Effect of Melatonin on some Inflammatory Markers in L-arginine Induced acute Pancreatitis in Rats

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ABSTRACT

Objective: Acute pancreatitis is an inflammatory disease of the pancreas resulting in significant morbidity and mortality, due to distant organ failure. Melatonin exerts complex physiological and pharmacological effects on multiple systems and organs. *Aim of the study:* *The present study was designed to investigate the effects of melatonin pre- and post-treatment with L-arginine (L-Arg.) on some inflammatory markers in acute pancreatitis in rats.* *Materials and methods: The present study was carried out on 75 white albino rats which were randomly classified into five equal groups;* *group I (control group);* *group II (L-arginine injected group);* *group III (melatonin pre-treatment with L-arginine);* *group IV (melatonin post-treatment with L-arginine) and* *group V (melatonin injected group). All groups were subjected to measurements of serum amylase and lipase activities; total antioxidant capacity (TAC) levels, serum and tissue TNF α levels; MPO and PON1 enzymes activities,* *in addition to* *histopathological examination of all pancreatic specimens.* *Results: L-arginine injected group showed severe necrotizing pancreatitis confirmed by histopathological changes and significant elevations in serum amylase and lipase activities relative to the control values. Also, significant increase of serum TAC, serum and tissue TNF-α levels and MPO enzyme activity and significant decrease of PON-1 enzyme activity were detected in L-arginine injected group as compared to control group. In melatonin injected groups, either pre- or post- treatment with L-arginine, serum amylase and lipase activities and both serum and tissue TNF-α levels, MPO activity were significantly decreased in both groups with significantly lower levels in rats given melatonin pre- treatment with L-arginine. Also, significant increase of both serum and tissue PON-1 were found in both groups.* *Conclusion: These data might confirm the protective effect of melatonin as an antioxidant in acute pancreatitis.*
Key wards: Acute pancreatitis (AP), tissue tumor necrosis α (TNF α), myeloperoxidase (MPO), paraoxonase-1 (PON-1)

INTRODUCTION

Acute pancreatitis is an inflammatory disease characterized by increasing incidence and associated with high morbidity and mortality[1].

The most important predictor of mortality is the development of persistent multiple organ failure[2]. The mechanisms involved in triggering distant organ inflammation are unclear, however, in addition to

the release of activated hydrolytic enzymes, different pathways have been reported, including cytokines [3], oxygen-derived free radicals[4], or activated complement [5].

Cytokines are a family of low molecular weight proteins which are not constitutively expressed in normal tissues and up-regulation is usually initiated following external stimuli such as injury or stress in various cell types. On a functional basis cytokines can be classified into two groups; the pro- and the anti-inflammatory cytokines[3]. Tumor necrosis factor α (TNF-α) is a pro-inflammatory cytokine involved in systemic inflammation and stimulate acute phase reaction. It exerts host-damaging effects in different autoimmune and inflammatory diseases. Dysregulation of TNF-α production has been implicated in a variety of human diseases, as well as cancer [6].

Neutrophils play a key role in tissue destructive conditions in various inflammatory diseases. Leukocyte activation, including neutrophils, is an important link in the pathogenesis of acute pancreatitis (AP) and associated lung injury[7]. When neutrophils become activated during phagocytosis, they undergo a process referred to as a respiratory burst. This respiratory burst causes production of superoxide, hydrogen peroxide, and other reactive oxygen species (ROS), which are all toxic to microbes[7]. Upon stimulation, neutrophils assemble and activate nicotinamide adenine dinucleotide phosphate (NADP) dependent oxidase, thus generating hydrogen peroxide, and degranulate, thereby releasing the proteins of azurophilic granules into the phagolysosome. Reactive oxygen radical formation and the liberation of proteolytic enzymes from the neutrophil granules are the two mechanisms of the damaging action of neutrophils [8].

One of the enzymes contained in azurophilic granules is myeloperoxidase (MPO) which in combination with halides and hydrogen peroxide is toxic for many cells, such as tumor cells, erythrocytes, platelets, leukocytes, and microorganisms [9].

The antioxidant status of tissues can be described by the analysis of single components in the defense systems against ROS, as well as by the determination of total antioxidant capacity (TAC). In contrary, the TAC measurement does not represent the sum of activities of antioxidants; it could be used for clinical diagnosis, as it is an easy and less time-consuming procedure [10].

Paraoxonase-1 (PON1) is expressed in the liver, released to the blood and is associated with the high-density lipoprotein (HDL) particle [11]. PON-2 is not present in blood, but is expressed widely in a number of tissues, including the liver, lungs, brain and heart[12]. Of the paraoxonase family, PON-1 is the most investigated and best understood member [11].

Melatonin is a hormone secreted by the pineal gland in the brain. It is a potent scavenger of ROS and highly effective protector of various tissues against the effects of ROS[13]. Melatonin is not only a non-enzymatic scavenger, but also an inducer of anti-oxidative enzymes such as superoxide

dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [14].

The present study was designed to investigate the effects of melatonin pre- and post-treatment with L-arginine (L-Arg.) on some inflammatory markers in acute pancreatitis in rats.

MATERIALS & METHODS

The present study was carried out on 75 white albino rats, their weight ranged 150-200 g.. During the study the animals were kept in wire mesh cages and were fed standard rat chew and allowed free access to water. They were kept under constant environmental conditions (25 ºC and 12 hours dark/light cycle). The animals were randomly classified into five equal groups.

1. **Group I (control group):** Rats received I.P injection of physiological saline in a dose of 3.2 ml/kg body weight.
2. **Group II:** In which acute pancreatitis was induced by I.P injection of 3.2 g/kg body weight L-arginine twice at an interval of one hour.
3. **Group III:** Rats were subjected to preliminary I.P injection of a single dose of melatonin (50 mg/kg body weight), 30 minutes prior to induction of pancreatitis with L-arginine.
4. **Group IV:** Rats received the same dose of melatonin one hour after the second injection of L-Arginine.
5. **Group V:** Rats received I.P injection of a single dose of melatonin (50 mg/kg body weight).

Chemicals used in the experiment including melatonin, L-arginine and paraoxon were obtained from Sigma chemical Co. St., Louis, MO, (USA). The doses of melatonin were chosen on the basis of previous studies [15]. All experiments were carried out according to the guidelines of the Ethical Committee of Tanta University, Faculty of Medicine.

■ **Sample collection:**

By the end of the experimental period (24 hours), all rats were sacrificed and blood samples were collected. Sera were separated and stored in aliquots at -70 °C till used. Then, the abdomen and the thorax were opened and the pancreases were dissected, washed three times in ice cold saline and blotted individually on ash-free filter paper. One piece was kept into 10% formalin solution and fixed for histopathological examination, and the remaining pieces were stored at -70 ºC till used for preparation of tissue homogenates. Specimens were weighed and homogenized with a potter-Elvenhjem tissue homogenizer (20-30 up and-down strokes). One part was homogenized in 1.15% KCl for estimation of myeloperoxidase activity and tumor necrosis factor α and the second one in Tris- HCl 100mM, pH 8 for estimation of PON1 activity and protein content. Tissue homogenate was centrifuged at 7,700 x g for 30 minutes at 4ºC, and the resultant supernatant was assayed for the different estimations.

All groups were subjected to measurements of the following;

1. Spectrophotometric determination of serum amylase[16] and lipase enzymes activities [17].
2. Spectrophotometric determination of serum total antioxidant capacity

using a commercial kit (Biodiagnostic Company Egypt) [18].

3. Estimation of serum and tissue tumor necrosis factor α levels: By using RayBiotech, Rat TNF-Alpha ELISA Kit [19].

4. Spectrophotometric determination of serum and tissues myeloperoxidase enzyme activity [20].

5. Determination of serum and tissue PON1 enzyme activity [21].

6. Estimation of protein content was performed for pancreatic tissue homogenates according to Lowry et al. (1951)[22].

7. Histopathological examination of formalin fixed pancreatic specimens.

Statistics:

Statistical data and analyses used in the present study were conducted, using range, the mean, standard deviation, analysis of variance [ANOVA] test followed by Tukey's post hoc test and linear correlation coefficient(r). P<0.05 was considered significant.

RESULTS

Tables 1 and 2 illustrate the statistical comparison between all studied parameters in both serum and pancreatic tissues in the studied groups. They showed significant increase of both serum amylase and lipase activities in L-arginine injected group (group II), when compared to the other groups (P<0.001). Their activities showed significant decrease in melatonin injected groups both pre- and post- treatment with L- arginine (groups III and group IV) (P <0.001), with the lowest levels in group III. There was no significant difference between the control group (group I) and melatonin injected group (group V) (P > 0.05).

Serum and tissue TNF-α levels and MPO enzyme activities were significantly increased in L-arginine injected group (group II) as compared to the other groups (P <0.001). Significant decrease in their values were detected in melatonin injected groups (groups III and group IV), with the lowest values in group III (P <0.001). There was no significant difference between the control group (group I) and melatonin injected group (group V) (P > 0.05).

Serum total antioxidant capacity level was significantly increased in L-arginine injected group (group II) when compared to the other groups (P <0.001). Significant increase of its level was also found in group III as compared to group I and group IV, with non significant difference between it and L-arginine injected group (group II) (P >0.05).

Serum and tissue PON1 enzyme activities showed significant decrease in L-arginine injected group (group II) when compared to the other groups (P <0.001). There was significant increase in their activities in both melatonin injected groups as compared to group I and group II (P <0.001), with non significant difference between them (P >0.05). There was significant increase in their activity in melatonin injected group (group V) when compared to the control (P- value <0.001).

Correlation studies showed significant positive correlation between serum TNF-α and serum amylase (r=0.97, P<0.001), lipase (r=0.929,P <0.001), serum TAC (r

=0.74, P<0.001), and serum MPO activity (r=0.863, P<0.001). Significant negative correlation was found between serum TNF-α and serum PON1 activity (r= -0.85, P<0.001) which showed significant negative correlation with serum levels of amylase (r=-0.863, P<0.001), lipase (r=-0.803, P<0.001), TAC (r=-0.591,P<0.001), and serum MPO activity (r=-0.729, P<0.001). MPO enzyme activities showed significant positive correlation with serum amylase (r= 0.858,& P<0.001), lipase (r= 0.882, P<0.001) and serum TAC (r=0.793, P<0.001). Significant positive correlation between TAC and serum amylase (r= 0.79, P<0.001), and lipase (r=0.762, P<0.001). Also, there was significant positive correlation between serum amylase and lipase activities (r =0.942, P<0.001), table 3.

Significant negative correlation between tissue TNF-**α** levels and tissue PON-1 activities (r=-0.914, P<0.001) with significant positive correlation between it and tissue MPO activities (r=0.837,P-<0.001). There was significant negative correlation between tissue PON-1activity and tissue MPO activity (r=-0.754, P <0.001), table 4.

Significant positive correlation was found between serum and tissue TNF-α levels (r=0.947, P<0.001) and between serum and tissue MPO activity (r=0.904, P<0.001. Also between serum and tissue PON-1 activity (r =0.813 & P <0.001) table 5.

Histopathological results showed no changes in the gross and histological appearance of the pancreases regarding pancreatic acini and normal pancreatic islets with normal vasculature from **control group**, as shown in Fig 1. However, sections of the pancreas of L-arginine injected group **(group II)**, showed severe inflammatory cellular infiltrate formed of lymphocytes and plasma cells associated with severe edema and focal necrotic acinar cells with hyperemia (vascular dilatation and congestion) (Fig 2). In melatonin injected group before L-arginine injection **(group III)** sections of the pancreas showed mild edema with minimal cellular infiltration mainly lymphocytic population with few plasma cells and normal pancreatic acini (Fig 3). Sections of the pancreas of melatonin injected group after L-arginine injection **(group IV)** showed moderate to severe edema and severe vascular dilatation with mild lymphocytic infiltration (Fig 4). Section of the pancreas in melatonin injected group **(group V)** showed normal pancreatic architecture regarding acini, islets and vasculature (Fig 5).

Table 1: Comparison between the studied serum parameters in all the studied groups.

	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F	P. value
Serum amylase (U/L)	63.33 $\pm$28.61	23973.33 $\pm$2673.94	13073.73 $\pm$1881.36	17427.80 $\pm$1164.51	71.33 $\pm$24.83	704.85	<0.001*
Serum lipase (U/L)	37.467 $\pm$15.684	1341.33 $\pm$338.75	750.60 $\pm$121.44	1090 $\pm$82.59	38.06 $\pm$18.23	196.031	<0.001*
Serum TNF α (pg/ml)	7.053 $\pm$1.303	192.31 $\pm$25.06	113.31 $\pm$14.312	143.98 $\pm$14.16	4.80 $\pm$0.80	507.447	<0.001*
Serum MPO (U/ml)	2.773 $\pm$0.779	13.360 $\pm$2.634	5.380 $\pm$0.814	11.380 $\pm$1.611	2.420 $\pm$0.704	167.724	<0.001*
Serum TAC (mM/l)	410.06 $\pm$75.06	1126.66 $\pm$258.31	1082.86 $\pm$127.32	703.93 $\pm$90.12	556 $\pm$65.64	75.114	<0.001*
Serum PON 1 enzyme activity (U/ml)	176.06 $\pm$17.84	78.933 $\pm$11.58	153.13 $\pm$14.26	163.200 $\pm$16.187	217.667 $\pm$29.645	106.311	<0.001*

Group I: Control group (no=15).
Group II: L-arginine injected group (no=15).
Group III: Melatonin injection pre-treatment with L-arginine (no=15).
Group IV: Melatonin injection post-treatment with L-arginine (no=15).
Group V: Melatonin injected group (no=15).

Table 2: Comparison between all tissue studied parameters in the studied groups.

	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F	P. value
Tissue TNF α (pg/mg protein)	25.047 $\pm$6.072	260.493 $\pm$29.547	133.753 $\pm$14.003	174.227 $\pm$14.488	16.047 $\pm$4.039	600.601	<0.001*
Tissue MPO (U/mg protein)	4.387 $\pm$0.736	14.693 $\pm$2.735	6.880 $\pm$0.880	13.027 $\pm$1.372	4.587 $\pm$0.690	157.112	<0.001*
Tissue PON1 enzyme activity (U/mg protein)	345.733 $\pm$84.380	145.533 $\pm$34.961	259.600 $\pm$27.841	252.533 $\pm$28.685	551.600 $\pm$59.202	129.046	<0.001*

Group I: Control group (no=15).
Group II: L-arginine injected group (no=15).
Group III: Melatonin injection pre-treatment with L-arginine (no=15).
Group IV: Melatonin injection post-treatment with L-arginine (no=15).
Group V: Melatonin injected group (no=15).

Table 3: Correlation between all studied parameters in the serum.

		Serum TNF-α (pg/ml)	Serum PON-I (U/ml)	Serum MPO (U/ml)	Serum TAC (mM/l)	Lipase (U/L)
Serum PON-1 (U/ml)	r	-0.85				
	P-value	<0.001*				
Serum MPO (U/ml)	r	0.863	-0.729			
	P-value	<0.001*	<0.001*			
Serum TAC (mM/l)	r	0.74	-0.591	0.793		
	P-value	<0.001*	<0.001*	<0.001*		
Lipase (U/L)	r	0.929	-0.803	0.882	0.762	
	P-value	<0.001*	<0.001*	<0.001*	<0.001*	
Amylase (U/L)	r	0.97	-0.863	0.858	0.79	0.942
	P-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

Table 4: Correlation between the studied parameters in pancreatic tissues

		Tissue TNF-α	Tissue PON-1
Tissue PON-1	r	-0.914	
	P-value	<0.001*	
Tissue MPO	r	0.837	-0.754
	P-value	<0.001*	<0.001*

Table 5: Correlation between studied parameters in both serum and tissues.

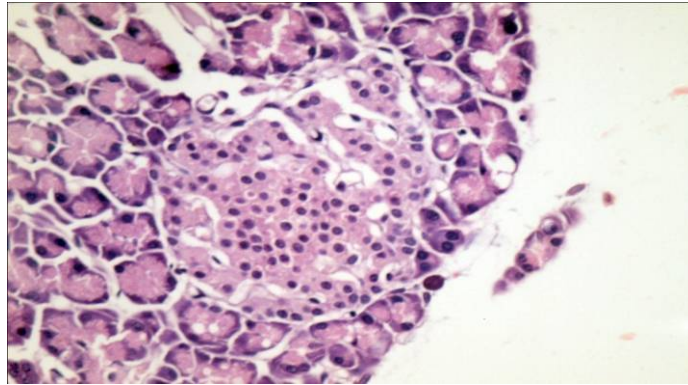|		Tissue TNF-α	Tissue MPO	Tissue PON1
Serum TNF-α	r	0.947		
	P-value	<0.001*		
Serum MPO	r		0.904	
	P-value		<0.001*	
Serum PON-1	r			0.813
	P-value			<0.001*

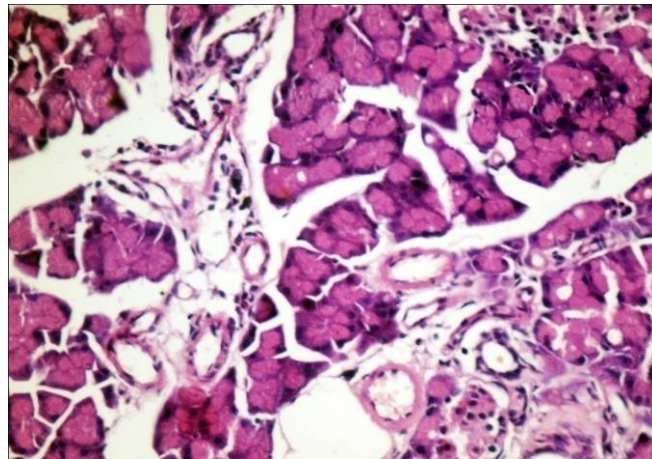**Fig 1: Section of the pancreas from group I (Hx. &E. X 200).**

Fig 2: Section of the pancreas from group II (Hx. &E. X 200).
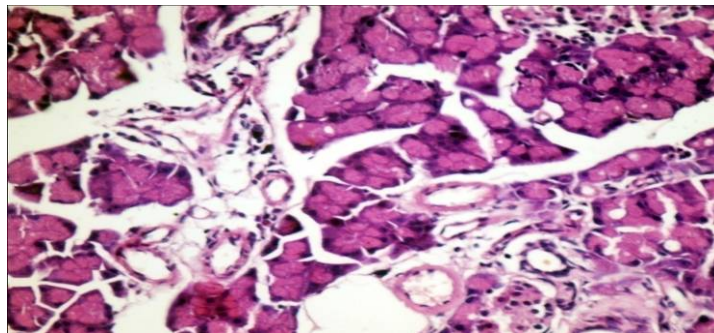

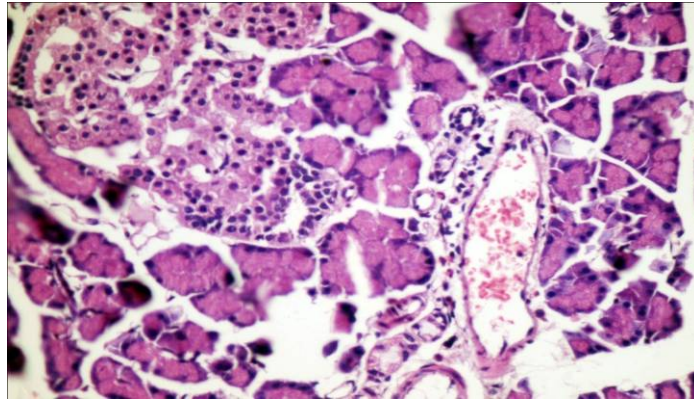**Fig 3: Section of the pancreas from group III (Hx. &E. X 200).**

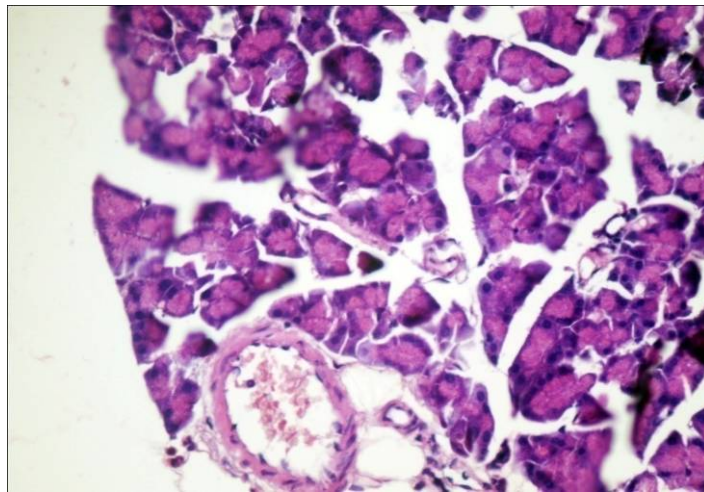**Fig. 4. : Section of the pancreas from group IV (Hx. &E. X 200).**

Fig. 5: Section of the pancreas from group V (Hx. &E. X 200).

DISCUSSION

Both experimental and clinical evidences have shown that pro-inflammatory cytokines and oxidative stress are critically involved in the development of local and systemic complications associated with acute pancreatitis [23].

Melatonin has received considerable attention because of its anti-oxidative property[24]. Accordingly, the present study was designed to investigate the effects of melatonin pre- and post-treatment with L-arginine (L-Arg.) on some inflammatory markers in acute pancreatitis in rats.

Our data showed significant increases of both serum amylase and lipase activities in L-arginine injected group. Significant decrease of their

activities was detected in melatonin injected groups. These results are in agreement with the results of **Matull et al.**[25] who reported an increase of serum amylase and lipase enzyme activities in acute pancreatitis confirming their diagnostic roles[25]. This increase may be due to the excessive production of ROS by activated leukocytes and acinar cells together with a decreased capacity of intrinsic anti-oxidative defense system leading to peroxidation of lipid membranes and disintegration of the cytoskeleton. This disturbs the intracellular transport of lysosomal and digestive enzymes, causing their premature activation, auto-digestion and cell death [25,26].

The decreased serum amylase and lipase activities with melatonin injection may be due to the effective role of melatonin in decreasing pancreatic damage caused by ROS during AP by restoring the fine structure of acinar cells and restoring tissue antioxidant enzyme activities. Also, melatonin has unique role in its ability to protect various tissues against the damage caused by ROS[27].

Several research lines have suggested that TNF-α plays a pivotal role in the pathogenesis of different autoimmune and inflammatory diseases including acute pancreatitis[28]. It is a key regulator of other pro-inflammatory cytokines and of leukocyte adhesion molecules and it is a priming activator of immune cells[29].

The important influence of TNF-α on microcirculatory disturbance in AP may be mediated through the up-regulation of the effect of Intercellular adhesion molecule I (ICAM I) on vascular endothelial cells, promotion of leukocyte adhesion, and increased capillary permeability and plasma extravasation. Also, the down-regulation of the expression of thrombomodulin in endothelial cells, activation of the coagulation system, and promotion of microcirculatory stagnation and thrombosis; and direct impairment of vascular endothelial cells, which significantly increases pancreatic capillary permeability [30].

Our study showed significant increase of both serum and pancreatic tissue TNF-α levels in L-arginine injected group. Significant decrease of their levels in melatonin injected groups either pre- or post-L-arginine injection (group III and group IV) with the lowest levels group III.

These results are in harmony with other previous studies that documented an increased blood TNF-α and its soluble receptors concentrations in rat models of acute edematous pancreatitis and acute necrotizing pancreatitis [29,31]. Also, an increased TNF-α in the serum of patients with acute pancreatitis has been reported and these levels appear to be correlated with the severity of pancreatic inflammation [32].

Su et al.[33] found an increase in TNF-α protein level in the pancreatic tissue of experimentally induced pancreatitis in rats than those of the control group. They suggested that TNF-α is a pivotal factor in pathogenesis of pancreatitis, it cooperates with amylase and lipase to intensify the leisure in pancreatic tissue resulting in bleeding and necrosis[33]. It has been reported that the overproduction of TNF-α in acute pancreatitis is pivotal in the induction

of inflammatory genes, cell death, endothelial up-regulation, and in the recruitment and activation of immune cells and it is regarded as one of the major mediators of systemic progression and tissue damage in severe form of the disease [34].

The results of the current study also confirm the notion that melatonin is capable of reducing elevated TNF-α concentrations in acute pancreatitis. These results are in agreement with those of **Gülben et al.**[35] who concluded that exogenous melatonin, and that produced from L-tryptophan, attenuates pancreatic damage in rats with acute pancreatitis and such effect could be attributed to the reduction in oxidative stress, damaging effects and TNF-*α* release [35].

Melatonin could limit the propagation of the inflammatory process and in this way could reduce pancreatic tissue damage. Also, melatonin-induced protection of the pancreas depends not only on the anti-oxidative effect of that indole, but probably also on its immune-modulatory effect[36]. Another possible mechanism of the protective effect of melatonin is that melatonin exerts an anti-inflammatory effect by inhibiting the activation of nuclear factor kappa B (NF-κB) a transcription factor with a central role in the development of inflammatory diseases. Blocking the activation of NF-κB eliminates the induced over-expression of inflammatory cytokines (TNF- *α*) in acute pancreatitis [37].

Oxidative stress and TNF-α seem to potentiate each other, generating a vicious circle in the course of AP. TNF-α intensifies oxidative stress through different mechanisms;

conversion of xanthine dehydrogenase to xanthine oxidase (XO) in endothelial cells; increasing the mitochondrial production of ROS, and promoting chemotaxis and activation of neutrophils [38]. MPO is one of the enzymes contained in azurophilic granules. Increased MPO blood levels reflect neutrophil activation[9]. Derivatives of myeloperoxides oxidation can evoke the liberation of vasoactive amines from platelets and mast cells [39].

Our results showed significant increase of both serum and tissue MPO enzyme activities in L-arginine injected group with significant decrease in their activity in melatonin injected groups (group III and group IV) with the lowest activities in group III. These results are in agreement with those of **Chooklin et al.**[40] who reported an increased MPO activity in acute pancreatitis patients which was more prominent in severe pancreatitis than in mild pancreatitis[40]. They also, showed that prolonged high MPO activity points to continued neutrophil activation with the liberation of biologically active substances (cytokines and ROS) [40].

Okutan et al.[41] **and Kaçmaz et al.**[42] reported that MPO activity, which is an index of tissue neutrophil infiltration, is inhibited by melatonin treatment, this finding suggests its anti-inflammatory effect. Melatonin also inhibits the generation of free radicals and the accumulation of neutrophils in the damaged tissues. Melatonin's antioxidant action is also derived from its protective effects on antioxidant enzymes [41,42].

Serum TAC levels were significantly increased in L-arginine

injected group. Also, significant increase of its level was found in melatonin pre-treatment with L-arginine injection (group III). Significantly lower level was found in melatonin injected group after L-arginine injection (group IV). Our results are in agreement with the results of **Chuang et al.**[10] who reported that the increased serum TAC levels in patients with acute pancreatitis reflected clinical severity of sepsis in those patients and serum TAC levels became elevated as APACHE II scores rose [10].

Serum and tissue PON1 enzyme activities showed significant decrease in L-arginine injected group, but significant increase in their activities were detected in both melatonin injected groups (group III and group IV), with no significant difference between them. Our results are in agreement with the results of **Franco-Franco-Pons et al.**[43] and **Xing-liang et al.**[44] who reported significant decrease in serum PON1 activity during severe acute pancreatitis [43,44].

The observed decrease in serum PON1 activity may be due to several factors including; proteolytic degradation of PON1 by the effect of circulating hydrolytic enzymes released in the early stages of acute pancreatitis, or through oxidative inactivation and modification of the enzyme[43]. Also, it could be related to an inhibition in hepatic PON1 synthesis. The latter possibility is related to the fact that liver is the main source of PON1 [45].

On the other hand, it has been reported that during the early stages of acute pancreatitis, a sort of acute-phase response occurs resulting in changes in the expression of different proteins in the liver. In addition, during pancreatitis, concentrations of IL-6 increases significantly, and this interleukin acts as inhibitor of liver expression of PON1 [45].

Melatonin seems to function *via* a number of means to reduce oxidative stress. Experimental evidence supports its actions as a direct free radical scavenger of peroxyl radicals, hydroxyl radicals, and other reactive oxygen species[46]. **Sahin et al.**[47] reported that melatonin has an indirect antioxidant action by stimulating the activity of several enzymes related to the anti-oxidative defense system including the activity of PON and arylesterase [47].

It also, protects the anti-oxidative enzymes from oxidative damage, and increases the efficiency of mitochondrial electron transport chain (ETC), thereby lowering electron leakage and reducing free radical generation [48].

Conclusion: All the above findings might confirm the protective effect of melatonin as an antioxidant in acute pancreatitis.

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تأثير الميلاتونين على بعض دلالات الالتهاب فى التهاب البنكرياس الحاد المسبب من أرجنين إل في الجرذان

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مقدمة البحث :

يعد مرض التهاب البنكرياس الحاد من الامراض الشديدة الخطورة و التي تؤدى للوفاة،و من العوامل الهامه التى أدت الى خطورة هذا المرض هو انتشاره من موضعه و تاثيره علي أعضاء أخرى وبخاصة الرئة و بالرغم من ان الاليات التى تسبب انتشار المرض غير واضحة تم ذكر بعض الاسباب و منها افراز الانزيمات الهاضمة و نشاط السيتوكينات و مشتقات الاكسجين الحرة. ان الضرر التاكسدى الناتج عن الخلية يتم التغلب عليه بالعديد من اليات الدفاع و منها مضادات الاكسدة و التى هى مجموعة من المركبات الطبيعية أو المخلقة حيث تقلل من تكون الشقائق الحرة كما تقلل من تفاعلها مع المركبات الهامة و مضادات الاكسدة قد تكون انزيمية اولا انزيمية. الميلاتونين (منتج الغدة الصنوبرية) و الذي يلعب دورا هاما فى تنظيم ايقاع الساعة البيولوجية وتنظيم التكاثر فى بعض الانواع قد جذب الانتباه نحوه مؤخرا لما له من نشاط مضاد للا كسدة و على الرغم من مستواه المنخفض فى الدم قد لوحظ وجود علاقة قوية بين هذا المستوى و نشاطة المضاد للاكسدة داخل الجسم او خارجه فهو قادر على ازالة اكسيد النيتروجين و مشتقات البيروكسيل و تنشيط العديد من الانزيملت الاخرى.

هدف البحث:

دراسة تاثير الميلاتونين كمضاد للاكسدةعلى التهاب البنكرياس المسبب من ارجنين ال فى الجرذان.

خطة البحث

وقد اجريت الدراسة على خمس مجموعات من جرذان التجارب كل منها خمسة عشر جرذا:

المجموعة الاولى : (مجموعة ضابطة) : حقنت بمحلول فسيولوجى بنفس كمية حقن (ارجنين ال)

المجموعة الثانية : تم احداث التهاب بنكرياسى حاد بها عن طريق حقنها فى التجويف البريتونى ب (ارجنين ال) بمعدل ٣.٢جم/كجم و ذلل مقسما علي جرعتين يفصل بينهما ساعة واحدة

المجموعة الثالثة :تم حقنها فى التجويف البريتونى بجرعة واحدة من الميلاتونين بمعدل ٥٠جم/كجم و ذلك قبل حقن الجرعة الاولى من (ارجنين ال) بنصف ساعة.

المجموعة الرابعة:تم حقنها بنفس الجرعة من الميلاتونين و ذلك بعد حقن الجرعة الثانية من (ارجنين ال) بساعة واحدة.

المجموعة الخامسة: تلقت جرعة واحدة من الميلاتونين.

و كل هذة المجموعات تم فيها القياسات الاتية:

فى المصل:

- تقدير مستوى انزيمات الليبيز والاميليز.   - قياس نشاط مضادات الاكسدة

- تقدير نشاط انزيم المايلوبيروكسيديز.   - تقدير نشاط انزيم الباراوكسينيز

ـ مستوى عامل نخر الورم الفا.

فى الانسجة البنكرياسية:

- ـتقدير نشاط انزيم المايلوبيروكسيديز.   ـتقدير نشاط انزيم الباراوكسينيز

ـ عامل نخر الورم الفا.

وتم أخذ عينات من بنكرياس كل مجموعات الدراسة للفحص الهستوباثولوجى

و قد اسفرت نتائج البحث عن:

قد اسفرت الدراسة الحالية ان هناك زيادة ذات دلالة احصائية في انزيم مستوى الاميليز و الليبيز كدلالة على حدوث التهاب البنكرياس الحاد فى الفئران المحقونة ارجنين ال و هذا نتيجة تحلل و تدمير خلايا البنكرياس نتيجة افراز انواع الاكسجين التفاعلية.

و كـان قد وجد ايضـا زيادة ذات دلالـة احصـائية فى مستويات عامل نخر الـورم الفـا فـى المصـل وفـى انسـجة البنكريـاس فى نفس المجموعة وقد انخفض انخفاضا ذو دلالة احصـائية عند استخدام الميلاتونين امـا قبـل او بعـد الحقن بارجنين ال و لكن كان اكثر انخفاضا فى مجموعة حقن الميلاتونين قبل ارجنين ال.

وكان نشاط انزيم المايلوبيروكسيديز فى المصـل و انسـجة البنكريـاس فى المجموعـة الثانيـة قد اظهر زيادة ذات دلالـة احصـائية ايضـا وقد انخفض انخفاضا ذو دلالة احصـائية عند استخدام الميلاتونين امـا قبـل او بعـد الحقن بارجنين ال و لكن كان اكثر انخفاضا فى مجموعة حقن الميلاتونين قبل ارجنين ال.

كما اسفرت النتائج ايضا عن وجود زيادة ذات دلالة احصـائية في مستوى قياس مضـادات الاكسدة فى الفئران المحقونة فى ارجنين ال لانه يتم تنشيط العمليات المضـادة للالتهابات لموازنة مستويات مفرطة من السيتوكينات الموالية للالتهابات او الاكسدة.

و من ناحية اخرى فان نشاط انزيم البارااوكسينيز فى المصـل و انسجة البنكريـاس كانت انخفضت انخفاضا ذو دلالة احصـائية نتيجة لنشاط التحلل البروتينى المرتبط بالتهـاب البنكريـاس الحـاد او مـن خلال تعطيل الاكسدة و تعديل الانزيم و قد لوحظ زيادة ذات دلالة احصـائية فى السيرم و انسجة البنكرياسـى ايضـا عند استخدام الميلاتونين اما قبل او بعد الحقن بارجنين ال. وجميع هذة النتائج تؤكد التاثير الوقائى للميلاتونين كمضاد للاكسدة فى التهاب البنكرياس الحاد.