NTU Journal of Pure Sceinces (2023) 2 (2) : 6-14
DOI: https://doi.org/10.56286/ntujps.v2i2

IRAQI Academic Scientific Journals

ISSN: 2789-1089 EISSN: 2789-1097
NTU Journal of Pure Sciences
Available online at: https://journals.ntu.edu.iq/index.php/NTU-JPS/index

Anti-inflammatory and Protective Effects of Melatonin on Rats Exposed to Anticancer Drugs

Salwan W. yousif[1] , Ghada A. Taqa[2] Ameer M. Taha[3]
1.,2. Department of Dental Basic Sciences, College of Dentistry. University of Mosul , Mosul, Iraq, 3.Department of Biology, College of Education for Pure Science, University of Mosul , Mosul , Iraq

Article Informations

Received: 07-08- 2022,
Accepted: 26-02-2023,
Published online: 30-06-2023

Corresponding author:
Name: Salwan Wadala Yousif
Affiliation : University of Mosul
Email: salwan@uomosul.edu.iq

Key Words:
Melatonin,
Anticancer,
AST,
ALT,
CRP,
Anti -inflammatory .

A B S T R A C T

This study was aimed to investigate the effects of Melatonin at (10mg/kg) on liver function enzymes AST,ALT,ALP activity and determination the inflammatory marker CRP activity and total counting of WBC on rats exposed to anticancer drugs Gemcitabine, and protective effects of melatonin in the liver tissue.
Materials and Methods: sixty four adult male albino rats were used in this study, weighing (250-350) gm at temperature 22±20C , The animals were feeding and drinking water as needed . the animals were divided into 5 main groups as follow : Group A(Control group n=8) given a daily D.W. Group B:(melatonin group n=8)given a daily melatonin 10mg/kg/orally. Group C (Gemcitabine n=16) this group was divided into two group according to Gemcitabine dose (25 and 50 mg/kg). Group D (protective group n=16): this group was divided into two group according to Gemcitabine dose (25 and 50 mg/kg) together with given a daily melatonin in dose (10mg /kg) orally. Group E (protective and treatment group n=16) : this group was divided into two group according to Gemcitabine dose (25 and 50 mg/kg). This group was given a daily melatonin in dose (10mg /kg) orally as a treatment. The results showed that melatonin reduced the activity of AST, ALT enzymes compare with gemcitabine treated groups, While increased the activity of ALP in A, B groups compare with gemcitabine treated groups , that induced by chemotherapy drug ,While decreased the activity of CRP inflammatory marker in A, B groups compare with another groups that treated with anticancer drug. Melatonin given protective effect on liver enzymes and tissues from damaged that happened by Gemcitabine in two different dose (25,50 mg/kg) .

Introduction

Inflammation is an the important response to tissue damaged by chemical and physical or biological factors, then Increased production of free radical and oxidative stress rather than increased of liver damage and hepatotoxicity can be induced by a variety of factors such as exposure to drugs and chemotherapy treatment [1].

Melatonin (N,acetyle-5-methoxytryptamin), is a natural hormone produced in different organs mainly secrets by the pineal gland and other organs, it has many physiological function in the body like regulation circadian rhythm and antioxidant effects by scavenges free radical especially by inhibits mitochondrial permeability transition pore and activation the antioxidant enzyme [2]. Melatonin has contractility biological mechanisms function used to treatment of cancer patients by prophylaxis of the both cancer advancement related to symptoms and chemotherapy-spontaneous toxicity [3]. Many studies showed that melatonin have important protective role in different types of liver injury and fibrosis and melatonin have shown that it regulate the activation of the immune system, reducing chronic and acute inflammation, Experimental and clinical data suggest that melatonin exerts its anti-inflammatory effects by modulating both pro- and anti-inflammatory cytokines in various pathophysiological situations [4, 5]. Hepatocellular enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are released following liver damage. is markedly elevated in response to chemotherapy drugs [6] .

Evidence refers that melatonin exerts a variety of anticancer properties at different stages of tumor progression and metastasis , Moreover, the combination of melatonin and chemotherapies has been reported to Melatonin is considered to act directly at the mitochondrial level, where it reduces free radical formation and also protects ATP synthesis, by stimulating key enzymatic complexes , Direct scavenging of free oxygen and nitrogen species is also present at the mitochondrial level, by this activity the membrane against disruption and supporting the continuity of the electron chain and to protect mitochondrial DNA [7].

improve the effectiveness of anticancer drugs, Melatonin significantly enhanced the cytotoxicity of the Oxidative Medicine and Cellular Longevity chemotherapy drugs against cancer cells. Consistently, each of the chemotherapy drugs with melatonin increased the ratio of cells entering mitochondrial apoptosis due to ROS overproduction, mitochondrial membrane depolarization, and highly expanded DNA fragmentation [8,9].

The aim of this study was to investigate about the melatonin ability to reduce inflammatory mechanisms during chemotherapy treatment by estimation inflammatory marker like CRP, total WBC and its protective effects by decreases the levels of liver function enzymes like AST, ALT, and to inform about the protective of melatonin on tissues like liver.

Materials and methods

Medication :

-Melatonin : the dose of orally melatonin (10 mg) dissolve in (1ml) distilled water to reach the final dose 10mg/kg of body weight.

-Gemcitabine : the dose of injected Gemcitabine was prepared from vial contain (1000 mg/10ml) diluted in distilled water to prepare final doses (25 and 50 mg/kg) of body weight .

Animals : sixty four adult male albino rats were used in this study ,weighing (250-350)gm. with temperature 22 ± 2^0C , The animals were feeding and drinking water as needed, the animals were divided into 5 main groups as following :

Group A(control n=8): act as the pattern control group and was given a daily Distal water in dose (1ml/kg) orally through the gavage tube for 21 days and from 19^{th} day until 21^{th} day of experiment an intraperitonial (I.P) injection of distilled water (D.W) at volume (1ml/kg) was given one hourly after oral D.W management.

Group B (melatonin group n=8): was given a daily melatonin in dose (10mg/kg) orally (the dose of melatonin was prepared daily by dissolving in distilled water) for 19 days and I.P of distilled water (1ml/kg) for 3 successive days from each one 19^{th} day hoed 21^{th} day of experiment one hourly after distilled water management.

Group C (gemcitabine n=16): this group was divided into two group according to gemcitabine dose (25 and 50 mg/kg). This group was given a daily distal water in dose(1ml/kg) orally for 19 days and I.P of gemcitabine(25 and 50 mg/kg)for 3 consecutive days from 19^{th} day until 21^{th} day of experiment one daily after distal water management.

Group D (protective group n=16):this group was divided into two group according to gemcitabine dose (25 and 50 mg/kg). This group was assumption a daily melatonin in dose (10mg /kg) orally for 19 days and from 19^{th} day until 21^{th} day of experiment I.P of gemcitabine dose (25 and 50 mg/kg)was assumption one daily after melatonin dose.

Group E (protective and treatment group n=16) :this group was divided into two group according to gemcitabine dose (25 and 50 mg/kg). This group was assumption a daily melatonin in dose (10mg /kg) orally for 19 days and from 19th day until 21th day of experiment I.P of gemcitabine at dose (25 and 50 mg/kg) was given one hourly after melatonin dose. Then this group continuous treatment by melatonin till day 28.

At day 21and two hours next the last treatment , total animals of all group (Except group E) at 4 weeks were located below light Ether anesthesia in for the blood sample assemblage for biochemical analysis and organs (liver) were scratch and placed in 10% buffered formalin for histological examination.

Blood collecting

Blood samples were collected by withdrawn from retro-orbital venous plexus under light ether anesthesia using micro hematocrit capillary tubes and gathered in Eppendorf tube and prevented to clot at room temperature for 15 minute and centrifuged at 3500 rpm for 20 minute to derive readily apparent serum which were stored in a deep freezer at (-20°C) for subsequent measurements.

Biochemical and blood marker measurement

the activity of AST,ALT, was determination according to **[10],** when ,WBC measured by Auto Hematology Analyzer ,CRP inflammation marker was measured by Enzyme linked Immunosorbent assay (ELISA)KIT from sigma Aldrich company.

Histological tissues Examination:

At the end of experimentation all rats of each group were immolation (Exception)last group (protective and treatment group) at 4 weeks from stated experiment)by cervical dislocation, specimens from liver, liver were derived and then refined for light microscopically testing as follows:

The samples of tissue fixation in 10% buffered formalin then preparation with water and dehydrated through heaving alcohol concentration (70%/24hrs, 80%/1hr, 90%/1hr and 100% for two exchanges 1hr /each step), The samples were absolved by two exchanges of xylene, 10 minutes / each alteration ,Penetrate of the samples with clear white paraffin wax in an oven at 58°C,two exchanges 2hrs/ each pace then inserted as an integral part in paraffin wax that was plugged in the tissue container, Then sliced the paraffin block by arotary microtome at 4-6μm sections, then put in the circular water bath at 45C° [11].

Statistical analysis

Data analysis performed by using SPSS version 19 for windows software the differences between groups were statistically analyzed by one-way analysis at variance (ANOVA), the differences were considered significant at $P \leq 0.05$.

RESULTS

Figure (1, 2) show significantly decreases in AST and ALT in A and B groups at $P \leq 0.05$ compared with gemcitabine at dose (25,50) mg/kg in group D treated melatonin 10mg/kg more than 2 weeks protective group before administration of gemcitabine we found that AST,ALT enzymes concentration reduced in comparison to group C that treated with gemcitabine alone .also we found that the melatonin in 10mg/kg pre and post gemcitabine in (25,50)were more significantly reduction in value of AST and ALT.

Figure (3) show significantly increased in total WBC count in Aand B groups at $P \leq 0.05$ compared with gemcitabine at dose (25,50) mg/kg when in D protective group treated with melatonin 10mg/kg we found WBC count increased compare with Cgroup treated with gemcitabine alone, also we found melatonin in 10mg/kg pre and post treated with gemcitabine (25,50) has more significantly increased in WBC value.

Figure (4) show significantly decreased in CRP inflammatory marker in A and B groups at $P \leq 0.05$ compare with gemcitabine at dose (25,50) mg/kg when in group D treated melatonin 10mg/kg for 2weeks before administration of gemcitabine we found CRP decreased compare with C group treated melatonin 10mg/kg for 2 weeks before administration of gemcitabine we found CRP value decreased with C group treated with gemcitabine alone ,also we found melatonin in 10mg/kg pre and post treated with gemcitabine (25,50) has more significantly decreased in CRP value.

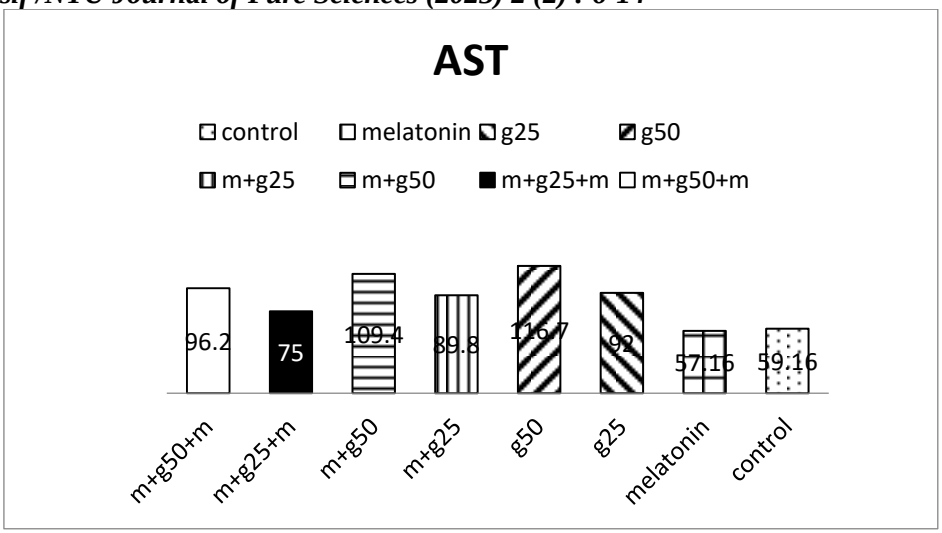

Figure 1. Effect melatonin on aspartate aminotransferase AST p≤0.05.

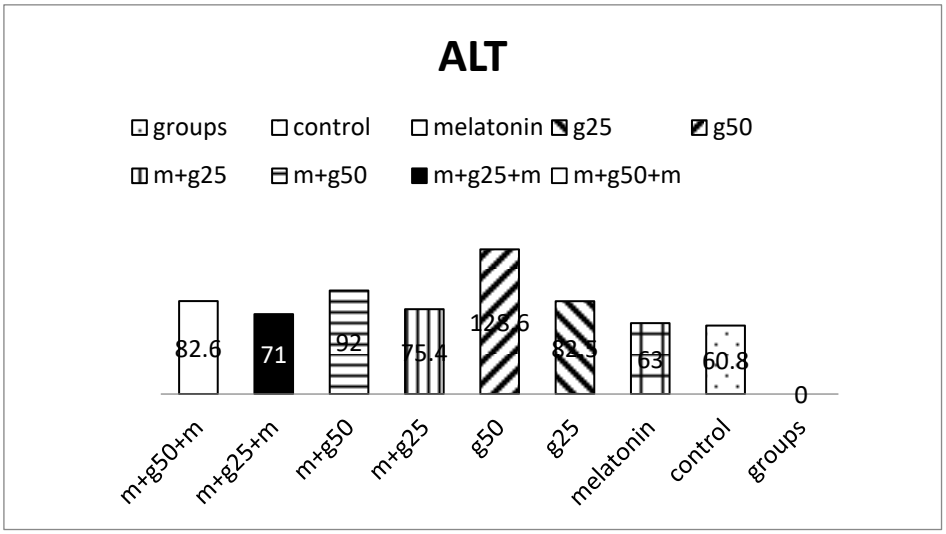

Figure 2. Effect melatonin on alanine aminotransferase ALT p≤0.05.

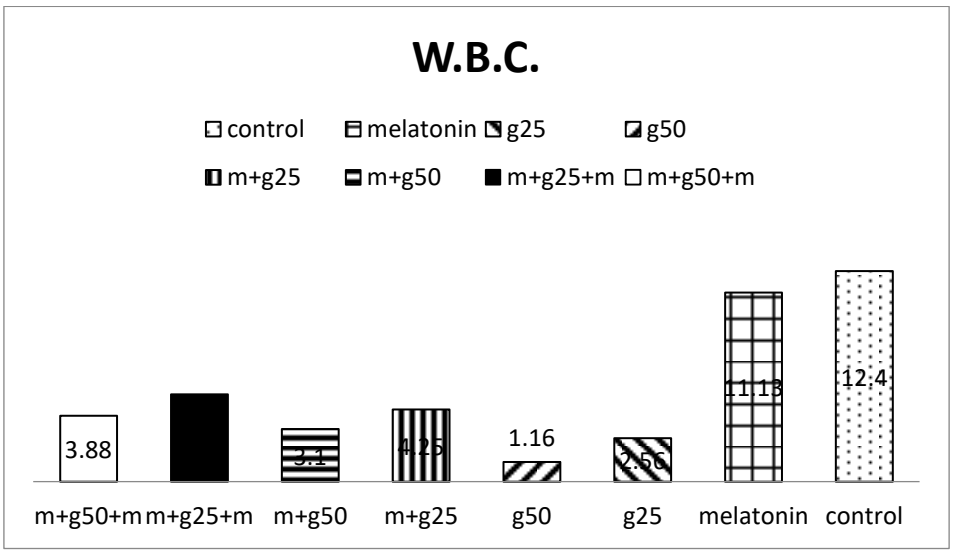

Figure 3. Effect melatonin on total white blood cells WBC p≤0.05

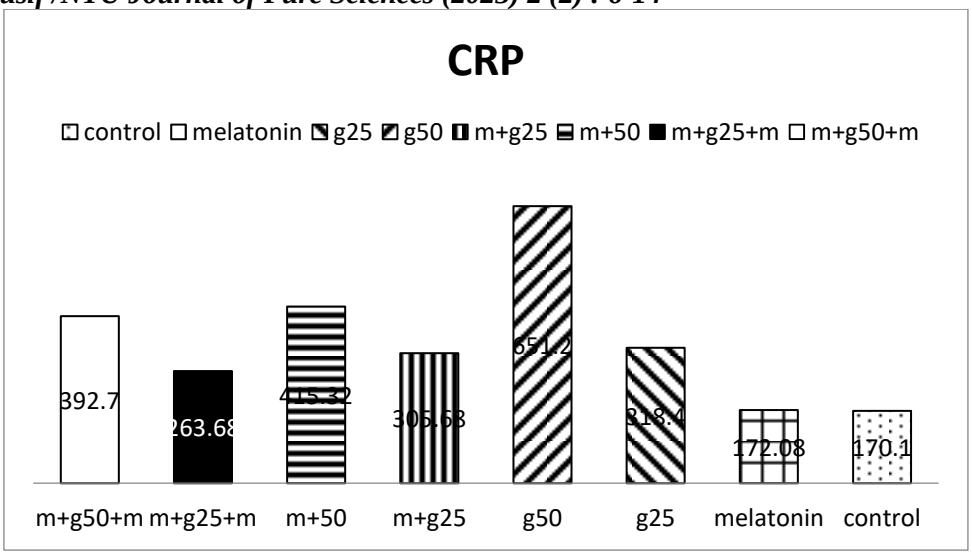

Figure 4. Effect melatonin on C reactive protein CRP p≤0.05

Effect of melatonin treatment on Histopathological examination:

The microscopically examination of liver section from control and melatonin groups showed no evidence of histological abnormalities and show regular hepatocytes and architecture details and normal portal areas no pathological changes observed .

In group C gemcitabine (25,50) mg/kg show many changes noticed in the sections included congested in the in portal veins and infiltrations of inflammatory cells and vacuolization of cytoplasm ,necrosis and dilatation of sinusoids and filtration of lymphocytic inflammatory cells were noticed, then in group D protective of melatonin and gemcitabine (25,50) mg/kg show moderate degradation and congestion of hepatocytes, there was moderate filtration and congestion and hemorrhage of hepatic tissue, group E protective and treatment of melatonin show mild and few changes observed in architectural details in liver sections compared with chemotherapy and melatonin groups slight necrosis and degenerative of hepatocyte .

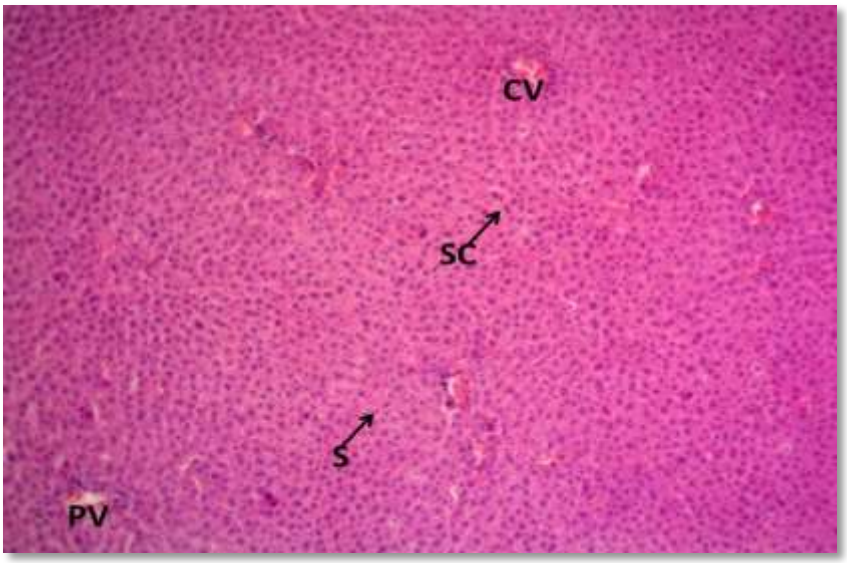

Figure 1. Histological section of rat liver from the control group showing normal histological features represented by hepatocytes (H), sinusoids (S), portal region (P), and central vein (CV). Hematoxylin-eosin stain, 100X

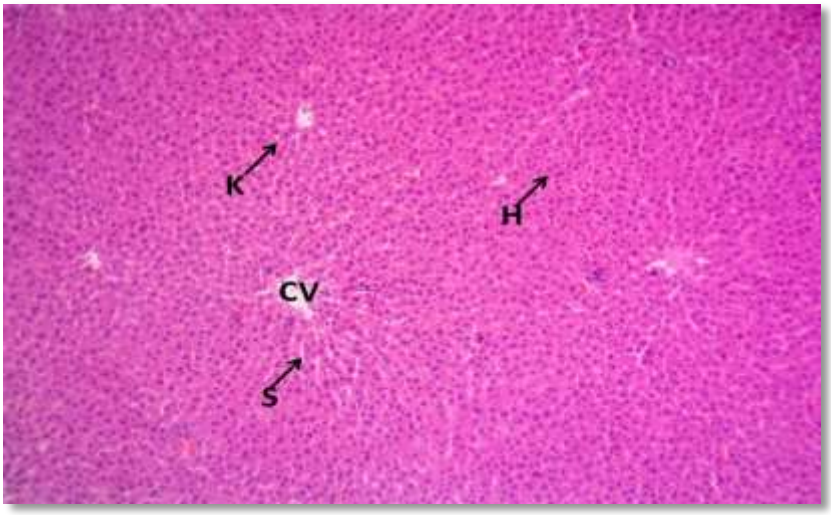

Figure 2. Histological section of a rat liver from the group treated with melatonin only M at a dose of 10 mg showing the normal histological features represented by hepatocytes (H), sinusoids (S), portal region (P) and central vein (CV). Hematoxylin-eosin stain, 100X

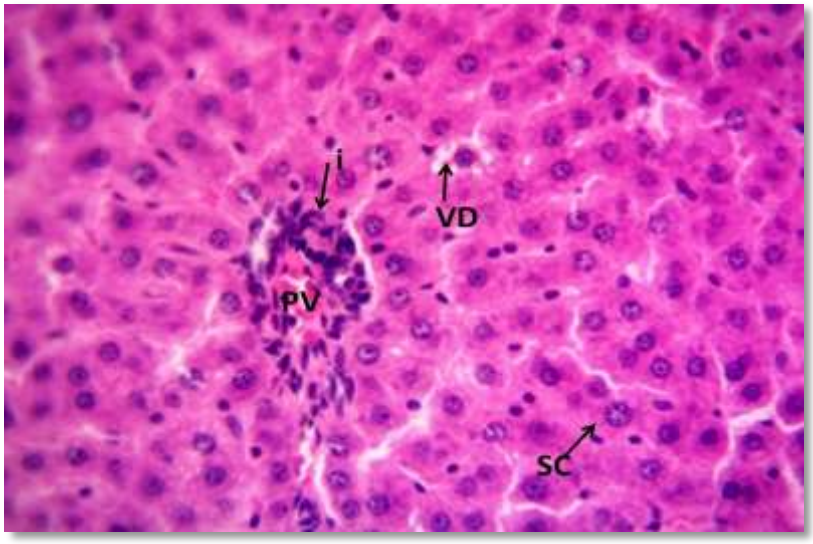

Figure 3. Histological section of a rat liver from the drug-only group J at a dose of 25 mg showing cloudy degeneration (cellular swelling), vacuolar degeneration (SC) of hepatocytes (VD), inflammatory cell infiltration (i) and portal vein congestion (PV). Hematoxylin-eosin stain, 400X

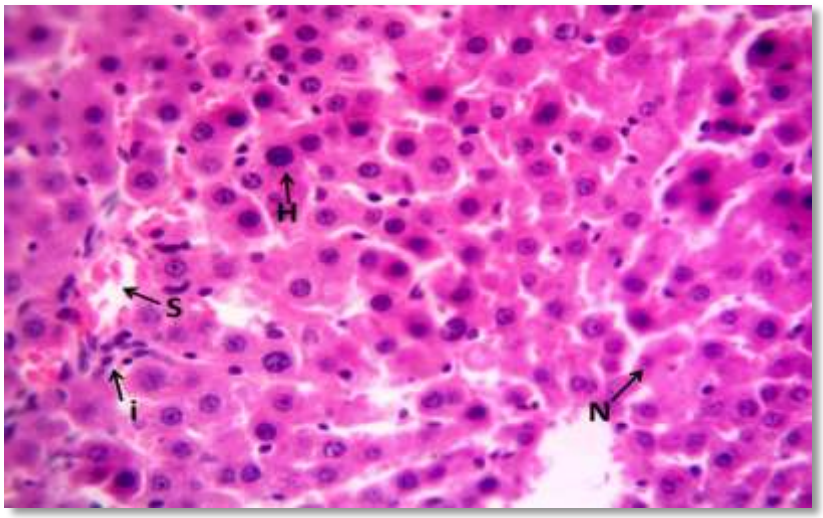

Figure 4. Histological section of the liver of a rat from the group treated with drug only J at a dose of 50 mg showing thrombo necrosis of hepatocytes (N), hypertrophy of each other (H), dilatation and congestion of the sinusoids (S) and infiltration of inflammatory cells (i). Hematoxylin-eosin stain, 100X

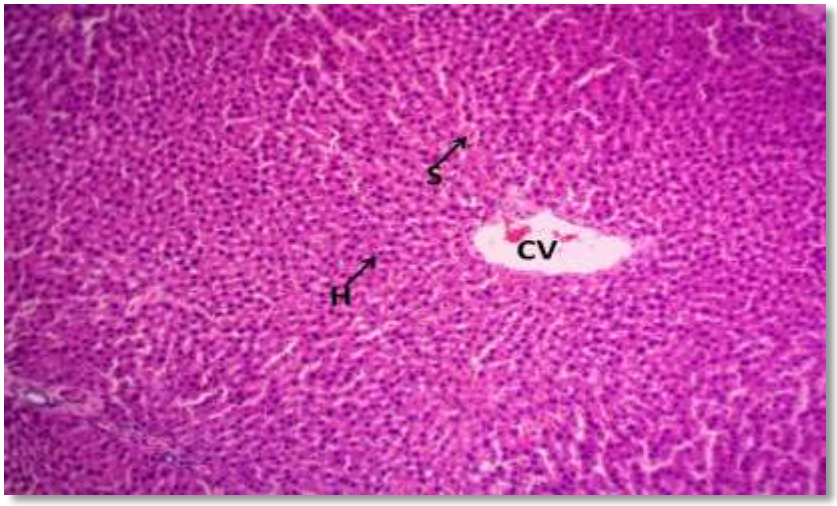

Figure 5. Histological section of the liver of a rat from the group treated with 10 mg of melatonin and J25 drug, showing the normal shape of hepatocytes (H) and slight congestion in the sinusoids (S) and central vein (CV). Hematoxylin-eosin stain, 100X

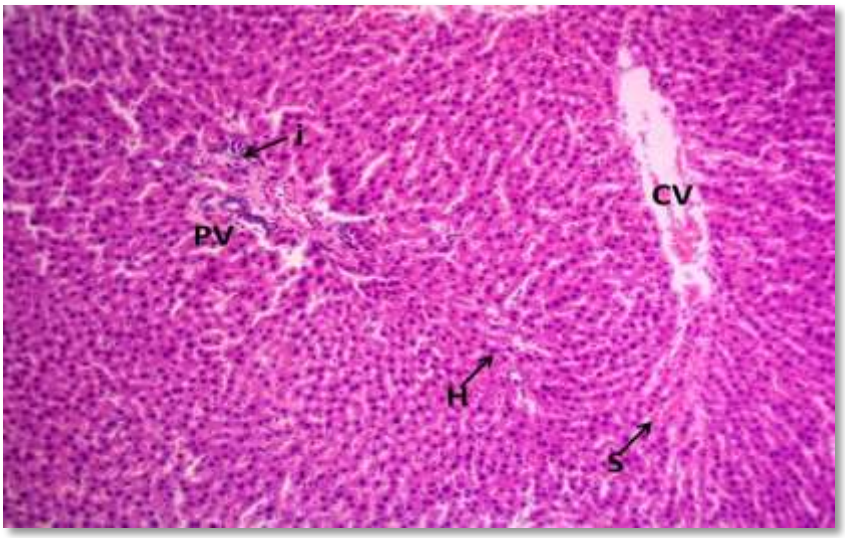

Figure 6. Histological section of a rat liver from the group treated with 10 mg of melatonin and J50, showing normal hepatocytes (H), congestion in sinusoids (S), central vein (CV), portal vein (PV), and inflammatory cell infiltration (i). Hematoxylin-eosin stain, 100X

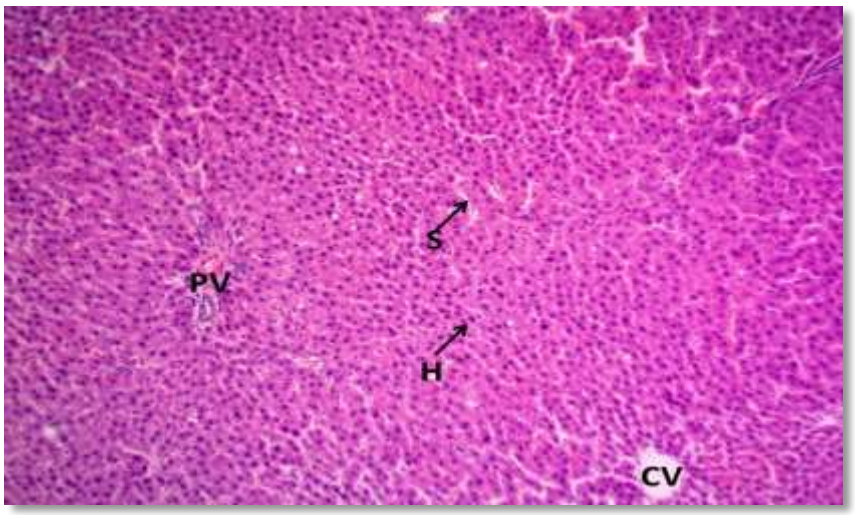

Figure 7. Histological section of the liver of a rat from the group treated with melatonin 10mg and the drug at a dose of 25 mg then melatonin M showing the normal shape of hepatocytes (H), sinusoids (S), central vein (CV) and portal vein congestion (PV). Hematoxylin-eosin stain, 100X

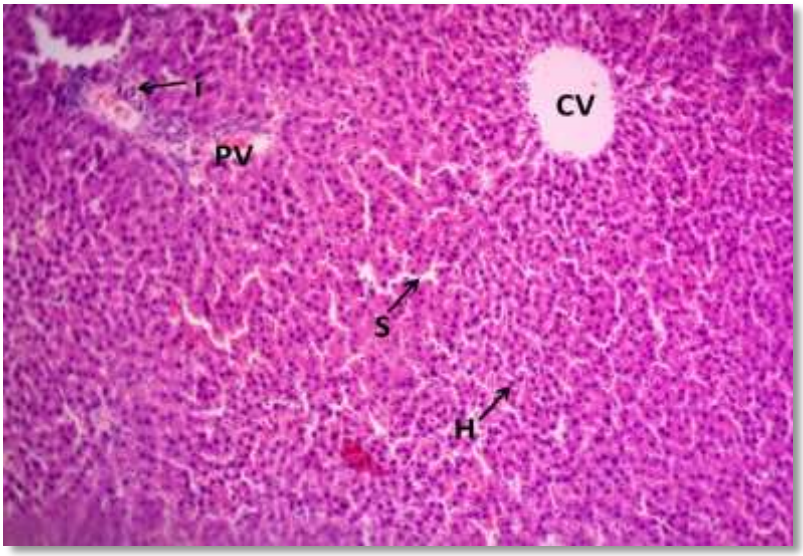

Figure 8. Histological section of the liver of a rat from the group treated with melatonin 10mg and the drug at a dose of 50 mg, then melatonin M, showing the normal shape of hepatocytes (H), central vein (CV), dilatation of the sinusoids (S), portal vein congestion (PV) and slight infiltration of inflammatory cells (i). Hematoxylin-eosin stain, 100X

DISCUSSION

The current study showed that chemotherapy drugs are the most relevant treatment options in cancer therapy, inspite of their documented efficacy, have many side effects which are closely related to inflammation, Chemotherapeutic drugs as gemcitabine can induced damaged in liver and another organs and can produced different changes in liver function and intracellular enzymes released in to circulation after necrosis and inflammation of hepatocytes like AST, ALT [12, 13].

Inflammation is an essential response to tissue injuries induced by physical and biological reasons, Several studies have indicated the role of melatonin in reducing the side effects and hepatotoxicity caused by the use of chemical treatments in humans and many laboratory animals. [13, 14]. melatonin has several important anti-inflammatory effects related to a direct interaction with specific binding sites location in lymphocytes and macrophages and blocking of the transcriptional factors that triggers pro-inflammatory cytokine production [13,14] , Melatonin, due to its anti-inflammatory property, has been considered as a protective agent in cancer therapy in several studies using both in vivo and in vitro [14, 15].

In this study melatonin in 10mg /kg proved ability to decreases the levels of liver function enzymes and reduction the inflammation marker CRP and this ability agree with [15, 16]. Melatonin may also exhibit anticancer and protective oncostatic activity through several mechanisms, including inhibition of cancer cell proliferation, decrease in oxidative stress, and increase in immune system activity. Melatonin exhibits potent anti-inflammatory, antioxidant and fibro suppressive activities against thioacetamide-induced hepatic fibrogenesis via the suppression of oxidative stress, DNA damage, pro-inflammatory cytokines, and fibrogenic gene Transcripts , Melatonin protects against lipid-induced mitochondrial dysfunction inhepatocytes and inhibits stellate cell activation during hepatic fibrosis in mice [17].

Chemotherapeutic drugs like gemcitabine can produce many histological alteration in the tissues causes lipophilic compounds ,metabolic pathways are include a series of steps that modify the parent molecule and induced irreversible cellular injury damaged recruitment of inflammatory cells, on the other hand acute necrosis of liver due to complication of gemcitabine administration in cancer patient and agreement with several studies like this [18, 19].

Melatonin in this study improved its ability to reduce the changes and histopathological damaged in liver tissues that happened by gemcitabine drug indifferent concentration ,and produces more protective, Melatonin has been shown to have anti-inflammatory effect through multiple mechanisms, It reduces macromolecular damage in all organs by scavenging free radicals ,These result finds are antecedently trace and supported by many studies [19, 20] and researchers who declared that use of antioxidant agents to normalizing the histological changes induced by chemotherapy anticancer drugs ,the role of antioxidant hormone melatonin is to inhibition the oxidative damage and prevent metastasis and cell proliferation [19, 20, 21]. Finally we investigate in this study the oral administration of melatonin to rats significantly diminished hepatic ALT and AST, and decreases the hepatocellular damaged, this results agreed with many studies [21, 22, 23].

References

[1] Agrawal, A., Darbari, S., Rai, T. P., Kukani, G.T. Role of melatonin in the pathophysiology of cancer, J. chr. D. 2016.7(1-6).http://www.chronotherapyjournal.net/

[2] Anwer, M.J., Muhammad, B.Y., Bader, A.A., Abdlghani, M., Mahmood, D., Haider, M., An insight in to scientific background and future perspectives for the potential uses of melatonin. Egyptian Journal of Basic and Applied Sciences. 2015. 2:139-152. https://doi.org/10.1016/j.ejbas.2015.05.003

[3] Eghbal,M.A., Eftikhari, A., Ahnadian, E.,Yadolla, A.Y., Parvizpor, A. A review of biological and pharmacological actions of melatonin :oxidant and prooxidant properties. Pharmaceutical Bioprocessing 2016. 4(4): 069-081. https://www.openaccessjournals.com/articles

[4] Taqa, G.A.& Albanna, S.S.. Histological and Clinical Finding of Systemic Melatonin on Wound Healing after Tooth Extraction in Rabbits. Journal of Applied Veterinary Sciences 2022. 7(1), 18-23. https://dx.doi.org/10.21608/javs.2021.98538.1107.

[5] Mohammed, S.M., Sulaiman, M.S., Taqa, G. A. Effects of Melatonin on Primary Oral Wound Healing in Rats. Journal of Applied Veterinary Sciences. 2022. 7(1), 24-30. https://dx.doi.org/10.21608/javs.2021.98771.1108.

[6] Pandi–perumal, S.R., Trakht, I., Srinivasan, V., Spence, D.W., Maestroni, G.J., Zisapeli, N.,Gardinali, D.P. Physiological effects of melatonin :Role of melatonin receptors and signal transduction pathway. Progress in Neurobiology. 2008. 85(3):335-353. https://doi.org/10.1016/j.pneurobio.2008.04.001

[7] Chitimus, D.M., Poperson, M.R., Voiculescu, S.E., Panaitescu, A.M., Pavel, B., Zagrean, L., Zagrean, A.M. Melatonin :reducing The Toxicity and increasing the efficacy of drugs. Journal of Pharmacy and Pharmacology 2020. *10*(9), 1211; https://doi.org/10.3390/biom10091211 .

[8] Reiter, R.J., Tan, D.X., Sainze, R.M., Mayo, J.C., Lopez-burello, S. 2002. Effects of melatonin on fatty liver disease: The role of NR4A1/DNA-PKcs/p53 pathway, mitochondrial fission, and mitophagy. Journal of pineal Research. 54(10):1299-1321.https://doi.org/10.1211/002235702760345374.

[9] Zhou, H, Du, W., Li, Y., Shi, C., Hu, N., Ma, S., Wang, W., Ren, J. 2018. Effects of melatonin on fatty liver disease: The role of NR4A1/DNA-PKcs/p53 pathway, mitochondrial fission, and mitophagy. J. pine. Res..6(1).https://doi.org/10.1111/jpi.12450.

[10] Tietz, N.W., 1995. Clinical Guide to Laboratory tests. 3rd ed. Philadelphia. WB. Saunders, 268-273.http://www.sciepub.com/reference/141231.

[11] Luna, L. G., 1968. Manual of histological stainingmethods of the forces institute of pathology 33th. ed. the Blackstone Division ,McGraw.Hill Book Company New York ,USA., p 471 -480.https://www.worldcat.org/title

[12] Roghani, M., Kalantar, H., Khodaier, M.J., Khorsandi, L.M., Kalantar, M., Goudarzi, M., Kalantar, H., 2020. Alleviation of liver Dysfunction , oxidative stress and inflammation underlies the protective effects of ferulic acid in Methotrexate-induced hepatotoxicity. Drug Design, Drug Desig., Dev. & ther.. (14): 19331941. https://dx.doi.org/10.2147%2FDDDT.S237107.

[13] Najafi, M., Shirazi, A., Motevaseli, E., Rezaeyan, A.H., Salajgeheh, A., Rezapoor, S., 2017. Melatonin as an anti-inflammatory agent in radiotherapy. Inflamm. pharm. 25(4):403-413. https://dx.doi.org/ 10.1007/s10787-017-0332-5

[14] Maitra, S., Baidia, D.K., Khanna, P., 2013. Melatonin in perioperative medicine :Current perspective. Sau. J. Anaes. 7(3):315-321.https://doi.org/10.4103/1658-354x.115316.

[15] Aziz, F.M., Maulood, I.M., Chawsheen, M.A.H.. 2012. Effects of melatonin ,vitamin C and E alone or in combination on lead –induced injury in liver and kidney organs of rats. IOSR J. Pharm. 2 (5): 13-18. http://dx.doi.org/10.9790/3013-25201318.

[16] Joshoa, H., Saumya, B., Carale, H., Michael, R.. 2021. Anti-inflammatory effects of melatonin :Asystemic review and Meta-analysis of clinical trails. Bra. Beh. & Immu..93:245-253.https://doi.org/10.1016/j.bbi.2021.01.034.

[17] Lopez, A.S., Ortiz, G.G., Moises, F.P., Ramirez, M.M., Qvintero, O.B., Lara, D.C., Jirano, L.R., Brizuela, I.V., 2018. Efficacy of melatonin on serum pro-inflammatory cytokines and oxidative stress markers in relapsing remitting multiple sclerosis. Archiv. Med.Res..49(6):391-398.http://dx.doi.org/10.1016/j.arcmed.2018.12.004.

[18] Habibi, N. N., Monfared, A. S., Gorji, K. E., Karimi, M., Moghadamni, A. A., Tourani, M., Niksirat, F., 2019. The protective effects of melatonin on blood cell counts of rectal cancer patients following radio-chemotherapy: a randomized controlled trial. Clin.Trans. Onc., 21(6), 745- 752. https://doi.org/10.1007/s12094-018-1977-2.

[19] Dawood, G.A.,Taqa, G.A., Alnema, M.M., 2020. Histological Effect of COQ10 on Liver and Buccal Mucosa in mice. J. App. Vet. Sci.. 5(2):15.https://dx.doi.org/10.21608/javs.2020.85543.

[20] Zhai, Z., Xie, D., Qin, T., Zhong, Y., Xu, Y., & Sun, T. (2022). Effect and Mechanism of Exogenous Melatonin on Cognitive Deficits in Animal Models of Alzheimer's Disease: A Systematic review and Meta-analysis. Neuroscience.

[21] Pfeffer, M., von Gall, C., Wicht, H., & Korf, H. W. (2022). The Role of the Melatoninergic System in Circadian and Seasonal Rhythms—Insights From Different Mouse Strains. Frontiers in Physiology, 665.

[22] Khatab, L. A., Abdel-Raheem, I. T., & Ghoneim, A. I. (2022). Protective effects of melatonin and L-carnitine against methotrexate-induced toxicity in isolated rat hepatocytes. Naunyn-Schmiedeberg's Archives of Pharmacology, 395(1), 87-97.

[23] Sulimani, E. S., Yousef, J. M., & Mohamed, A. M. (2021). Molecular target mechanisms of celecoxibinduced liver damage in rats and the potential prophylactic roles of melatonin and/or quercetin. Journal of Pharmacy & Pharmacognosy Research, 9(4), 397-408.