Melatonin Ameliorates Hepatic Damage Induced by Cyclophosphamide in Rats [+]

تأثير الميلاتونين في تقليل الضرر الكبدي المستحدث بالسايكلوفوسفامايد عند الجرذان

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Abstract:

The present study investigated the protective effect of melatonin on the tissue peroxidative damage in cyclophosphamide (CP) induced hepatotoxicity. Male albino rats of 250±20 gm were categorized into three groups. Two groups were administered CP (150 mg/kg/day intraperitoneally for 2 days) to induce hepatotoxicity; one of these groups received melatonin treatment (5 mg/kg intraperitoneally for 2 days 30 minutes prior CP administration). Group one served as the control. The extent of liver damage in CP-induced rats was evident from the increased activities of serum aminotransferases (ALT), alkaline phosphatase(ALP) and lactate dehydrogenase(LD); whereas melatonin pretreatment prevented the rise in these marker enzymes. Evaluation the changes of non enzymatic antioxidants glutathione along with malondialdehyde levels in the experimental groups. In CP-administered rats the antioxidant molecules showed depleted levels ($p<0.05$), in comparison with the control group. However the extent of lipid peroxidation and the abnormal antioxidant status were normalized in melatonin pretreated rats. Melatonin exerted cytoprotection towards liver organ at a dose of 5mg/kg/day. Histopathological examination also confirmed the protective efficacy of melatonin which may be efficacious as a hepatoprotectant in cyclophosphamide-induced liver toxicities.

Keywords: **Cyclophosphamide; melatonin; Hepatotoxicity**

المستخلص :

أظهرت هذه الدراسة الحديثة التأثيرات الوقائية للميلاتونين على الأنسجة المتضررة من فرط الأكسدة الناتج من استخدام السايكلوفوسفامايد والذي يؤدي إلى تسمم الكبد. تم استخدام ثلاثة مجاميع من ذكور الجرذ بوزن ٢٥٠±٢٠ غرام، أثنين من المجاميع تم إعطائهم السايكلوفوسفامايد بجرعة ١٥٠ملغم/كغم عن طريق البريتون ليومين لاستحداث تسمم الكبد. تم أعطاء الميلاتونين لأحد هذه المجاميع بجرعة ٥ملغم/كغم عن طريق البريتون ليومين قبل ثلاثين دقيقة من استخدام السايكلوفوسفامايد. تم استخدام مجموعة أخرى من الجرذ كمجموعة سيطرة. تم تحديد مدى ضرر الكبد عن طريق اختبار وظائف الكبد وحساب مقدار الكلوتوثايون وامالونديلديهايد، كذلك الفحص النسيجي للكبد. يمكن الاستنتاج من هذه الدراسة إن استخدام الميلاتونين له القابلية على منع الضرر النسيجي الناتج من استخدام السايكلوفوسفامايد باستخدام الميلاتونين، كمان له القابلية في تحسين مستويات وظائف الكبد والكلوتوثايون اضافة الى خفض مستوى المالونديلديهايد.

[+]Received on 19/10/2009 ,Accepted on 19/5/2010

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Introduction:

Melatonin, the pineal gland hormone known for its ability to modulate circadian rhythm, has recently been studied in its several functions. It is believed to inhibit cancer growth, to stimulate the immune system and to act as an antioxidant. In particular, the antioxidant activity of melatonin is ascribed to two different mechanisms: (i) melatonin stimulates radical detoxifying enzymes, such as glutathione peroxidase [1]; (ii) melatonin directly scavenges $OH^{\cdot}$ radicals with an efficacy greater than vitamin E or mannitol [2]. These data, coupled with those relative to its inhibitory effects on cytochrome P-450, actually render melatonin one of the most studied agents in the field of antimutagenesis/anticarcinogenesis research [3].

In the present work melatonin was analysed for its protective activities both as an antioxidant agent and as a hepatoprotectant against cyclophosphamide, which is a well-known alkylating chemotherapeutic agent with immunosuppressive activities. Cyclophosphamide is effective against a wide spectrum of malignancies, such as, leukemia, lymphoma, breast, lung, prostate, and ovarian cancers [4]. The parent compound is inactive *in vivo* and *in vitro* and exerts its biological activities through metabolites, mainly phosphoramide mustard, by hepatic microsomal enzymes [5]. The alkylating metabolite(s) can bind to a variety of molecules including amino acids, proteins, and peptides, but the most important binding site is DNA where cross- linking occurs [6].

The hepatotoxicity of cyclophosphamide was observed in the increased therapeutic dose of cyclophosphamide. Phospharamide mustard and acrolein are 2 active metabolites of cyclophosphamide produced by the liver microsomal enzymes [7]. Cyclophosphaide's antineoplastic effects are associated with phosparamide mustard, while acrolein is linked with its toxic side effects [8]. Increased deposition of the extracellular matrix components, particularly collagen, is a central phenomenon in liver fibrosis. Stellate cells, the central mediators in the pathogenesis of fibrosis are activated by free radicals, and synthesize collagen [9].

To avoid this toxic side effect, some antioxidant agents should detoxify the toxic acrolein. Melatonin is a potent physiological scavenger of hydroxyl radicals [10]. Melatonin has also been shown to be involved in the inhibitory regulation of collagen content in tissues. The present study investigated the protective effect of melatonin on the tissue peroxidative damage and abnormal antioxidant levels in cyclophosphamide induced hepatotoxicity.

Materials & Methods:

Animals

Thirty six male rats (*Rattus norvegicus*) are used in the present study, weighing 120-140 g, housed in the animal house of the College of Pharmacy, University of Baghdad. All animals received human care according to the criteria outlined in the guide for the care and use of laboratory animals prepared by the national academy of sciences and published by the national institutes of health.

Drug Administration

Animals were divided into three groups and were provided food and water ad libitum. Two groups were administered cyclophosphamide(150 mg/kg /day intraperitoneally for 2 days) to induce hepatotoxicity; one of these groups received melatonin treatment(5mg/kg/day intraperitoneally for 2 days prior to the cyclophosphamide administration). A vehicle (saline) control group was also included.

Biochemical Analysis

At the end of treatment period, the rats were sacrificed by an overdose (100mg/kg) of thiopental (twenty-four hour after the last injection). Laparotomy was performed to obtain livers for the assessment of tissue damage. After animals were sacrificed, blood samples were obtained by heart puncture and immediately placed into plane tube to obtain the serum for analysis of (ALT, AST and ALP). In the plane tube, blood allowed to clot and serum was separated after centrifugation for (15-20) minutes at 3000 rpm and the resulted serum was kept frozen at (-18ºC) unless immediately analyzed.

Histological Examination

Livers were excised from each animal immediately, placed in chilled saline phosphate buffer solution, blotted with filter paper and accurately weighed. A 10% (W/V) tissue homogenate was prepared for measurement of MDA and GSH in phosphate buffer at 4^{o}C, using metal head tissue homogenizer which was adjusted at set 3 for one minute. All samples were kept frozen at (-18 ^{o} C) unless analyzed immediately.

Specimens from liver were prepared for histopathological examination according to the method of Bauer [11], using paraffin sections technique.

Statistical analysis

The significance of differences between mean values was calculated using unpaired Student's ***t***-test and analysis of variance (ANOVA). *P* values less than 0.05 were considered significant for all data presented in the results.

Results:

Cyclophosphamide administration induced severe biochemical changes as well as oxidative damage. Table 1 shows the significant elevation ($p<0.05$)in MDA levels in liver tissues after exposure of animals to 150mg/kg cyclophosphamide compared with control group and with those animals treated with 5mg/kg melatonin. Animals pre-treated with melatonin showed significant reduction ($p<0.05$) in the level of MDA when compared with animals only treated with cyclophosphamide. Treatment of rats with 150mg/kg cyclophosphamide significantly reduces GSH levels in liver ($p<0.05$) compared with control animals. Meanwhile treatment with 5 mg/kg melatonin, administered thirty minutes prior cyclophosphamide results in significant elevation of GSH in the studied tissues ($p<0.05$) compared with cyclophosphamide and saline treated animals (table-1).

Exposure of animals to intraperitoneal injections of cyclophosphamide (150 mg/kg) for two days produces significant elevation in the serum levels of hepatic enzymes activity (AST, ALT, ALP)($p<0.05$) compared with control treated animals. Administration of melatonin in a dose of 5 mg/kg significantly reduces enzymes activities both with respect to cyclophosphamide and saline treated animal group and between each other (table-2).

Histopathological studies showed that sections prepared from livers of rats, previously treated with cyclophophamide 150 mg/kg showed diffuse marked swelling of hepatocytes and narrowing of sinusoidal spaces (fig-1b). All these pathological conditions were restored in the melatonin –treated rats (fig-1c). These restorations may be due to the protective effect of melatonin against tissue damage and oxidative stress induced by cyclophosphamide.

(Table-1) Effect of 5mg/kg melatonin on the levels of malondialdehyde (MDA) and glutathione (GSH) in livers of rats previously treated with 150mg/kg cyclophosphamide

Treatment groups	MDA nmol/g tissue	GSH µmol/g tissue
Saline (n=12)	50.9 ± 1.11^{a}	7.9 ± 0.14^{a}
Cyclophosphamide (150 mg/kg) (n=12)	148.3 ± 4.36^{b}	3.6 ± 0.13^{b}
Cyclophosphamide (150 mg/kg)+ Melatonin (5mg/kg) (n=12)	72.5 ± 2.1^{c}	6.8 ± 0.1^{c}

Data are expressed as mean ± SEM; n= number of animals; values with non-identical superscripts (a, b, c) within the same variable considered significantly different (P<0.05).

(Table -2) Effects of 5 mg/kg melatonin on the liver enzymes (AST, ALT, and ALP) of rats previously treated with 150 mg/kg cyclophosphamide.

Treatment groups	Liver enzymes level (U/L)		
	AST	ALT	ALP
saline (n=12)	57.0 ± 1.53^{a}	34.0 ± 1.20^{a}	91.3 ± 2.27^{a}
Cyclophosphamide (150 mg/kg) (n=12)	147.2 ± 3.87^{b}	122.7 ± 2.6^{b}	187.9 ± 3.44^{b}
Cyclophosphamide (150 mg/kg)+ Melatonin (5mg/kg) (n=12)	85.8 ± 2.71^{c}	57.4 ± 2.7^{c}	112.5 ± 3.33^{c}

Data are expressed as mean ± SEM; n= number of animals; values with non-identical superscripts (a, b, c) within the same variable considered significantly different (P<0.05).

a

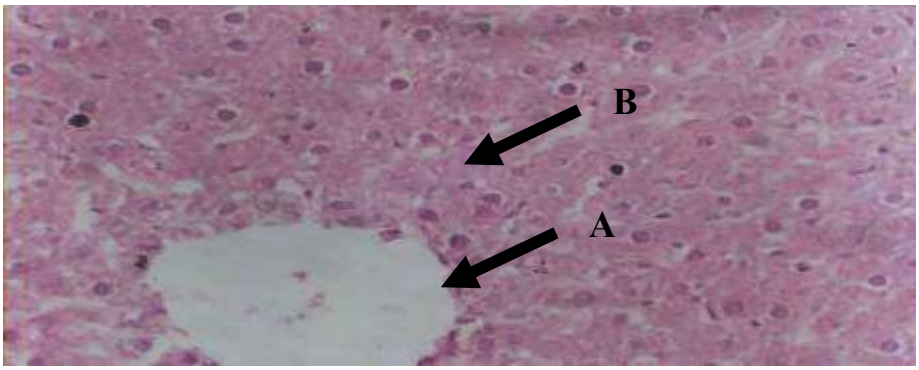

Fig.1 (a) Control liver showing normal architecture, the central hepatic vein (arrow A) and arrangement of hepatocytes around it (arrow B) (H&E X 200).

b

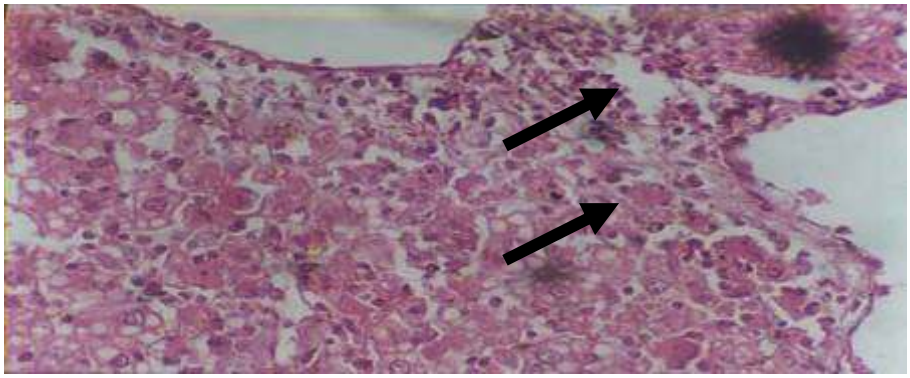

Fig .1 (b) Cyclophosphamide-administered liver showing diffuse marked swelling and narrowing of sinusoidal spaces of hepatocytes (H&E X 200).

c

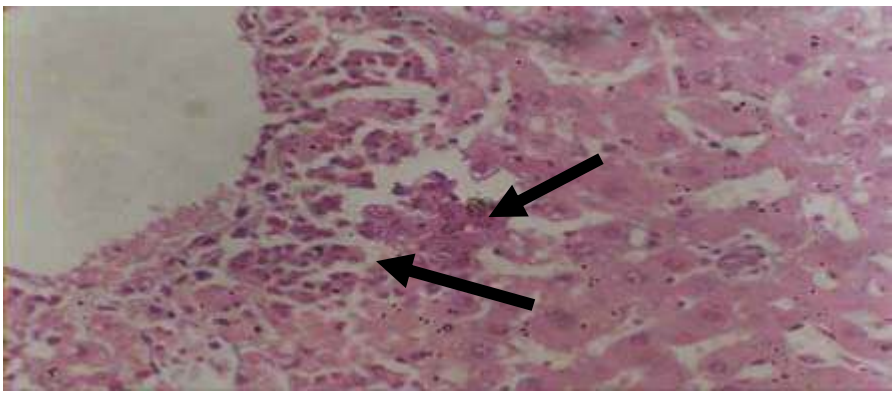

Fig .1 (c) Cyclophosphamide–administered and melatonin-treated liver showing almost normal architecture with moderate cell swelling around central vein (H&E X 200).

Discussion:

Any pathological state that leads to increased production and/or ineffective scavenging of reactive oxygen species may play a crucial role in determining tissue injury [12]. High doses of cyclophosphamide can cause death within 10 days of its administration [13]. Administration of intermittent massive doses of cyclophosphamide has been found to be advantageous in chemotherapy [14]. Cellular mechanism of toxicity is mediated by an increase in free radicals through intracellular phosphoramide mustard and acrolein, the principle alkylating metabolites of cyclophosphamide [15]. In the present study, the administration of cyclophosphamide damages the liver, and this observation is consistent with previous reports [16, 17]. Tissue damage due to cyclophosphamide might be alleviated due to

the antioxidant property and membrane stabilizing property of melatonin [18, 19 and 20]. This suggests the protective role of melatonin towards liver.

In the present study, the activities of AST, ALT and ALP are increased in the serum which considered a marker of liver damage. Hepatic dysfunction was the most common regimen-related toxicity reported in patients treated with cyclophophamide and total body irradiation [21]. Hepatic tissues were the primary sites for the microsomal activation of the drugs. Hepatic activation of cyclophosphamide leading to the formation of toxic metabolite caused damage to liver tissues as shown by increased liver enzymes in serum. The restoration of the levels of these marker enzymes in those animals intoxicated with cyclophosphamide and pre-treated with melatonin indicates the protective activity of melatonin towards livers. This might be due to scavenging activity of melatonin against the toxic metabolite that was produced during the activation of the cyclophosphamide by liver microsomal enzymes.

Histopathological studies proved that cyclophosphamide causes damage to the liver, and this was evidenced by the marked swelling and narrowing of sinusoidal spaces of liver tissues. This might be due to membrane damaging potential of the cyclophosphamide's metabolites. These pathological changes correlated well with the altered enzyme activities, these findings are compatible with other previous study [22]. During melatonin treatment these abnormal pathological findings of tissue injury and necrosis were reduced and tissues were protected from oxidative damage. The histopathological observations suggested the possibility of the melatonin being able to protect the tissues and thus decreasing the leakage of the enzymes (AST, ALT and ALP) into the circulation.

From these observations, it's possible to conclude that cyclophosphamide administration result in the pronounced oxidative stress and tissue damage due to its toxic metabolite. It is hypothesized that melatonin protects the liver tissue by scavenging the toxic metabolite. Further studies for the protective role of the melatonin in cyclophosphamide – induced toxicities are in progress.

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