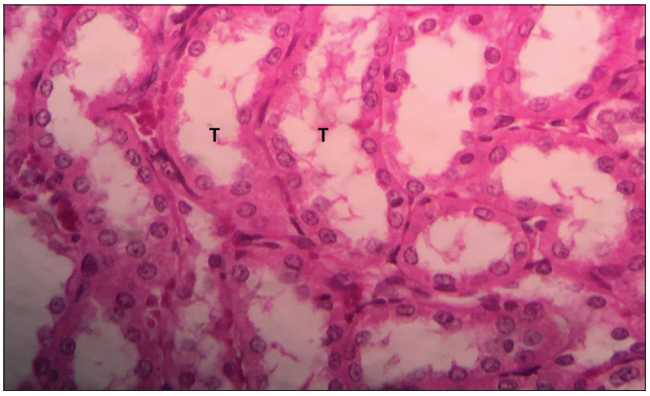

Figure 15: Normal tubules (T) showed no degeneration, necrosis, or inflammation. (H and E stain 600×)

changes of hepatic and renal tissue. congestion and inflammatory cells infiltration in the hepatic parenchyma and portal area, degeneration and necrosis of hepatocytes with increase in the number of kupffer cells, disorganization of hepatic tissue with hyperemia, and congestion of central vein, sinusoids, and blood vessels of portal area. These results coincide with Hailan *et al*. [20] findings who studied the damaging role of gemcitabine on liver tissue in mice and they found that gemcitabine lead to DNA fragmentation due to cessation of DNA synthesis, in addition to stimulation of apoptotic process in the tissue.

Hryciuk *et al.* [21] reported that four patients suffered from toxicity after gemcitabine administration seen as liver cell necrosis, microvascular degeneration, intracellular cholestasis, and focal necrosis.

Mascherona *et al*. [22] reported that many cases of liver injury in patients received cyclic treatment of gemcitabine and their pathological changes included portal hepatitis and biliary duct damage, with fibrosis around sinusoids.

Renal sections of this group showed various pathological changes including severe degeneration and necrosis of the renal tubules, thickening of blood vessel wall with thrombus formation, mononuclear inflammatory cells infiltration with cyst formation in the interstitial tissue, lobulation of glomeruli, and expansion of Bowman's space with hyaline cast formation in the renal tubules. These results were in agreement with Mortazavi *et al.* [16] study who noticed that gemcitabine treated rat group suffered from severe renal tissue damage including degeneration and necrosis of renal tubules with inflammatory cells infiltration in interstitium of kidney.

Saad *et al*. [23] found that administration of gemcitabine to rats before cisplatin can worsen the renal tissue damage such as tubular necrosis and atrophy.

Group C

The sections of this group (received gemcitabine and melatonin) showed less tissue damage. The pathological changes of liver sections characterized by apoptosis in some hepatocytes with pyknosis and necrosis of other hepatocytes. Kidney sections showed improvement in the histological changes except epithelial cells swelling with vascular congestion.

Melatonin controls glutathione peroxidase and improves glutathione level. In addition, its protective role is not only reduction of radicals but also prevention of radicals formation by its action on mitochondria [24].

These results were in agreement with Hardeland [25] study, who studied the defensive effect of melatonin on tissue of mice and he found that this is because melatonin enhances the flux of electrons and ATP availability, it decreases the leak of electrons due to its high affinity at binding site Complex I. Hence, it stops the blockage caused by reactive nitrogen intermediates.

The results of this study were also in agreement with Mortazavi *et al*. [16] results who studied the effect of melatonin on protecting the renal tissue in rats treated by single high dose of gemcitabine and found some remarkable little pathological changes in renal tissue.

Ohta *et al*. [26] reported how melatonin acts against (alpha-naphthylisothiocyanate) toxin which specifically leads to hepatic damage in treated rats and they noted that its protective action is due to its antioxidant action in addition to inhibition neutrophils infiltration in the hepatic parenchyma in rats.

Galley *et al*. [27] stated that melatonin can reduce the mitochondrial swelling and damage in rats administered paclitaxel keeping its membrane intact by its antioxidant action.

Reiter *et al*., [28] found that melatonin enhances the mitochondrial function which has a direct effect on regulating the all the intercellular reactions and processing functions.

Group D

Hepatic and renal sections of animals of this group (melatonin only) showed normal looking tissue. As melatonin induces antioxidant enzymes and stimulates mitochondrial action [29].

Conclusion

Gemcitabine can lead to damage of both liver and renal tissue of male rats. Melatonin can protect these organs and reduce damage. More studies concerning modification of doses and duration of treatment with gemcitabine and melatonin are recommended.

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