

Effect of Melatonin on Experimentally Induced Acute Pancreatitis and Associated Hyperlipidemia

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Abstract

Melatonin (MEL) has potent antioxidant and tissue-protective effects against oxidative stress, so it attracted scientific attention. This study evaluated the effects of MEL on acute pancreatitis (AP) in male rats. Blood samples were collected for assessment of lipid profile, amylase, and lipase activity. Inflammatory cytokines, caspase-3, lipid peroxidation products (MDA), as well as the levels of the antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH), were measured in pancreatic tissue. AP was proven by histopathological evaluation and by serum elevation of amylase and lipase enzymes. MEL significantly minimized histological alterations of AP, diminished serum amylase, and lipase activity, and improved lipid profile. MEL dramatically reduced MDA levels, stopped the onset of oxidative stress, and maintained SOD, CAT, GPx, and GSH levels in the pancreas of AP rats demonstrating its antioxidant capacity. Additionally, MEL halted the rise in pro-inflammatory cytokines TNF- α , IL-6, and IL-1 β . Furthermore, MEL enhanced the anti-inflammatory cytokine IL-10 indicating its anti-inflammatory effect. Finally, it played a cytoprotective role by preventing apoptosis through decreasing elevated levels of caspase-3 and keeping cell viability. In conclusion, MEL has protective effects on the pancreas which are attributed to its anti-hyperlipidemic, antioxidant, and anti-apoptotic effects as well as its anti-inflammatory properties.

Keywords: melatonin, pancreatitis, oxidative stress, inflammatory cytokines, hyperlipidemia.

1. Introduction

Acute pancreatitis (AP) is a pancreatic inflammatory disorder that ranges in severity from minor, self-limiting disease to severe one associated with multiple organ dysfunction syndrome, high morbidity, and mortality (Hines and Pandol, 2019). It has a high incidence and a 3 to 15% death rate globally, which can be a major issue for doctors (Pădureanu et al., 2022).

One of the major factors contributing to death in AP is the emergence of systemic inflammatory response syndrome. Within 24 to 72 hours, this condition may lead to multi-organ failure (Bruno, 2001). Moreover, recurring AP attacks may give rise to chronic pancreatitis and pancreatic cancer (Robbins, 2002).

The pathogenesis of AP is most believed to entail oxidative stress, early zymogen activation, tissue

autodigestion, and a series of local and systemic inflammatory responses (Meher et al., 2015). In the pancreatic parenchyma, the autodigestive activity of acinar cells triggers an inflammatory response, resulting in neutrophil and macrophage infiltration, cytokine, interleukin 1, 6, and 8, as well as other inflammatory mediators production (Anchi et al., 2017). Moreover, the excessive formation of reactive oxygen species (ROS) in AP and the lack of scavengers' ability to destroy those ROS result in accumulation in pancreatic tissue, which causes pancreatitis (Assi et al., 2017).

Since Speck identified the link between hyperlipidemia and AP in 1865, extensive research has shown that hyperlipidemia is linked to AP in 12-38% of cases over the world (Gan et al., 2006). Research has focused on dyslipidemia that occurs during AP and the connection between AP and hypertriglyceridemia (Cheng et al., 2015; Gillies et al., 2017). Due to the frequent observation of secondary lipid abnormalities in individuals with alcohol-induced AP, as well as diabetic, pregnant, and obese patients, hyperlipidemia may be an epiphenomenon to AP (Lin et al., 2004). However, up to 7% of cases of AP are caused by primary lipid abnormalities, particularly hypertriglyceridemia or chylomicronemia (Gan et al., 2006).

Despite improvements in the supportive management of AP over the past two decades, there is still a need for specific and efficient pharmacological therapies due to the poorly understood pathophysiology of the illness (Moggia et al., 2017). Because of its complex etiology and clinical course, new methods and the use of unconventional medicinal agents are still being researched.

Melatonin (5-methoxy-N-acetyl tryptamine; MEL) is a powerful endogenous antioxidant produced and mostly secreted by the pineal gland and declines with aging. Several physiological functions linked to the daily cycle of light and darkness are under the influence of MEL. The pineal gland, plasma, and pancreas all exhibit a diurnal rhythm from this antioxidant hormone (Abdulwahab et al., 2021). MEL is recognized as a powerful tissue protector that successfully prevents oxidative stress and shields against damage brought on by toxic radicals (Reiter et al., 2016). This is correlated with its dual antioxidant actions, which include: (1) direct scavenging of harmful ROS and reactive nitrogen species; and (2) Indirect impact via the activation

of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), or glutathione reductase (Reiter et al., 2016; Goc et al., 2017).

Additionally, MEL modifies the inflammatory defense by downregulating the pro-inflammatory cytokines like interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 22 (IL-22), and tumor necrosis factor α (TNF- α) and boosting the anti-inflammatory cytokine interleukin 10 (IL-10) (Jaworek et al., 2017). It also has an anti-apoptotic effect (Abdulwahab et al., 2021). Moreover, MEL supplementation was found to be significantly related to lower levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), and elevated levels of high-density lipoprotein cholesterol (HDL-C), in a recent meta-analysis of eight randomized controlled trials (Tamura et al., 2008; Ozkalayci et al., 2021).

L-arginine-induced AP is one of the most widely used animal models. It is employed to investigate both biochemical and histopathological abnormalities caused by AP. L-arg can cause AP in rats through a number of processes, including the release of digesting enzymes, cellular proteins, and higher amounts of inflammatory mediators due to the release of oxygen and nitrogen free radicals that harm the zymogen granules' cell membranes (El-Ashmawy et al., 2018).

We hypothesized that pro-inflammatory cytokines and oxidative stress promote each other and play a major role during AP. Based on the antioxidant, anti-inflammatory, anti-apoptotic, and hypolipidemic properties of MEL, we suggest that MEL might change their production during AP. To test this hypothesis, the current study was applied to verify the potential beneficial influences of MEL in L-arg induced-AP.

2. Materials and methods

2.1. Drugs and Chemicals

Melatonin was acquired from SinaClon BioScience Company (Iran). Alpha Global Search (India) provided L-arg. Normal saline (0.9% NaCl) was obtained from the Bio-diagnostic Company, Egypt.

Wuhan EIAab Science Co. Ltd. (China) provided the kits for the testing of TNF- α , IL-6, and IL-1 β measurement kits were purchased from Elabscience Biotechnology Inc. (USA). Cusabio Technology LLC (USA) provided Kits for the determination of IL-10 and caspase-3. From the Bio-diagnostic Co

(Egypt), kits for determination of SOD, CAT, GPx, GSH (reduced glutathione), and MDA (malondialdehyde) were acquired. Kits for estimation of pancreatic function; amylase and lipase enzymes were obtained from Reactivos GPL (Barcelona, Spain). Kits for measurement of TC, TG, and HDL-C were obtained from Human Gesellschaft Für Biochemica and Diagnostica mbH (Wiesbaden, Germany). All chemicals were of analytical grade.

2.2. Animals

A total number of 32 adult male albino rats weighing 180-200g were obtained from the animal house, Faculty of Medicine, Sohag University, Egypt. Rats were kept in conventional cages with normal light/dark cycles, and a temperature of (24±2°C). The animals were fed a commercial pellet diet and had unrestricted access to food and water. Prior to the experimental procedure, rats were held for a week to allow for adaptation. The experimental technique was implemented and approved in accordance with the rules of the Medical Research Ethics Committee of the Faculty of Medicine at Sohag University in Egypt (Approval No: # S20-152).

2.3. Experimental design

Four groups of eight rats each were divided at random, and each group received the following:

Control group: rats were given normal saline intraperitoneally (i.p.) twice at one-hour interval.

MEL-treated group: rats were given MEL (50 mg/kg.i.p.) (Szabolcs et al., 2006) twice at one-hour interval.

L-arg-treated group: rats received two intraperitoneal injections of L-arg (2g/kg) (Sidhu et al., 2010) at one-hour interval.

L-arg+MEL-treated group: rats were given two intraperitoneal injections of L-arg (2g/kg) at one-hour interval and received an intraperitoneal injection of MEL (50 mg/kg) 30 min before each L-arg dose.

2.4. Samples collection

Rats from all groups were weighed and then treated with light ether anesthesia 24 hours after the last treatment. To extract the serum for analysis of the lipid profile and pancreatic enzymes, direct blood samples from the heart were obtained and centrifuged. Afterward, cervical dislocation was

used to kill animals and separate the tissue samples. The pancreas was swiftly removed and weighed after being perfused with cold normal saline (0.9% NaCl) and dried on filter paper. In phosphate-buffered saline (pH 7.4), a portion of the pancreas was weighed and homogenized. The resulting homogenate was centrifuged for 15 minutes at 4°C at 4000 rpm to separate the supernatant which was kept at -80 for further evaluation of TNF- α , IL-6, IL-1 β , IL-10, Caspase-3, SOD, CAT, GPx, GSH and MDA. The remaining part was promptly preserved in 10% formalin and examined histopathologically.

2.5. Evaluation of pancreatic edema

The pancreatic weight (P.W.) to body weight (B.W.) ratio was estimated as a basic indicator of pancreatic edema (Biradar and Veeresh, 2013).

2.6. Biochemical analysis

2.6.1. Estimation of serum pancreatic biomarkers

Serum amylase and lipase were measured spectrophotometrically (photometer 5010, Germany).

2.6.2. Estimation of a lipid profile

Total cholesterol, TG, and HDL-C were measured spectrophotometrically (photometer 5010, Germany), while LDL-C, and VLDL-C (very low-density lipoprotein-cholesterol) levels were determined using Friedewald's Formula (Friedewald et al., 1972):

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \text{TG}/5$$

$$\text{VLDL-C} = \text{TG}/5$$

2.6.3. Estimation of oxidative stress biomarkers

Superoxide dismutase was measured using Nishikimi et al. (1972) method. The data were described as U/g tissue. CAT was assayed consistent with the method of Aebi (1984). The data were reported as U/g tissue. GPx was assayed utilizing Paglia and Valentine's (1967) approach. The data were described as U/g tissue. GSH was assayed according to Beutler et al. (1963). GSH concentration was expressed in mg/g tissue. Using the Ohkawa et al. (1979) approach, the amount of MDA in pancreatic tissue was measured to assess the degree of lipid peroxidation. Data were presented as nmol/g tissue. All parameters were

estimated by a colorimetric method.

2.6.4. Estimation of inflammatory biomarkers

Following the manufacturer's recommendations, the concentrations of TNF- α , IL-6, IL-1 β , and IL-10 were measured using the enzyme-linked immunosorbent assay (ELISA). Inflammatory markers were stated as pg/g tissue.

2.6.5. Estimation of apoptotic biomarker

Caspase-3 was assessed by ELISA using the manufacturer's recommended procedure. Caspase-3 was reported as ng/g tissue.

2.7. Histopathological studies

Pancreatic specimens from various groups were embedded in paraffin wax, after being fixed in 10% formalin, then sectioned into five μ m thick sections and stained with hematoxylin and eosin (H & E). At 200x and 400x magnification, the histopathological alterations were seen using an Olympus light microscope (Tokyo, Japan).

Pathological score

As previously mentioned (Schmidt et al., 1992), a scoring system from 0 (absent) to 4 (extensive) was used to assess the pancreatic injury. The sum of the values for edema, acinar cell necrosis, inflammatory cell infiltration, and hemorrhage is known as the total pathological scores.

2.8. Statistical analysis of data

Findings were stated as mean \pm SE. The data processing software utilized was SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were used for the statistical analysis. $P < 0.05$ was regarded as significant.

3. Results

3.1. Effect of MEL on pancreatic edema (PW/BW Ratio) in the rat model of AP

According to **Figure (1)**, the PW/BW ratio significantly increased ($P < 0.05$) in the L-arg-treated group when compared to the control group. While in MEL pretreatment (L-arg+MEL group), there was a significant decrease ($P < 0.05$) in PW/BW ratio when compared to the L-arg-treated group. Moreover, there was a non-significant ($P > 0.05$) difference between the MEL-treated and L-arg +

MEL-treated groups, and the control group.

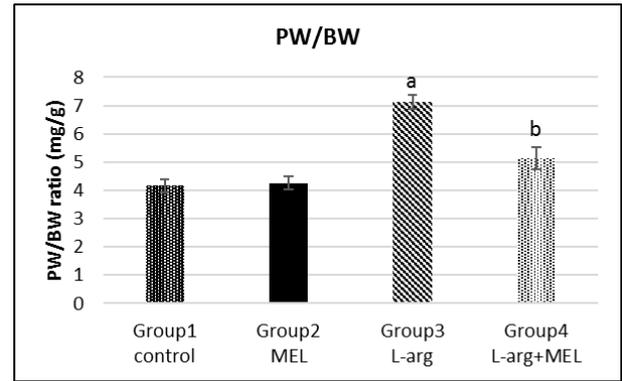


Figure 1: Effect of melatonin (50 mg/kg i.p 30 min before each L-arg injection) on PW/BW ratio in L-arginine (2g/kg i.p twice at one-hour interval)-induced acute pancreatitis. The results were analyzed with a one-way ANOVA, then subjected to a Tukey post-hoc test, and shown as mean \pm SE (n=8). MEL=Melatonin, L-arg=L-arginine, PW/BW =Pancreatic weight/Body weight. (a) $P < 0.05$ compared to the control group, (b) $P < 0.05$ compared to the L-arg group.

3.2. Effect of MEL on serum pancreatic biomarkers in the rat model of AP

The data from **Table (1)** showed that, as compared to the control group, the serum levels of amylase and lipase in the L-arg-treated group significantly increased ($P < 0.05$). Amylase and lipase significantly decreased ($P < 0.05$) in the MEL pretreatment (L-arg+MEL group) compared to the L-arg-treated group. For the MEL-treated and L-arg+MEL-treated groups, and the control group, there was no statistically significant difference ($P > 0.05$).

3.3. Effect of MEL on Lipid profile in the rat model of AP

The results shown in **Table (2)** revealed a substantial increase ($P < 0.05$) in serum levels of TC, TG, LDL-C, and VLDL-C and a significant drop ($P < 0.05$) in HDL-C in the L-arg-treated group as compared to the control group. When compared to the L-arg-treated group, there was a substantial decrease ($P < 0.05$) in TC, TG, LDL-C, and VLDL-C, as well as an increase ($P < 0.05$) in HDL-C in the MEL pretreated group (L-arg+MEL group). Moreover, there was a significant increase ($P < 0.05$) in the serum level of LDL-C in the L-arg+MEL group compared to the control group. Also, there was no statistically significant difference ($P > 0.05$) between the MEL-treated and L-arg+MEL-treated groups, and the control group.

Table 1. Effect of melatonin (50 mg/kg i.p 30 min before each L-arg injection) on serum pancreatic biomarkers in L-arginine (2g/kg i.p twice at one-hour interval)-induced acute pancreatitis:

Groups	Amylase (U/L)	Lipase (U/L)
Group1 Control	63.25±3.79	27.88±1.39
Group2 MEL	64.25±3.66	28.25±1.26
Group3 L-arg	284.5±7.9 ^(a)	128.38±5.21 ^(a)
Group4 L-arg+MEL	82.38±3.25 ^(b)	37.75±1.06 ^(b)

The results were analyzed with a one-way ANOVA, then subjected to a Tukey post-hoc test, and shown as mean±SE (n=8). MEL=Melatonin, L-arg=L-arginine. ^(a) $P<0.05$ compared to the control group, ^(b) $P<0.05$ compared to the L-arg group.

Table 2: Effect of melatonin (50 mg/kg i.p 30 min before each L-arg injection) on lipid profile in L-arginine (2g/kg i.p twice at one-hour interval)-induced acute pancreatitis:

Groups	TC mg/dl	TG mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl
Group1 control	147.13±6.17	98.75±4.01	74.38±2.9	53±2.75	19.75±0.8
Group2 MEL	148.88±5.9	100.5±4.24	73.75±2.8	55.03±3.55	20.1±0.85
Group3 L-arg	261.88±9.47 ^(a)	286.5±12.96 ^(a)	34.88±1.52 ^(a)	169.7±8.64 ^(a)	57.31±2.6 ^(a)
Group4 L-arg+MEL	168.50±5.27 ^(b)	112±3.85 ^(b)	67.63±2.17 ^(b)	78.47±5.1 ^{(a)(b)}	22.4±0.77 ^(b)

The results were analyzed with a one-way ANOVA, then subjected to a Tukey post-hoc test, and shown as mean±SE (n=8). MEL=Melatonin, L-arg=L-arginine, TC=Total cholesterol, TG=Triglycerides, HDL-C=High density lipoprotein-Cholesterol, LDL-C= Low density lipoprotein-Cholesterol, VLDL-C= Very low-density lipoprotein-Cholesterol. ^(a) $P<0.05$ compared to the control group, ^(b) $P<0.05$ compared to the L-arg group.

3.4. Effect of MEL on pancreatic oxidative stress biomarkers in the rat model of AP

3.4.1. Effect of MEL on SOD, CAT, GPx, and GSH activity

As indicated in **Table (3)**, there were significantly lower levels ($P<0.05$) of pancreatic SOD, CAT, GPx, and GSH in the L-arg-treated group than in the control group. In contrast to the L-arg-treated group, a substantial increase ($P<0.05$) in pancreatic SOD, CAT, GPx, and GSH was observed in the L-arg+MEL group. Furthermore, there was a non-significant difference ($P>0.05$) between the MEL-treated and L-arg+MEL-treated groups and the control group.

3.4.2. Effect of MEL on MDA activity

As indicated in **Figure (2)**, there was a significantly elevated level ($P<0.05$) of pancreatic MDA in the L-arg-treated group when compared to the control group. On the contrary, there was a significant drop ($P<0.05$) in pancreatic MDA in MEL pretreated group (L-arg+MEL group) compared to the L-arg-treated group. Moreover, there was no statistically significant difference ($P>0.05$) between the MEL-treated and L-arg+MEL-treated groups and the control group.

Table 3: Effect of melatonin (50 mg/kg i.p 30 min before each L-arg injection) on pancreatic SOD, CAT, GPx, and GSH in L-arginine (2g/kg i.p twice at one-hour interval)-induced acute pancreatitis:

Groups	SOD U/g tissue	CAT U/g tissue	GPx U/g tissue	GSH mg/g tissue
Group1 control	102.38±5.77	142.25±4.99	17.46±0.58	21.4±0.95
Group2 MEL	99±7.17	144±5.7	17.87±0.59	21.78±0.64
Group3 L-arg	41.38±2.09 ^(a)	67.38±2.97 ^(a)	5.75±0.29 ^(a)	6.06±0.29 ^(a)
Group4 L-arg+MEL	89.00±4.97 ^(b)	134.38±7.15 ^(b)	15.94±0.51 ^(b)	19.22±0.69 ^(b)

The results were analyzed with a one-way ANOVA, then subjected to a Tukey post-hoc test, and shown as mean±SE (n=8). MEL=Melatonin, L-arg=L-arginine, SOD=Superoxide dismutase, CAT=Catalase, GPx=Glutathione peroxidase, GSH=Reduced glutathione. ^(a) $P<0.05$ compared to the control group, ^(b) $P<0.05$ compared to the L-arg group.

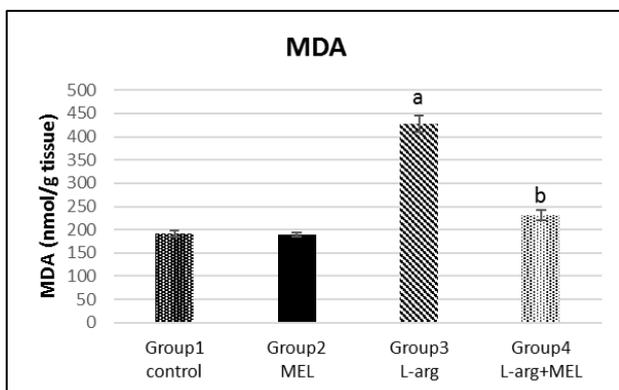


Figure 2: Effect of melatonin (50 mg/kg i.p 30 min before each L-arg injection) on pancreatic MDA in L-arginine (2g/kg i.p twice at one-hour interval)-induced acute pancreatitis. The results were analyzed with a one-way ANOVA, then subjected to a Tukey post-hoc test, and shown as mean±SE (n=8). MEL=Melatonin, L-arg=L-arginine, MDA=Malondialdehyde. ^(a) $P<0.05$ compared to the control group, ^(b) $P<0.05$ compared to the L-arg group.

3.5. Effect of MEL on pancreatic inflammatory biomarkers in the rat model of AP

The obtained data in **Table (4)** revealed a significant rise ($P<0.05$) in pancreatic TNF- α , IL-6, and IL-1 β and a significant decrease ($P<0.05$) in IL-10 in the L-arg-treated group as compared to the control group. TNF- α , IL-6, and IL-1 β levels were significantly lower ($P<0.05$) in the MEL pretreatment (L-arg+MEL group) compared to the L-arg-treated group, whereas IL-10 level was significantly increased ($P<0.05$). Also, there was a non-significant change ($P>0.05$) between the MEL-

treated and L-arg+MEL-treated groups and the control group.

3.6. Effect of MEL on pancreatic apoptotic biomarker caspase-3 in the rat model of AP

As seen in **Figure (3)**, there was a considerable rise ($P<0.05$) in pancreatic caspase-3 in the L-arg-treated group when compared to the control group. In contrast to the L-arg-treated group, the pancreatic caspase-3 level was significantly lower ($P<0.05$) in the MEL-pretreated group (L-arg+MEL group). For the MEL-treated, and L-arg+MEL-treated groups and the control group, there was no statistically significant difference ($P>0.05$).

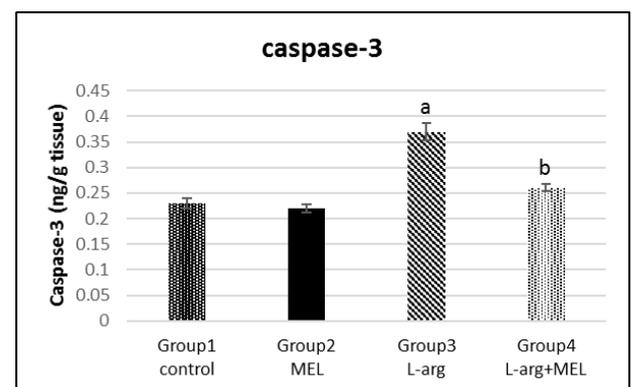


Figure 3: Effect of melatonin (50 mg/kg i.p 30 min before each L-arg injection) on pancreatic caspase-3 in L-arginine (2g/kg i.p twice at one-hour interval)-induced acute pancreatitis. The results were analyzed with a one-way ANOVA, then subjected to a Tukey post-hoc test, and shown as mean±SE (n=8). MEL=Melatonin, L-arg=L-arginine. ^(a) $P<0.05$ compared to the control group, ^(b) $P<0.05$ compared to the L-arg group.

Table 4: Effect of melatonin (50 mg/kg i.p 30 min before each L-arg injection) on inflammatory biomarkers in L-arginine (2g/kg i.p twice at one-hour interval)-induced acute pancreatitis:

Groups	TNF α Pg/g tissue	IL6 Pg/g tissue	IL1 β Pg/g tissue	IL10 Pg/g tissue
Group1 control	3.025 \pm 0.15	13.09 \pm 0.84	21.25 \pm 1.03	15.54 \pm 0.64
Group2 MEL	3.15 \pm 0.12	12.71 \pm 0.69	22.56 \pm 0.94	15.17 \pm 0.61
Group3 L-arg	12.9 \pm 0.53 ^(a)	32.32 \pm 1.53 ^(a)	54.71 \pm 2.32 ^(a)	3.97 \pm 0.21 ^(a)
Group4 L-arg+MEL	4.05 \pm 0.19 ^(b)	16.35 \pm 0.74 ^(b)	24.92 \pm 1.34 ^(b)	14.04 \pm 0.44 ^(b)

The results were analyzed with a one-way ANOVA, then subjected to a Tukey post-hoc test, and shown as mean \pm SE (n=8). MEL=Melatonin, L-arg=L-arginine. TNF- α =Tumor necrosis factor α , IL-6=Interleukin 6, IL-1 β = Interleukin 1 β , IL-10= Interleukin 10. ^(a) P <0.05 compared to the control group, ^(b) P <0.05 compared to the L-arg group.

3.7. Pancreatic histopathology

Microscopical analysis of the pancreatic tissue in the control and MEL-treated groups revealed normal-appearing acini, islets of Langerhans, and normal blood vessels. Histological evaluation of pancreas sections from rats treated with L-arg demonstrated tissue damage in comparison to the healthy control group, which is characterized by hydropic degeneration of islets of Langerhans, degenerated acinar cells with darkly pyknotic nuclei and densely eosinophilic cytoplasm, severely dilated and congested blood vessels, and inflammatory cell infiltrate of lymphocytes. Whereas the group given MEL and L-arg displayed pancreatic acini that seemed normal and had only mildly to moderately dilated and congested blood vessels (**Figure 4**).

Pathological score

When comparing the groups' overall pathological scores, the outcomes demonstrated that the group receiving L-arg had noticeably higher scores (P <0.05) than the control group, whereas the score of the MEL pretreatment group, was significantly (P <0.05) lower than the L-arg-treated group. A comparison of mean pathological scores is displayed in (**Figure 5**).

4. Discussion

Severe acute pancreatitis is accompanied by high morbidity and mortality. MEL is a strong

endogenous antioxidant and anti-inflammatory compound widely distributed in the body. It effectively controls pro- and anti-inflammatory cytokines under a variety of pathophysiological circumstances (**Su et al., 2022**). The present study revealed that melatonin has a suppressing effect against L-arg-induced AP. This effect is attributed to the anti-inflammatory, antioxidant as well as anti-apoptotic properties of MEL.

Our study aimed to elucidate the protective role of MEL on L-arg-induced AP. This study explored the effect of MEL on the PW/BW ratio that was significantly increased in the group treated with L-arg, which reflects pancreatic edema. Administration of MEL significantly reduced PW/BW ratio and alleviated all the histological alterations. This is in concordance with the results of **Jung et al., (2010)**. In addition, MEL significantly decreases pancreatic enzymes amylase and lipase. Generally, pancreatic digestive enzymes play a role in the necrosis of acinar cells at an early stage leading to acinar cell destruction, which in turn causes inflammatory processes and the release of cytokines into the pancreas and the body as a whole (**Pereda et al., 2006**). These results agree with previous studies (**Jung et al., 2010; Chen et al., 2018**).

Furthermore, it was reported that MEL exhibits a hypolipidemic effect in rats with AP. Comparing AP rats to the control group, it was shown that there was a substantial rise in the levels of the serum TC, TG, LDL-C, and VLDL-C along with a fall in the level of the serum HDL-C. In the current

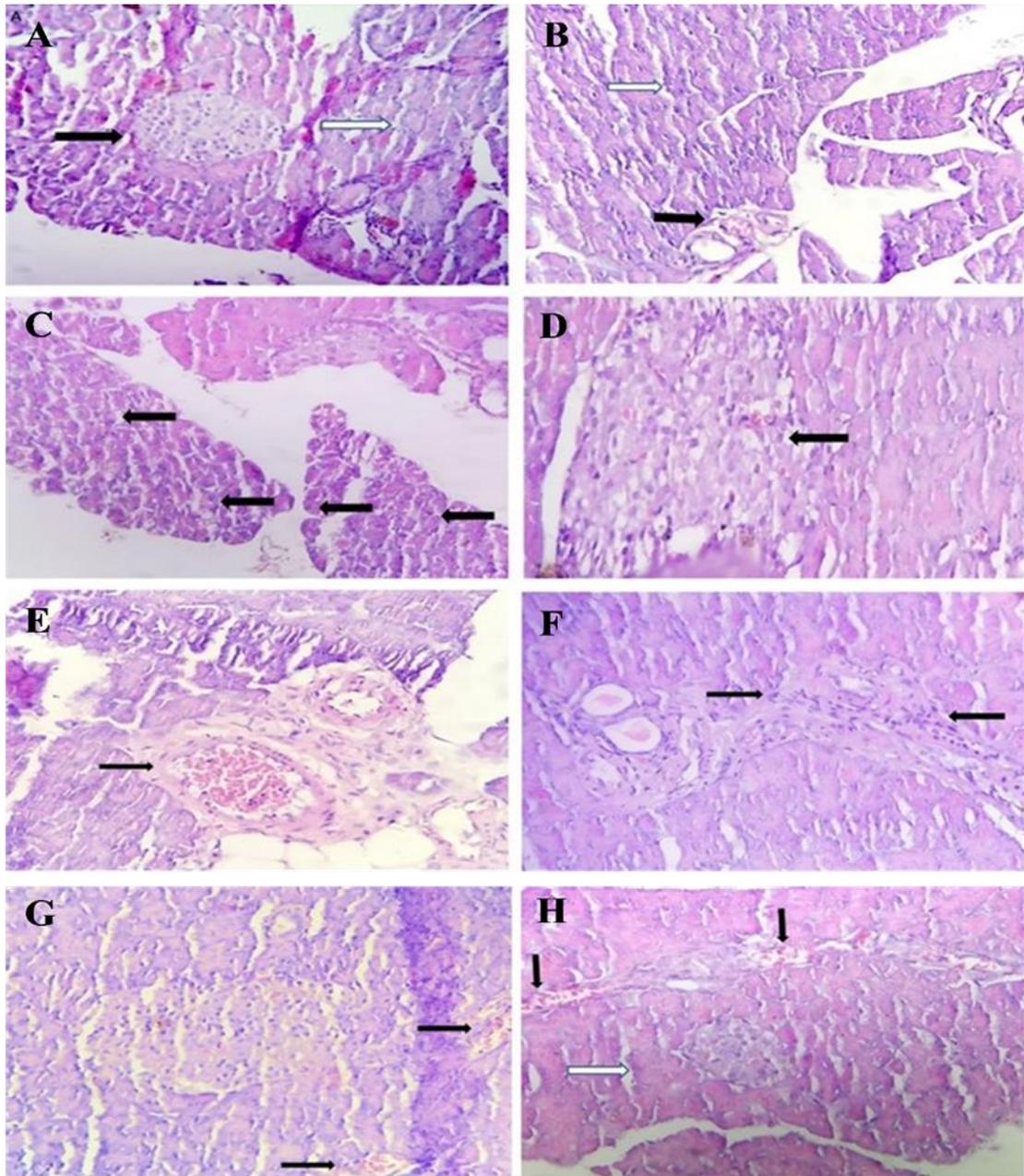


Figure 4. Photomicrographs of pancreatic sections of the Control group (A), the MEL group (B), the L-arg-treated group (C, D, E, F), and L-arg and MEL-treated group (G, H).

A) Pancreatic tissue with normally looking acini (white arrow) and normally appearing islets of Langerhans (black arrow) (H&E, 200x).

B) Pancreatic tissue with normally looking acini (white arrow) and normal blood vessels (black arrow) (H&E, 200x).

C) Degenerated acinar cells with darkly pyknotic nuclei and densely eosinophilic cytoplasm (black arrows) (H&E, 200x).

D) Pancreatic tissue showing hydropic degeneration of cells of islets of Langerhans (black arrow) (H&E, x400).

E) Severely Dilated and congested blood vessels (black arrow) (H&E, x400).

F) Inflammatory cell infiltrate of lymphocytes (black arrows) (H&E, 200x).

G) Normally looking pancreatic acini with slightly dilated and congested blood vessels (black arrows) (H&E, 200x).

H) Moderately dilated and congested blood vessels (black arrows) and relatively normal pancreatic acini and islets of Langerhans (white arrow) (H&E, 200x).

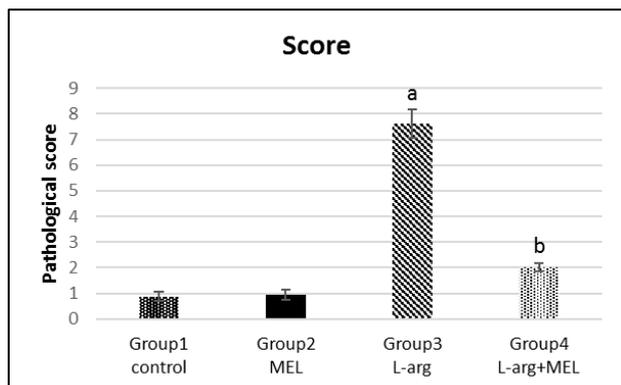


Figure 5: Effect of melatonin (50 mg/kg i.p 30 min before each L-arg injection) on pancreatic pathological score in L-arginine (2g/kg i.p twice at one-hour interval)-induced acute pancreatitis. The results were analyzed with a one-way ANOVA, then subjected to a Tukey post-hoc test, and shown as mean±SE (n = 8). MEL=Melatonin, L-arg=L-arginine. ^(a) $P < 0.05$ compared to the control group, ^(b) $P < 0.05$ compared to the L-arg group.

study, when MEL was administered to the AP group, the lipid profile significantly improved as compared to the L-arg-induced AP. These results are in harmony with those in which MEL enhanced the serum lipid profile in AP (Hadjzadeh et al., 2018; Abdulwahab et al., 2021; Yapislari et al., 2022). It's possible that MEL lowers cholesterol through reducing its intestinal absorption or by enhancing endogenous cholesterol elimination (Tengattini et al., 2008). MEL could prevent hyperlipidemia and improve HDL-C. Accordingly, the current investigation confirms that MEL has an anti-dyslipidemic effect.

Reactive oxygen species directly attack lipids and proteins in the biological membranes, leading to the formation of MDA (Dabrowski et al., 1988). In the current study, MDA, a lipid peroxidation product, significantly increased in the AP rats than in the normal control rats, indicating a state of high oxidative stress. The increased ROS production may be the cause of the elevated oxidative stress in AP rats. This sign is accompanied by a significant reduction in the SOD, CAT, GPx, and GSH. The present study describes MEL as an activator of antioxidant enzymes including the SOD, CAT, GPx, and GSH. The disturbed redox homeostasis was enhanced by MEL administration, as indicated by the lower MDA level and higher antioxidant levels in the pancreatic tissue of the treated group than in the untreated group. This finding indicates that MEL has a powerful

antioxidant capacity. This agrees with previous studies (Jung et al., 2010; Jaworek et al., 2012; Grupp et al., 2019).

Pro-inflammatory cytokines and oxidative stress promote each other, generating a vicious circle in AP that amplifies the inflammatory cascade (Pereda et al., 2006). Nuclear factor- κ B controls inflammatory reactions by up-regulating the pro-inflammatory cytokines expression (Nishida and Otsu, 2017). TNF- α is the most amplified cytokine and has a major impact in AP, which mediates both local and systemic inflammatory responses. In the present work, the tissue concentration of TNF- α , IL-6, and IL-1 β was significantly elevated but administration of MEL significantly reduced their tissue concentrations. While IL-10 was significantly reduced in the AP group and significantly increased after MEL supplementation. This suggests a heightened inflammatory response and a compromised immune system which improved after MEL administration. These results are in accordance with the earlier reports which demonstrated that the direct cause of AP induction is the pro-inflammatory cytokines (Jung et al., 2010; Jaworek et al., 2012; Chen et al., 2018; Grupp et al., 2019; Yapislari et al., 2022).

In the current study, MEL administration could reduce inflammatory damage, oxidative stress, and tissue injury. MEL played a cytoprotective role by preventing cellular apoptosis and restoring cell function in L-arg-induced AP. MEL has an anti-apoptotic effect which is mediated by decreased pancreatic tissue caspase-3 and these results are in concordance with previous studies (Col et al., 2010; Gülben et al., 2010; Bai et al., 2021). The sections of the pancreas of AP rats displayed striking damage in the form of acinar necrosis, dilated congested blood vessels, and inflammatory cell infiltration in the pancreatic tissue. These pathological changes agree with the previous study by Chen et al., (2016). The pathologic changes in pancreatic tissues declined considerably after MEL administration and this is in harmony with Chen et al., (2016) and Chen et al., (2018).

In conclusion, our findings are compatible with the available data from previous studies suggesting that administration of MEL attenuates the severity of L-arg-induced AP through suppressing pro-inflammatory cytokine production, exerting antioxidant defensive activity, and preventing

apoptosis. Considering its safety profile and efficacy we suggest that additive MEL supplementation in the AP could be beneficial.

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Conflict of interest

No overlapping interests.

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