



The Effects of Melatonin on Caspase-3 and Antioxidant Enzymes Activity in Rats Exposed to Anticancer Drug

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Abstract

This study aimed to investigate the effects of Melatonin at (10mg/kg) on programmed cell death (apoptosis) by measurements of Caspase-3 activity and antioxidant enzymes GSH, CAT, MDA, and Peroxynitrate activity on rats exposed to anticancer drugs Gemcitabine. Sixty-four adult male albino rats were used in this study, weighing (250-350)gm with a temperature of $22 \pm 2^\circ\text{C}$. The animals were feeding and drinking water *ad libitum*. The animals were divided into 5 main groups as follows: Group A (Control group n=8): given 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 groups according to Gemcitabine dose (25 and 50 mg/kg). Group D (protective group n=16): this group was divided into two groups according to Gemcitabine dose (25 and 50 mg/kg) together with given daily melatonin in dose (10mg/kg) orally. Group E (protective and treatment group n=16): this group was divided into two groups according to Gemcitabine dose (25 and 50 mg/kg). This group was given daily melatonin in dose (10mg/kg) orally as a treatment.

The results showed that melatonin reduced the activity of Caspase-3 enzyme (Apoptosis marker), MDA, and peroxynitrate, induced by chemotherapy drugs, while increased the activity of antioxidant enzyme GSH and CAT. Melatonin given a protective effect on liver and kidney tissues from damage that happened by Gemcitabine at two different doses (25, 50 mg/kg).

Keywords: Caspase-3, anticancer drugs, Antioxidant Enzymes, chemotherapy drugs, melatonin Antioxidant enzyme.

1. Introduction

Chemotherapy treatment-induced oxidative stress in biological systems, during this treatment many changes happened like lipid peroxidation that can be damaged and attached to many tissues and cellular targets, these processes can initiate apoptosis (programmed cell death), by activation of death receptors and inhibition Caspase enzymes activity [1], uses of antioxidant treatment during chemotherapy may increase the activity of medication by reducing the oxidative stress generation [2].

Melatonin (N,acetyl-5-methoxytryptamine), is a natural hormone produced in different organs mainly secreted by the pineal gland and other organs, it has many physiological functions in the body like regulation of circadian rhythm and antioxidant effects by scavenging free radicals, especially by inhibiting mitochondrial permeability transition pore and activating the antioxidant enzyme [3]. Melatonin has contractility biological mechanisms function used to treatment of cancer patients by prophylaxis of both cancer advancement related to symptoms and chemotherapy-spontaneous toxicity [4]. In several studies melatonin affects the levels of antioxidant

enzymes like glutathione (GSH), catalase (CAT), and Malondialdehyde (MDA), by increasing the activity of these enzymes [5]. Apoptosis can happen during cancer chemotherapy and is activated by the receptor – extrinsic pathway or by an activated intrinsic pathway in mitochondrial release of apoptogens and by activation of caspase-3 enzyme this can amplify death signaling [6]. Many studies suggest that melatonin reduces apoptosis through several mechanisms one of these is inhibition of caspase-3 enzyme activity [7,8]. The study aimed to investigate the melatonin ability to reduce apoptosis mechanisms during chemotherapy treatment and its protective effects by increasing the levels of antioxidant enzymes like GSH, CAT, and investigate the melatonin effects on several tissues like kidney and liver.

2. Materials and Methods

Medication :

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

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-Gemcitabine : the dose of injected Gemcitabine was prepared from a vial containing (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 a temperature of $22\pm 2^{\circ}\text{C}$, The animals were feeding and drinking water *ad libitum*, the animals were divided into 5 main groups as follows :

Group A(control n=8): acted as the pattern control group and was given daily Distal water in dose(1ml/kg) orally through the gavage tube for 21 days and from the 19th day until 21st day of experiment an intraperitoneal (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 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 19th day hoed 21st 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 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 the 19th day until 21st day of the experiment once 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 19th day until 21th 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 groups 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 21st day of experiment I.P of gemcitabine at a dose (25 and 50 mg/kg)was given one hourly after melatonin dose. Then this group continuous treatment by melatonin till day 28.

On day 21and two hours next to the last treatment , total animals of any group (Except group E) at 4 weeks from the started experiment were located below light Ether anesthesia in for the blood sample assemblage for biochemical analysis and then they were forfeited and organs (liver and kidney) were scratch and placed in 10% buffered formalin for histological examination.

Blood collecting and Biochemical tests

Blood samples were collected by with drawn 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 was stored in a deep freezer at (-20°C) for subsequent measurement of Caspase-3 , Malondialdehyde (MDA), glutathione enzyme (GSH), Catalase and peroxynitrate radical enzyme.

Determination of Caspase-3activity

Measured caspase-3concentration in serum by using rat caspase-3 ELISA Kit, by Nanjing Duly Biotech CO. Ltd. This method depended on converting color from blue to yellow by stopping solution and set of calibration received and analyzed at the same time as the samples and permit the operator to have a standard curve of optical density versus casp-3concentration,.

Determination of Malondialdehyde (MDA)activity

According to [9] in serum by using (TBA) thiobarbituric acid, the color formed was read at 532 nm.

Determination of glutathione enzyme (GSH)activity

By using rat Elisa Kit from (Elab science) ,the principle of this test is to competitive the micro Elisa plate provided in this Kit has been pre coated with GSH during the reaction, Reduced glutathione (GSH) can react with dithionitrobenzoic acid (DTNB) to produce thio-nitrobenzoic acid and glutathione disulfide. Nitromercaptobenzoic acid is a yellow compound which has the maximum absorption peak at 420 nm.

Determination of Catalase enzyme activity

Catalase enzyme was determined in serum by catalyzed the bivalent reduction of H₂O₂ to water [10], the color formed was read at 240 nm.

Determination of peroxynitrate radical enzyme activity/

This enzyme was measured in serum by nitration of phenol using the method of [11], The peroxynitrite radical (ONOO-) works on the nitration of phenol into nitrophenol whose absorption intensity can be read at a wavelength of (412) nm in spectrophotometer . The amount of nitrophenol formed and estimated in the blood serum reflects the level of the peroxynitrite radical generated in the serum.

Histological tissuesExamination .

At the end of the experimentation all rats of each group were decapitated except the last group (protective and treatment group) at 4 weeks from the stated experiment)by cervical dislocation, specimens from the liver,and kidney were derived and then refined for light microscopically testing followed by [12]as follows:

The tissue samples were fixed in 10% buffered formalin then prepared 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 then 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 a rotary microtome at 4-6µm sections, then put in the circular water bath at 45°C.

Statistical analysis

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

3. Result

1-Effect of melatonin treatment on Caspase-3 enzyme activity Fig (1) shows significant decreases in caspase-3 concentration in A and B groups at $p \leq 0.05$ compared with group gemcitabine at dose (25,50)mg/km In group D treated with melatonin at 10mg/kg for 2 week before administration of gemcitabine we found that the caspase-3 enzyme concentration was reduced in comparison to the C group treated with gemcitabine alone. Also we found that the melatonin treated group at 10mg/kg pre and post gemcitabine (25,50)mg/kg was more significantly reduced in value of caspase -3 enzyme.

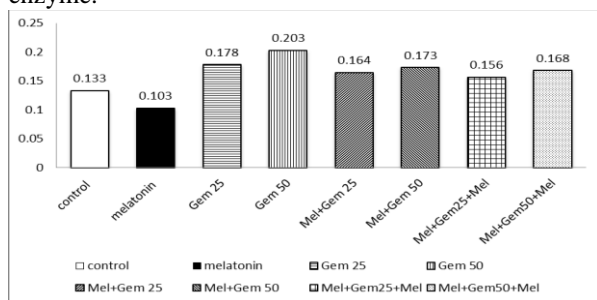


Fig.1. effect of melatonin on Caspase -3 enzyme activity in $P \leq 0.05$.

2-Effect of melatonin treatment on GSH and CAT activity

Fig (2,3) shows a significant increase in the GSH and CAT concentration in groups A and B at $p \leq 0.05$ compared with group gemcitabine at a dose (25,50) mg/km where the value was decreased, then in group D treated melatonin at 10mg/kg for 2 week before administration of gemcitabine we found that the GSH and CAT slightly increased then also we found that the melatonin at 10mg/kg pre and post gemcitabine (25,50)mg/kg highly increased compared to the gemcitabine treated group alone.

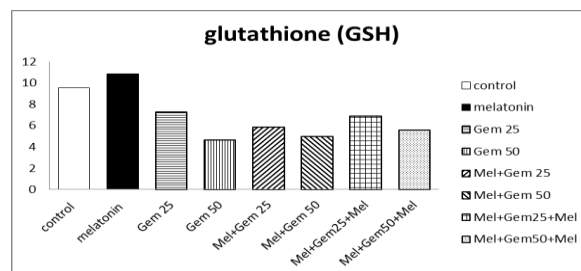


Fig.2. effect melatonin on glutathione (GSH) concentration $p \leq 0.05$.

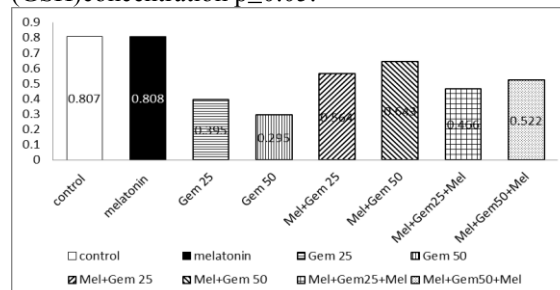


Fig.3. effect melatonin on catalase concentration $p \leq 0.05$.

3-Effect of melatonin treatment on MDA and peroxynitrate activity.

Fig (4,5) show significantly decreased in MDA and peroxynitrate concentration in groups A and B at $p \leq 0.05$ compared with group gemcitabine at dose (25,50)mg/km where the value was increased, then In group D treated melatonin 10mg/kg for 2 week before administration of gemcitabine we found that the MDA and peroxynitrate less decreased then also we found that the melatonin at 10mg/kg pre and post gemcitabine (25,50)mg/kg more decreased to improve this value compare with gemcitabine group alone.

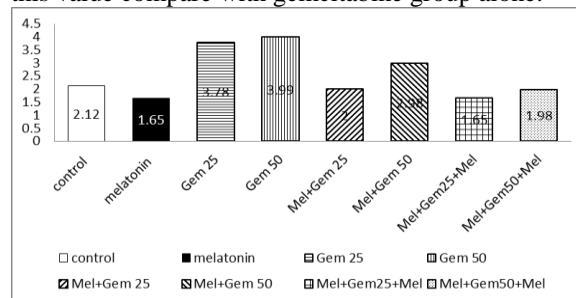


Fig.4. effect melatonin on Malondialdehyde (MDA) concentration $p \leq 0.05$.

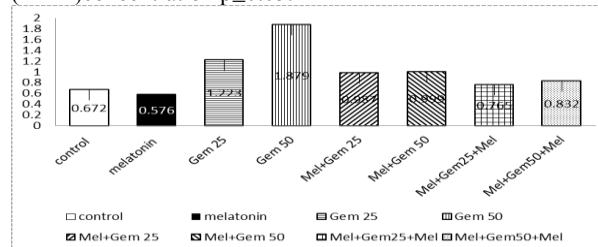


Fig.5. effect melatonin on peroxynitrate concentration $p \leq 0.05$.

Effect of melatonin treatment on Histopathological examination.

The microscopically examination of kidney sections from control and melatonin groups showed no evidence of histological abnormalities and showed regular cells and architecture of glomeruli nephrons and collecting tubules and no pathological changes were observed.

In group C gemcitabine (25,50)mg/kg showed congestion in the proximal convoluted tubule and showed mild and moderate infiltrations of inflammatory cells and vacuolization of cytoplasm.

Cloudy sever swelling in renal tubular cells with capillaries congestion and filtration of lymphocytic inflammatory cells were noticed, then in group D protective of melatonin and gemcitabine (25,50)mg/kg showed moderate degradation and congestion of cells, moderate filtration and congestion in glomeruli nephrons, then in group E protective and treatment of melatonin showed mild and few changes in architectural details in kidney sections compared with chemotherapy and melatonin groups.

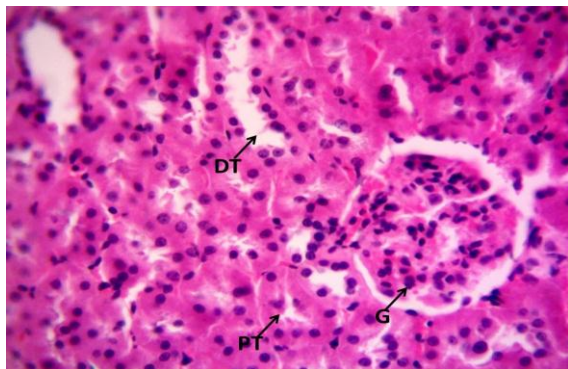


Fig.6. Histological section of a rat kidney from the control group showing the normal histological features represented by renal glomeruli (G), proximal renal tubule (PT), distal renal tubule (DT) and central vein (CV). Hematoxylin-eosin stain, 400X

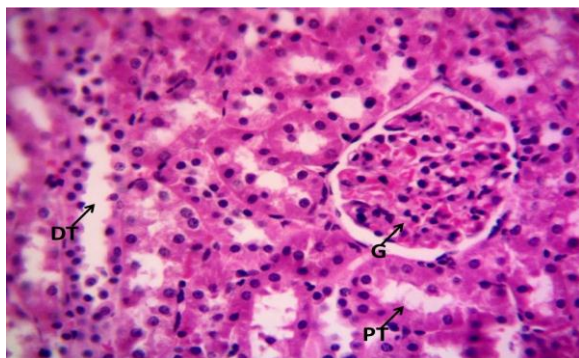


Fig.7. Histological section of a rat kidney from the group treated with melatonin only M at a dose of 10 mg showing the normal histological features represented by renal glomeruli (G), proximal renal

tubule (PT) and distal renal tubule (DT). Hematoxylin-eosin stain, 400X

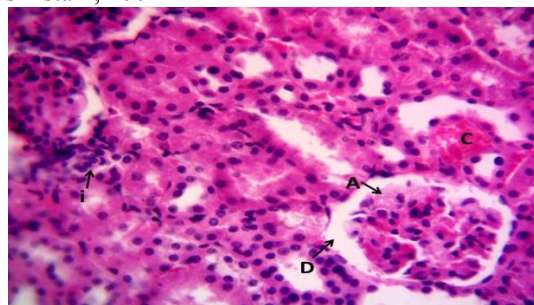


Fig.8. Histological section of a rat kidney from the drug-only group J at a dose of 25 mg showing renal glomerular atrophy (A), Bowman's capsular expansion (D), slight infiltration of inflammatory cells (I) and vascular congestion (C). Hematoxylin-eosin stain, 400X

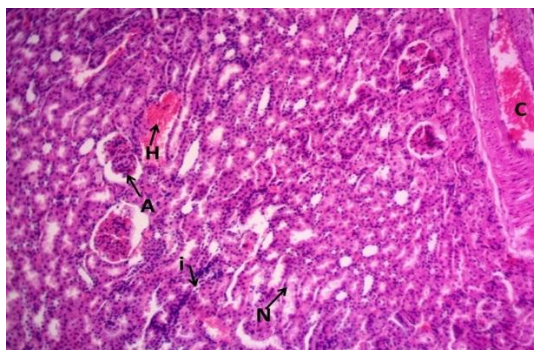


Fig.9. Histological section of a rat kidney from the drug-only group J at a dose of 50 mg showing renal glomerular atrophy, Bowman's capsular dilatation (A), necrosis of the epithelial cells lining the renal tubules (N), inflammatory cell infiltration (i), vascular congestion (C) and hemorrhage (H). Hematoxylin-eosin stain, 100X

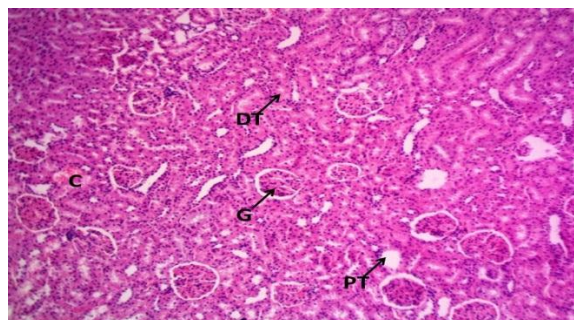


Fig.10. Histological section of a rat kidney from the group treated with the drug at a dose of 25 mg and melatonin M+J25, showing the normal histological features represented by renal glomeruli (G), proximal renal tubule (PT), distal renal tubule (DT) and slight congestion in blood vessels (C). Hematoxylin and eosin staining

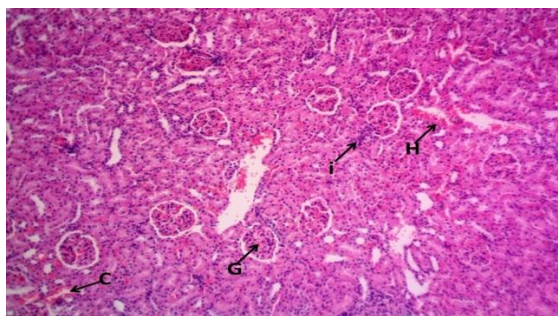


Fig.11. Histological section of a rat kidney from the group treated with the drug at a dose of 50 mg and melatonin M+J50 showing the normal histological features represented by renal glomeruli (G), slight infiltration of inflammatory cells (i), vascular congestion (C) and hemorrhage (H). Hematoxylin and Eosin Tincture, 100X

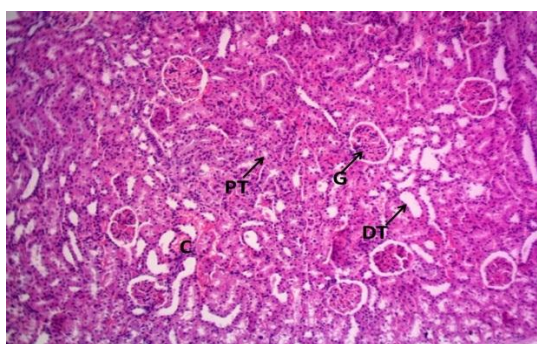


Fig.12. Histological section of a rat kidney from the group treated with melatonin and the drug at a dose of 25 mg then melatonin M+J25+M showing the normal histological features represented by renal glomeruli (G), proximal renal tubule (PT), distal renal tubule (DT) and slight congestion in blood vessels (C). Hematoxylin and Eosin Tincture, 100X

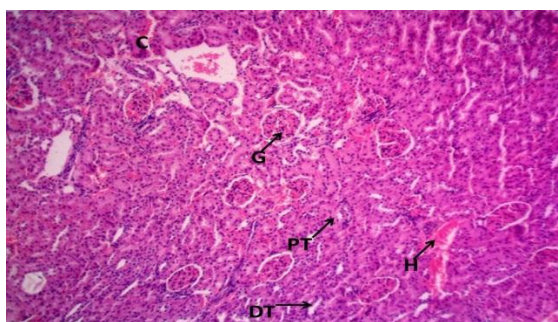


Fig.13. Histological section of a rat kidney from the group treated with melatonin and the drug at a dose of 50 mg then melatonin M+J50+M showing the normal histological features represented by renal glomeruli (G), proximal renal tubule (PT), distal renal tubule (DT), slight congestion in blood vessels (C) and hemorrhage (H). Hematoxylin and Eosin Tincture, 100X.

4. Discussion

Oxidative stress was produced from the treatment of cytotoxic drugs that are used for treatment of malignant diseases and caused hepatic and kidney problems [13]. This study focuses on gemcitabine side effects and the protective effect of antioxidant hormone of melatonin that are suppressor.

The cell damage that happened with chemotherapy treatment many studies suggest that melatonin has the ability to plays important role in the extrinsic pathway of apoptosis by modulation the expression of death receptors and decreases the concentration of caspase-3 enzyme activity and attenuate the expression level and elimination from the oxidation free radical of the cell then reduced the activity of caspase-3enzyme [14,15].

The antioxidant ability of melatonin on the levels of GSH,CAT proved the activation of antioxidant enzymes and its scavenging capacity and defense power of free radicals damaged and prevented the progression and incidence of disease[16]. The antioxidant effect of melatonin depended on cellular receptors that have scavenging action during many processes like interaction with hydroxyl radical(OH)and to detoxifying several (RNA) and (ROS) species melatonin metabolizes this toxic species to the molecules [17]. Chemotherapy treatment like gemcitabine has ability to increase lipid peroxidation products like MDA and peroxy nitrate radicals that are very harmful to living cells it can combine with other molecules like proteins lipids and DNA melatonin proved ability to protect DNA and other molecules by initiation gene expression of many enzymes that exert a crucial effect in maintaining oxidant-antioxidant homeostasis [18,19].

Chemotherapeutic drugs like gemcitabine can produce many histological alterations in the tissues causing lipophilic compounds ,metabolic pathways 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 renal tubule due to complication of gemcitabine administration in cancer patient and agreement with several studies like this [20-24].

Melatonin in this study improved its ability to reduce the changes and histopathological damag in kidney tissues that happened by gemcitabine drug indifferent concentrations ,and produces more protective effects by restoring the tissue normal appearance [28-31] .

These results are antecedently traced and supported by many researchers who declared that the use of antioxidant agents to normalized the histological changes induced by chemotherapy anticancer drugs [32]. the role of the antioxidant hormone melatonin is to inhibit the oxidative damage and prevent metastasis and cell proliferation[33-35].

5. Conclusion

Administration of anticancer drug gemcitabine increases the level of Caspase-3 enzyme and oxidative enzyme while Melatonin given produced protective effect on liver and kidney tissues from damage that happened by Gemcitabine

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