

Protective effects of melatonin and glucagon-like peptide-1 receptor agonist (liraglutide) on gastric ischaemia–reperfusion injury in high-fat/sucrose-fed rats

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Summary

Ischaemia–reperfusion (I–R) injury is a serious pathology that is often encountered with thrombotic events, during surgery when blood vessels are cross-clamped, and in organs for transplantation. Increased oxidative stress is the main pathology in I–R injury, as assessed in studies on the heart, kidney, and brain with little data available on gastric I–R (GI–R). Liraglutide is a GLP-1 receptor agonist that has insulinotropic and weight reducing actions, and melatonin that has been much studied as a chronotropic hormone; have also studied as being anti-oxidative stress agents. Herein, we aimed to explore the effects of liraglutide and melatonin on GI–R injury with high-fat/sucrose diet. Rats were divided into six groups; two diet-control, two melatonin- and two liraglutide-pretreated groups. All rats were subjected to 30 minutes of gastric ischaemia followed by 1 hour of reperfusion. Gastric tissues were assessed for the percentage of DNA fragmentation, myeloperoxidase activity, total oxidant status, total antioxidant capacity, oxidative stress index, BMI and histopathological examination. We showed that high-fat feeding for four weeks prior to GI–R significantly increased BMI, oxidative stress indices and decreased total antioxidant capacity, with a neutral effect on apoptosis compared to controls. Pretreatment with either melatonin (10 mg/kg per day orally) or liraglutide (25 µg/kg per day ip) reverses these effects. Furthermore, both drugs reduced weight only in HFS-fed rats. Both liraglutide and melatonin have nearly similar protective effects on gastric I–R injury through decreasing the oxidative stress and apoptosis.

KEYWORDS

apoptosis, gastric ischaemia–reperfusion, high-fat/sucrose diet, liraglutide, melatonin, oxidative stress

1 | INTRODUCTION

Accumulating evidence has documented that reactive oxygen species (ROS) play an important role in the ischaemia–reperfusion (I–R) injury as they are released during the reperfusion period that further destroys the tissues.¹ As a result of reperfusion

of ischaemic tissue, there is an imbalance between the rate of generation of ROS and the tissue's ability to detoxify these reactive species.² Excess cellular levels of ROS can cause damage to nucleic acids and mitochondrial membranes that can lead to the activation of apoptosis.³ DNA fragmentation is a hallmark of apoptosis that doesn't depend on the pathway.⁴ From another

point of view, increased oxidative stress is an important pathology in obesity that is a known risk factor for I-R injury.⁵ Melatonin is a hormone secreted mostly by the pineal gland in the brain which maintains the body's circadian rhythm. Interestingly, melatonin is produced by enterochromaffin-like cells in the gastrointestinal tract in an amount about 400 fold greater than detected in the pinealocytes.⁶ Melatonin was much studied for its marked antioxidant properties⁷ and its protective role against I-R injury in various organs including heart, liver, kidney, brain, intestine, lung, and testis.⁸ On the other hand, glucagon-like peptide-1 (GLP-1) is produced naturally in the intestine and brain in both humans and rats. Previous studies have revealed profound insulinotropic and antidiabetic effects of GLP-1,⁹ but it has a limited therapeutic use as it has a short half-life (1.5 minutes). Therefore, a series of long-acting receptor agonists of GLP-1 have been developed; one of those is liraglutide. Those agonists have a powerful insulinotropic, antioxidant activity¹⁰ and weight reducing effects.¹¹ Some available data reported a valuable effect of the GLP-1R agonist on protection against cardiac I-R injury¹² and on renal I-R injury¹³ but little data is available on its effect on the gastric I-R injury.

The present study aims to assess and compare the possible protective effect of GLP-1 receptor agonist (liraglutide) with that of melatonin on gastric I-R injury and their possible underlying mechanisms as well as to assess the effectiveness of GI-R as an experimental model for I-R.

2 | RESULTS

The impact of gastric ischaemia-reperfusion (GI-R) on % DNA fragmentation, myeloperoxidase activity, total oxidant status, total antioxidant capacity, oxidative stress index and the histopathological picture of gastric mucosa were investigated in standard chow diet-fed rats and high-fat diet-fed rats in untreated rats and in rats pretreated with liraglutide or melatonin.

2.1 | Body mass index

High-fat/sucrose diet (HFS) fed rats showed significant increase in body mass index (BMI) (g/cm^2) as compared to SCD control rats ($0.75 \pm 0.1 \text{ g}/\text{cm}^2$ vs $0.50 \pm 0.05 \text{ g}/\text{cm}^2$, $P < .05$). Either melatonin-treated or liraglutide-treated SCD-fed rats exhibited insignificant change in the BMI (g/cm^2) in relation to SCD control ($0.49 \pm 0.04 \text{ g}/\text{cm}^2$ and $43 \pm 9.5 \text{ gm}$ vs $0.50 \pm 0.05 \text{ g}/\text{cm}^2$, $P > .05$), respectively. Melatonin-treated HFS-fed rats insignificantly changed the BMI ($0.64 \pm 0.08 \text{ g}/\text{cm}^2$ vs $0.75 \pm 0.1 \text{ g}/\text{cm}^2$, $P > .05$) in comparison to SCD control. While liraglutide-treated HFS-fed rats significantly decreased BMI compared to untreated HFS-fed rats ($0.45 \pm 0.02 \text{ g}/\text{cm}^2$ vs $0.75 \pm 0.1 \text{ g}/\text{cm}^2$, $P < .05$). Moreover, liraglutide-treated HFS-fed rats significantly decreased BMI when compared to melatonin-treated HFS-fed rats ($0.45 \pm 0.02 \text{ g}/\text{cm}^2$ vs $0.64 \pm 0.08 \text{ g}/\text{cm}^2$, $P < .05$) (Figure 1).

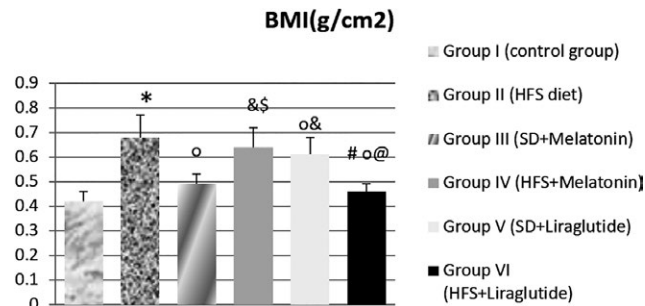


FIGURE 1 Mean \pm SD of body mass index (BMI) in all studied groups. *, $P < .05$ compared to group I (control); #, $P < .05$ compared to group II (HFS); o, $P < .05$ compared to group IV (HFS+melatonin); @, $P < .05$ compared to group V (SCD + liraglutide); &, $P < .05$ compared to group VI (HFS + liraglutide)

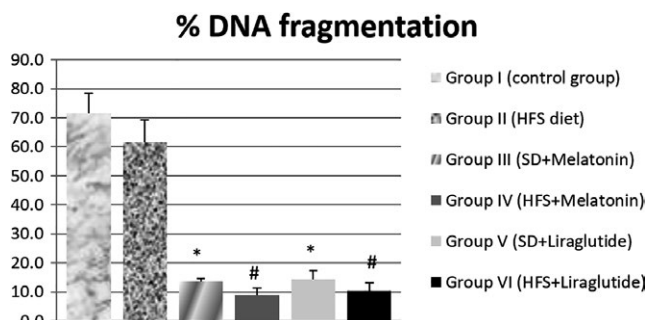


FIGURE 2 Mean \pm SD of %DNA fragmentation in all studied groups. *, $P < .05$ compared to group I (control); #, $P < .05$ compared to group II (HFS)

2.2 | Percentage DNA fragmentation

There was a significant decrease in the percentage of DNA fragmentation in the HFS group compared to the SCD control group ($61.7 \pm 9.2\%$ vs $71.4 \pm 6.6\%$, $P > .05$) respectively. Melatonin-treated or liraglutide-treated SCD-fed rats showed a significant decrease in % DNA fragmentation in comparison to the control group ($13.6 \pm 3.1\%$ and $14.4 \pm 3.4\%$ vs $71.4 \pm 6.6\%$, $P < .05$), respectively. Similarly, either melatonin-treated or liraglutide-treated HFS-fed rats showed a significant decrease in the % of DNA fragmentation in comparison to the untreated HFS group ($8.3 \pm 3.9\%$ and $10.4 \pm 3.7\%$ vs $61.7 \pm 9.2\%$, $P < .05$), respectively (Figure 2).

2.3 | Myeloperoxidase

Our results demonstrated that melatonin treatment (group III) prior to I-R showed a significant decrease in the level of myeloperoxidase (MPO) in comparison to untreated control group (group I) with mean values of $0.89 \pm 0.21 \text{ mU}/\mu\text{L}$ vs $2.67 \pm 0.61 \text{ mU}/\mu\text{L}$, $P < .05$, respectively. Also, liraglutide treatment (group V) showed a significant decrease in myeloperoxidase level in comparison to the untreated control group with mean values of $1.52 \pm 0.38 \text{ mU}/\mu\text{L}$ vs $2.67 \pm 0.61 \text{ mU}/\mu\text{L}$, $P < .05$, respectively. Melatonin treatment (group III), however,

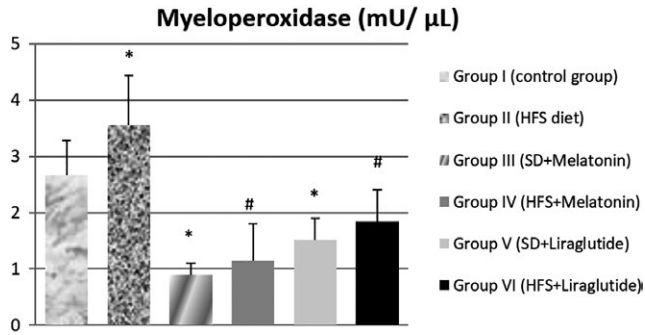


FIGURE 3 Mean ± SD of myeloperoxidase in all studied groups. *, $P < .05$ compared to group I (control); #, $P < .05$ compared to group II (HFS)

significantly decreased MPO (0.89 ± 0.21 mU/ μ L vs 1.52 ± 0.38 mU/ μ L, $P < .05$) as compared to liraglutide treatment (Figure 3). On the other hand, high-fat/sucrose diet (HFS) group (group II) showed a significant increase in the myeloperoxidase level in comparison to standard chow diet (group I) with mean values of 3.56 ± 0.8 mU/ μ L vs 2.67 ± 0.61 mU/ μ L, $P < .05$, respectively. Both melatonin or liraglutide administration combined with high-fat diet showed a significant decrease in the levels of MPO compared to the HFS group with mean values of 1.14 ± 0.66 mU/ μ L vs 3.56 ± 0.8 mU/ μ L, $P < .05$, and 1.84 ± 0.57 mU/ μ L vs 3.56 ± 0.8 mU/ μ L, $P < .05$, respectively (Figure 3).

2.4 | Total oxidant status

Melatonin-treated rats (group III) significantly decreased the gastric tissue level of total oxidant status (TOS) in comparison to the untreated controls (group I) with mean values of 16.81 ± 4.95 μ mol/L vs 29.66 ± 7.05 μ mol/L, $P < .05$, respectively. Similarly, liraglutide intake showed a significant decrease in TOS in comparison to untreated control with mean values of 16.70 ± 2.86 μ mol/L vs 29.66 ± 7.05 μ mol/L, $P < .05$, respectively (Figure 4).

On the other hand, untreated HFS-fed rats (group II) significantly increased the level of TOS (μ mol/L) compared to the untreated-SCD-fed group (group I) with mean values of 40.70 ± 7.57 μ mol/L vs 29.66 ± 7.05 μ mol/L, respectively. While melatonin-treated HFS-fed rats (group IV) significantly decreased the level of TOS compared to untreated HFS-fed rats (group II) with mean values of 18.77 ± 5.94 μ mol/L vs 40.70 ± 7.57 μ mol/L, $P < .05$, respectively. Similarly, liraglutide-treated HFS-fed rats (group VI) showed a significant decrease in the TOS in comparison to untreated-HFS-fed rats (group II) with mean values of 17.60 ± 2.77 μ mol/L vs 40.70 ± 7.57 μ mol/L, $P < .05$ (Figure 4).

2.5 | Total antioxidant capacity

The present results showed that in SCD-fed rats, melatonin-treated rats (group III) significantly increased total antioxidant capacity (TAC) compared to untreated controls (group I) with mean values

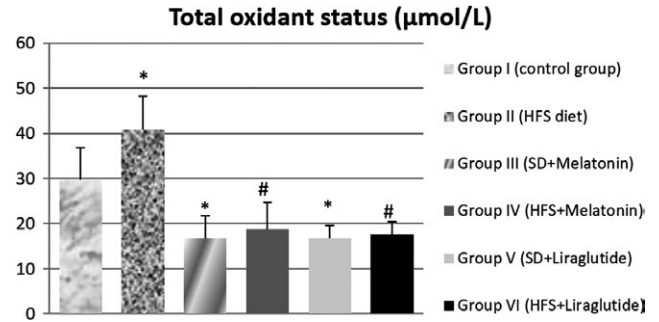


FIGURE 4 Mean ± SD of total oxidant status in all studied groups. *, $P < .05$ compared to group I (control); #, $P < .05$ compared to group II (HFS)

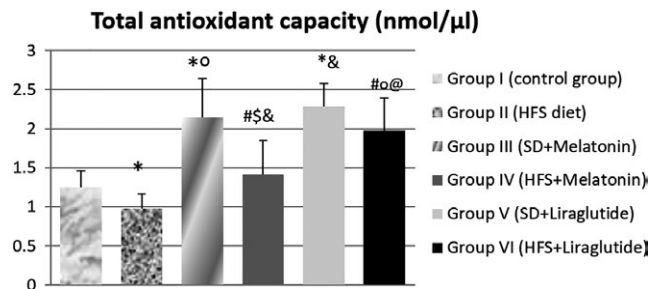


FIGURE 5 Mean ± SD of total antioxidant status in all studied groups. *, $P < .05$ compared to group I (control); #, $P < .05$ compared to group II (HFS); o, $P < .05$ compared to group IV (HFS + melatonin); @, $P < .05$ compared to group V (SCD + liraglutide); &, $P < .05$ compared to group VI (HFS + liraglutide)

of 2.14 ± 0.50 mmol/L per μ L vs 1.25 ± 0.21 mmol/L per μ L, $P < .05$, respectively. Similarly, liraglutide-treated rats significantly increased TAC in comparison to untreated control (group I) with mean values of 2.28 ± 0.30 mmol/L per μ L vs 1.25 ± 0.21 mmol/L per μ L, $P < .05$, respectively (Figure 5). Moreover, liraglutide treatment significantly increased TAC 2.28 ± 0.30 mmol/L per μ L vs 2.14 ± 0.50 mmol/L per μ L, $P < .05$, as compared to melatonin treatment (Figure 5).

In HFS-fed rats, the TAC was significantly increased in melatonin-treated rats (group IV) 1.41 ± 0.44 nmol vs 0.98 ± 0.18 μ mol/L, $P < .05$, compared to untreated-HFS-fed rats (group II). Liraglutide administration (group VI) showed a significant increase in the TOS in comparison to group II with mean values of 1.98 ± 0.41 μ mol/L vs 0.98 ± 0.18 μ mol/L, $P < .05$. Moreover, liraglutide-treated rats (group VI) significantly increased TAC 1.98 ± 0.41 mmol/L per μ L vs 1.41 ± 0.44 mmol/L per μ L, $P < .05$, compared to melatonin-treated rats (group IV) (Figure 5).

2.6 | Oxidative stress index

Our results showed that with melatonin administration (group III), there was a significant decrease in the oxidative stress index (OSI) in comparison to group I (SCD control); $0.72 \pm 0.27\%$ vs $2.45 \pm 0.74\%$, $P < .05$, respectively. Similarly, on liraglutide administration, there

was a significant decrease in OSI in comparison to group I (SCD control); $0.76 \pm 0.15\%$ vs $2.45 \pm 0.74\%$, $P < .05$, respectively. Rats on HFS (group II) showed a significant increase in the OSI when it was compared to standard chow diet (group I) with mean values of 4.36 ± 0.89 vs 2.45 ± 0.74 , $P < .05$, respectively. Melatonin treatment in HFS-fed rats (group IV) showed a significant decrease in the OSI as compared to group II with mean values of $1.34 \pm 0.31\%$ vs $4.36 \pm 0.89\%$, $P < .05$, respectively. On the other hand, liraglutide treatment in HFS diet-fed rats (group VI) showed a significant decrease in the OSI; $0.94 \pm 0.33\%$ vs $4.36 \pm 0.89\%$, $P < .05$, as compared to the HFS diet group (group II). There was a significant increase of the OSI in melatonin-treated HFS-fed rats (group IV) in comparison with melatonin-treated SCD-fed rats (group III); $1.34 \pm 0.31\%$ vs $0.72 \pm 0.27\%$, $P < .05$, respectively. Figure 6 compares liraglutide treatment in SCD-fed rats (group V) and in HFS-fed rats (group VI).

2.7 | Histopathological results

In the present work, gastric I–R in the control group (group I) showed marked oedema and disruption of gastric mucosal architecture with marked necrosis and apoptosis of the fundic glands and marked inflammatory cellular infiltration in the whole thickness of the mucosa (Figure 7A). Administration of either melatonin or liraglutide in rats fed on SCD (group III and group V) for 4 weeks before the induction of gastric I–R, reduced the oedema and moderately preserved the normal mucosal architecture with necrotic changes and sloughing off of the deep mucosa with some apoptotic changes in the upper (superficial) mucosa. They also decreased the inflammatory cellular infiltration that was limited to the basal mucosa (Figure 7B,C).

Gastric I–R in HFS group (group II) showed marked oedema and marked disruption of gastric mucosal architecture with areas of erosions (total loss of architecture) marked necrosis and sloughing of the whole thickness of the mucosa and marked inflammatory cellular infiltration that involves the mucosal thickness (Figure 7D). Administration of either melatonin or liraglutide in rats fed on HFS diet (group IV and group VI) for four weeks before the induction of gastric I–R, restored the normal mucosal architecture in comparison to HFS group (group II), where there were some necrotic changes and sloughing of the basal mucosa but only some apoptotic changes in the upper (superficial) mucosa. They also decreased inflammatory cellular infiltration that was limited to the basal mucosa (Figure 7E,F).

3 | DISCUSSION

Accumulating evidence has documented that total interruption of blood flow is often necessary during organ surgery, this interruption of blood flow is called warm ischaemia and upon revascularization, when molecular oxygen is reintroduced, the organ undergoes a process called reperfusion injury that causes cellular damage, tissue injury and deterioration of organ function.¹⁴ Patients with obesity have an increased risk of ischaemia–reperfusion injury as organ

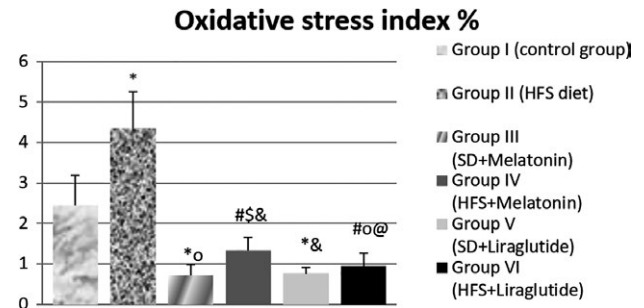


FIGURE 6 Mean \pm SD of oxidative stress index (%) in all studied groups. *, $P < .05$ compared to group I (control); #, $P < .05$ compared to group II (HFS); o, $P < .05$ compared to group IV (HFS+melatonin); @, $P < .05$ compared to group V (SCD + liraglutide); &, $P < .05$ compared to group VI (HFS + liraglutide)

steatosis can lead to mitochondrial dysfunction and signal transduction alterations.¹⁵

Moreover, obesity is commonly complicated by type 2 diabetes mellitus that is an added risk factor for I–R injury. Therefore, it is of major importance to investigate new treatment options that decrease body weight as well as decreasing the harmful effect of I–R injury by influencing the oxidative stress. Herein in the present work, we tested the hypothesis by which liraglutide can prevent I–R injury in high-fat diet-fed rats.

The results of the present study revealed that a high-fat/sucrose diet (HFS) significantly increased BMI, TOS, oxidative stress index (OSI) and myeloperoxidase (MPO) and a significant decrease in TAC, while significantly decreased TAC in comparison to SCD control, together with severe histopathological damage of gastric mucosa with areas of erosion and marked inflammatory cellular infiltration. This may be an effect of HFD irrespective of I–R injury¹⁶ or primarily due to HFD and aggravated by the ischaemic injury.¹⁷ Takeuchi et al¹⁸ explained one of the possible mechanisms that could be involved in the pathogenesis of the histopathological insult associated with I–R injury of the intestine, which is binding of endogenous corticotrophin releasing factor to its receptor.

Matsuzawa-Nagata et al¹⁹ reported that HFD-induced an increase in the oxidative stress even before the development of obesity. Furthermore, pathways for ROS production are up-regulated in both the liver and adipose tissue of HFD-fed mice even before the elevation of free fatty acids and tumor necrosis factor-alpha in the plasma or liver.¹⁹ In a similar work performed on the hepatic I–R injury; HFD increased acute mitochondrial oxidative stress, increased apoptosis and increased markers of inflammatory cellular infiltration.²⁰ HFD increases ROS production by decreasing the activities of both isoforms of the mitogen-activated protein kinase (MAPK) that is important for inhibition of mitochondrial ROS production. Moreover, MAPK α 2 is the physiological suppressor of the ROS produced by NADPH oxidase (Nox) pathway. Furthermore, the inhibition of MAPK results in an increase in the endoplasmic reticulum stress, which triggers a set of transducer proteins that stimulate the unfolded protein response that in turn trigger a low-grade inflammatory response.²¹

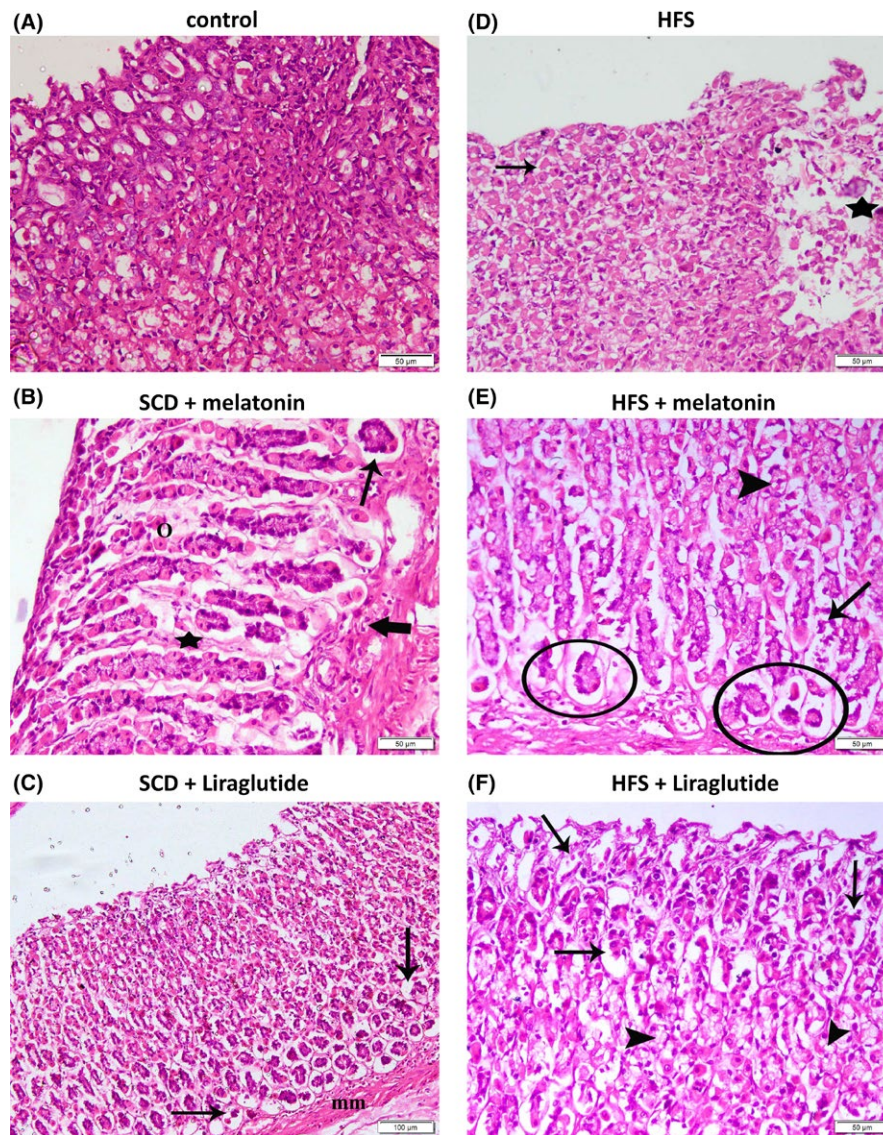


FIGURE 7 Histopathological photomicrograph of sections in the stomach of gastric ischaemia–reperfusion (I–R) rats of different groups. (A) Control group I showing a marked loss of architecture of the fundic glands with marked inflammatory cellular infiltration in the whole mucosal thickness (thick arrow) (H&E $\times 50$). (B) Group III (SCD + melatonin) showing some oedema of the mucosa (star), sloughing of the cells of the basal parts of the glands into the lumen (thin arrow) and decreased inflammatory cellular infiltration limited to the lamina propria (thick arrow) (H&E $\times 50$). (C) Group V (SCD + liraglutide) showing desquamation of mainly the basal part of the mucosa (arrow), and there is mild inflammatory cellular infiltration (thick arrow) (H&E $\times 100$). (D) Group II (HFS) showing marked distortion of the architecture of the fundic glands with areas of erosion (star) and marked desquamation all through the mucosa (arrow) (H&E $\times 50$). (E) Group IV (HFS + melatonin) showing desquamation of the basal part of fundic glands into the lumen (encircled area) and some apoptotic changes in the upper part of the fundic glands in the form of vacuolation of the cytoplasm the of the oxyntic cell (arrow head) (H&E $\times 50$). (F) Group VI (HFS + liraglutide) showing mildly preserved the architecture of the fundic glands, as there is still some desquamation of glandular cells into the lumen (arrow) and the non-desquamated glands show marked apoptotic changes in the form of cytoplasmic vacuolation of the of oxyntic cell (arrow head) (H&E $\times 50$)

The present work showed that high-fat diet insignificantly changed percentage DNA fragmentation and accordingly apoptosis compared to SCD control. This observation was reported in similar works on cardiac I–R injury.²² HFD protection against I–R injury was mediated by influencing anti-apoptotic and pro-autophagic pathways namely nuclear factor kappa B (NF- κ B) and beclin-1, a key initiator of autophagosome formation. It was also observed that the time course of high-fat-diet-induced increased autophagy and

decreased apoptosis in ischaemic hearts, correlates with the time course of cardioprotection observed as a result of high-fat feeding.²³ Furthermore, cardioprotection observed with HFD was associated with improvement in mitochondrial oxidative phosphorylation and reduced mitophagy.²⁴

In the present work, using melatonin in both SCD-fed rats and HFS-fed rats resulted in obvious improvement in GI–R-induced oxidative stress as detected by a significant decrease in the level of the

total oxidant, OSI, myeloperoxidase, and decrease apoptosis as detected by a decrease in percentage of DNA fragmentation, while total antioxidant status was significantly increased. Histopathologically, it decreased gastric mucosal injury and preserved gastric mucosal architecture, also there was decreased inflammatory cellular infiltration. Melatonin exerted similar results in a study done on renal I-R injury.²⁵ Melatonin has a direct scavenging action,²⁶ it reduces electron leakage and free radical generation within the mitochondria, by stimulating complex I and complex IV of the mitochondrial respiratory chain. This is referred to as radical avoidance.²⁷ Melatonin's indirect anti-oxidative effects were mediated through various intracellular signalling pathways, including Janus kinase 2/signal transducers and activators of transcription 3 (JAK2/STAT3), nitric oxide synthase (NOS), Silent information regulator 1 (SIRT1).²⁸ In addition, melatonin induces AMP-activated protein kinase (AMPK) phosphorylation and activation in hepatic cells.²⁹ SIRT3 serves as a downstream target of AMPK-activated peroxisome proliferator-activated receptor (PPAR γ) coactivator-1 α (AMPK-PGC-1 α) signalling, which plays a key role in the regulation of mitochondrial biogenesis and the deacetylation of mitochondrial anti-oxidative enzymes. It was found that AMPK-PGC-1 α -SIRT3 signalling is important in melatonin cardioprotective actions.²⁸

In the present work, we observed another mechanism by which melatonin can protect against GI-R injury via reducing apoptosis. In the present study, percentage DNA fragmentation was significantly reduced in melatonin-treated groups. Melatonin's antiapoptotic effect was reported in cardiac I-R injury.³⁰ Increased oxidative stress and endoplasmic reticulum stress that occur in I-R injury are a mainstay in activating apoptosis mainly through the caspase-dependent pathway.¹³

In the present work, liraglutide resulted in obvious improvement in the GI-R induced oxidative stress in SCD-fed rats and HFS-fed rats as was detected via the significant decrease in the level of the total oxidant, OSI, myeloperoxidase and percentage DNA fragmentation, while it significantly increased total antioxidant status, and improved gastric mucosal injury on histopathological examination. Our results are in accordance with Steven et al³¹ who detailed that liraglutide is a promising antioxidant and anti-inflammatory therapeutic compound. Conversely, Kiec-Klimczak et al³² announced that high-fat diet stimulates the long-lasting release of incretins, which is associated with a parallel increase of the markers of low-grade inflammation associating obesity in metabolic syndrome.

The effect of liraglutide on I-R injury is still unclear, some report protective effects,³³ while others report equivocal action.³⁴ Much fewer data are available on the effect of liraglutide on I-R injury in the context of high-fat feeding. Hossein et al³⁵ reported that treatment with glucagon-like peptide-1 analogue in HFD-induced-cardiac dysfunction, activates several cardioprotective pathways, prevents HFD-induced inflammation, reduces monocyte vascular adhesion, and improves cardiac function in vivo by activating AMPK. Moreover, liraglutide improved myocardial infarction through stimulation of pro-survival pathways in the normal and diabetic mice hearts, independent of weight loss.³⁶ On cortical neurons, the

activation of GLP-1 receptor protected against oxidative DNA damage via a signalling pathway involving DNA repair by activating the cyclic AMP response element binding protein.³⁷ Additionally, the antiapoptotic effect of GLP-1 may be through the activation of the reperfusion injury survival kinases (RISK) pathway and mediated through the inhibition of mitochondrial permeability transition pores (MPTP) opening, the blockade of calcium overload, and the activation of phosphatidylinositol 3-phosphate kinase (PI3K) pathway.³⁸ Therefore, we can conclude that GLP-1 protect against I-R injury by stimulating autophagy and suppressing apoptosis.³⁹

The present results detected that melatonin has a neutral effect on BMI in both SCD-and HFD-fed rats. Our results are in contrast with Prunet-Marcassus et al⁴⁰ who reported that melatonin appears to exert their weight reducing effect only when energy balance is disturbed. Moreover, Kaskar detected that melatonin treatment significantly reduced body weight in both SCD and HFD groups.⁴¹ Melatonin has a central effect on gene expression of some feeding behaviour signals as neuropeptide Y and leptin.⁴² Moreover, it regulates energy expenditure through controlling the size and activity of the brown adipose tissue as well as the browning process of the white adipose tissue.⁴³ However, liraglutide induced a weight reducing effect only in HFS-fed rats. This weight loss effect of liraglutide may be via a central effect, as liraglutide showed shifted food preference (increased chow/decreased candy consumption).⁴⁴ In addition, the high-fat diet may alter gut-brain communication.⁴⁵ On the other hand, it was reported that liraglutide's significant attenuation of the weight gain in both chow diet and HFS diet may be due to a reduction in food intake.⁴⁶

In conclusion, the results of the present study provide evidence that administration of either melatonin hormone or liraglutide drug prior to GI-R has a similar protective effect on gastric tissue against oxidative stress and apoptosis in male albino rats fed on standard chow diet or high-fat/sucrose diet. Moreover, liraglutide has a weight reducing effect only in HFS-fed rats, but not in SCD-fed rats.

4 | MATERIAL AND METHODS

4.1 | Experimental animals and design

Sixty male albino rats belonging to the local strain and weighing 100-150 g were included in the present study. They were obtained and inbred, with veterinary care, in the animal house of the Ophthalmology Research Institute. The animals were housed in wire mesh cages at room temperature with normal light-dark cycles and maintained on standard rat chow diet or high-fat/sucrose diet and tap water during the four-week experimental period. They were allowed to acclimatize to their environment for 1 week before the start of the experiments which were conducted in accordance with the ethical guidelines of the committee of the faculty of medicine, Cairo University.

The rats were divided randomly into six experimental groups of 10 rats each.

Group I (control group). The rats were fed on a standard chow diet for 4 weeks before the induction of GI-R.

Group II (HFS group). The rats were fed on high-fat/sucrose diet⁴⁷ for 4 weeks before induction of GI-R.

Group III (SCD + melatonin). Standard chow diet-fed rats were treated with melatonin at a dose of 10 mg/kg per day orally for 4 weeks before the induction of GI-R.⁴⁸

Group IV (HFS + melatonin). High-fat/sucrose-fed rats were treated with melatonin in the same regimen as group III.

Group V (SCD + liraglutide). Standard chow diet-fed rats were treated with liraglutide in a dose of 25 µg/kg per day intraperitoneally for 4 weeks before the induction of GI-R.^{36,47}

Group VI (HFS + liraglutide). High-fat/sucrose-fed rats were treated with liraglutide in the same regimen as group V.⁴⁷

4.2 | High-fat/sucrose diet

The high-fat diet was prepared as 10% fat of animal origin (10 g of fat/100 g of rat chow) that was combined with 10% sucrose added to drinking water⁴⁷ only in the high-fat diet-fed groups, to prevent hypoglycaemia, a possible cause of mortality with liraglutide.⁹

4.3 | Drugs

Liraglutide (Victoza, rDNA liraglutide), manufactured by Novo Nordisk (Bagsvaerd, Denmark) was obtained as a 3 mL-injection pen (6 mg/mL). Melatonin, 20 mg tablets, is a product manufactured by Puritans Pride, USA.

4.4 | Induction of gastric ischaemia-reperfusion

At the end of the 4-week experimental periods, rats were anaesthetized with urethane anaesthesia at a dose of 1.25 gm/kg ip.⁴⁹ Then the abdomen was opened by a longitudinal incision, and the coeliac artery was exposed then clamped with a disposable vascular clip to induce ischaemia for 30 minutes, then reperfusion was done for 1 hour.⁵⁰ Then, all rats were killed and stomachs were rapidly excised and divided longitudinally into two halves, the first half was preserved in formalin for histopathological examination and the second half was frozen for further assessment of percentage (%) DNA fragmentation, myeloperoxidase activity, TOS and total antioxidant activity.

4.5 | Determination of body mass index

The weights and nose-anus lengths measured at the end of the experimental period were used to calculate BMI according to the formula.⁵¹

$$\text{Body mass index (BMI)} = \text{body weight (g)} / \text{square length (cm}^2\text{)}$$

4.6 | Biochemical measures

Measurements of %DNA fragmentation was done using diphenylamine colorimetric assay⁵² and TOS in gastric tissues were

done using assay kit from Rel Assay Diagnostics Clinical Chemistry Solutions (product code: RL0024, Turkey).⁵³ Myeloperoxidase activity in gastric tissue was estimated using a colorimetric assay for MPO activity from Northwest Life Science Specialities (NWLSS; Product NWK-MPO03, Amsbio, Cambridge, MA, USA).⁵⁴ while TAC in gastric tissue was estimated using the TAC assay kit from Cell Biolabs (product code: STA-360, San Diego, CA, USA).⁵⁵ Determination of oxidative stress index (OSI) was calculated as the percentage ratio of TOS to TAC according to the formula: $\text{OSI} = (\text{TOS } \mu\text{mol/TAC } \mu\text{mol}) \times 100$.⁵⁶

4.7 | Histopathological procedures

The gastric mucosal injury was assessed according to the method described previously by Wada et al⁵⁷ Briefly, the tissue from the gastric mucosa of each animal was fixed with 4% formaldehyde, dehydrated ethanol, and embedded in paraffin wax. Sections were cut at 5 µm, mounted on clean glass slides, and dried overnight at 37°C. Sections were cleared, hydrated, and stained with hematoxylin-eosin for light microscopic observation. The blind analysis was performed on all samples in an Olympus BH-2 microscope for characterization of histopathological changes.

4.8 | Statistical analysis

Results were expressed as mean ± SD. The data were evaluated with Statistical Package for the Social Sciences (spss) 13.0. The statistical significance of differences for each parameter among the groups was evaluated by one-way ANOVA, followed by post hoc test. *P* values of ≤.05 were considered to be statistically significant.

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CONFLICT OF INTEREST

No conflict of interest to be declared.

AUTHOR CONTRIBUTION

All authors on this paper shared in the design, data analysis and interpretation, drafting of the manuscript, critical revision of the manuscript and approval of the article. Dina H. Merzeban shared in the concept and the design, acquisition of data, data analysis and interpretation, drafting of the manuscript. Hanan A. Mubarak, Manal M. Mahmoud, Heba S. Shoukry shared by critical revision of the manuscript and approval of the article. Laila A. Rashed shared by performing biochemical labs. Safinaz S. Sayed shared by data analysis, interpretation and critical revision of the histopathological part.

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