BENHA MEDICAL JOURNAL bmj
**Print ISSN** 1110-208X
**Online ISSN** 2357-0016

Original article

# Possible Therapeutic Effect of Ghrelin and Exosomes Derived from Mesenchymal Stem Cells on Induced Chronic Pancreatitis of Adult Albino Rats (Histological and Immunohistochemical Study)

Walaa S. Attaia, Mohammed M. Yousef, Nahla E. El-Azab, Nessrein E. Salem

Department of Histology and Cell Biology, Faculty of Medicine, Benha University, Egypt.

**Correspondance to:** Walaa S. Attaia, Department of Histology and Cell Biology, Faculty of Medicine, Benha University, Egypt

**E-mail:**

walaa-salim@fmed.bu.edu.eg

**Received:** 3 January 2022

**Accepted**: 6 June 2022

## Abstract

**Background:** Chronic pancreatitis (CP) is a disease characterized by permanent damage of the pancreas. **Objective:** evaluate the possible therapeutic effect of ghrelin and exosomes derived from mesenchymal stem cells in the treatment of chronic pancreatitis. **Materials and Methods:** Sixty-two adult male albino rats were randomly divided into six groups. Group I (control group). Group II (affected group): Rats were injected intraperitoneally by l-arginine at a dose of 500 mg/100g body weight that is 2.5 ml of l-arginine solution/100g body weight five times at three days intervals, dissolved in citrate buffer. Group III (recovery group). Group IV: CP treated with ghrelin. Group V: CP treated with MSCs derived exosomes. Group VI: CP treated with ghrelin and MSCs derived exosomes. Pancreatic specimens were taken and prepared for histological and immunohistochemical examination. **Results:** Group II and III showed signs of degeneration. There were distorted acini with cytoplasmic vacuolation, decrease number of cells of islets, empty spaces between islets, dilated congested blood vessels and cellular infiltration. Furthermore, there was intense positive collagen fibers deposition, *iNOS* and CD8 immunostaining. Groups IV and V showed improvement of some histological microscopic changes in group II, significant decrease ($P < 0.01$) of group IV, V, VI compared with group II. While group VI showed histological architecture near to control group. **Conclusion:** ghrelin and exosomes can treat chronic pancreatitis. However, better results can be obtained when exosomes were given with ghrelin.

**Key Words:** CP, ghrelin, exosomes, Masson trichrome, *iNOS* and CD8.

## Introduction

Chronic pancreatitis (CP) is characterized by chronic inflammation of the acinar cells leading to progressive fibrosis of the pancreas leading to destruction of the exocrine functions of the pancreas. There are factors responsible for the development of CP involve hereditary factors, alcoholism, autoimmune injury and smoking (1).

Current therapeutic options, such as lipase, acetaminophen or NSAIDs, octreotide can relief the pain. Surgical methods will use for patients when medical approach failed (2).

Ghrelin is a 28 amino acid peptide. Oxyntic glands of gastric mucosa responsible for its secretion. It was endogenous ligand of the growth hormone (GH). Ghrelin can regulate food intake and energy expenditure (3).

Exosomes are small sized (30–120 nm) extracellular vesicles (EVs) (4). They are differing from microvesicles in size (50–1500 nm) and shed from the budding of the plasma membrane. Exosomes play an important role in cell-to-cell communication by its contents, including lipids, RNAs, DNAs and proteins (5,6). Exosomes when reach recipient cells deliver their cargoes, which can induce the intracellular signaling and affect the physiological or pathological status of recipient cells (7).

The aim of this study was to evaluate the efficacy of ghrelin and exosomes derived from mesenchymal stem cells on L-arginine induced chronic pancreatitis on adult albino rats.

## Material and Methods

### Drugs and Chemicals

**L-arginine:**

Highly purified L-arginine hydrochloride in white powder form was obtained from (*El Ezaby Pharmacies*, Heliopolis, Cairo, *Egypt)*. L-arginine solution was prepared by dissolving of L-arginine (2 g) in saline (8 ml) and then adjust volume to 10 ml with saline, so each 1 ml contained 200 mg of l-arginine (8).

**Ghrelin:**

Ghrelin was obtained from (Sigma, St. Louis, MO, USA). Ghrelin solution was prepared by dissolving 1 mg of ghrelin acetate in 1 ml of Phosphate Buffer Solution (PBS) and then adjust volume to 25 ml with PBS. Ghrelin will be administered intraperitoneally with a single dose of ghrelin (20 μg/kg) for successive three days (9).

**Exosomes derived from MSCs:**

Exosomes were prepared in the stem cell and molecular biology unit, central lab, Cairo Faculty of Medicine. Exosomes were isolated from conditioned media of rat bone marrow derived MSC. Rats were injected intravenous with a single dose of 100 µg BMSC-EX diluted in 0.5 ml PBS (10).

**Animals and Diet:**

Sixty-two healthy adult male albino rats of weight range (180-200) grams were purchased and housed at the Animal Research Laboratory Unit, Kasr Al-Ainy Faculty of Medicine, Cairo University. All care and cleaning measures were followed to keep them in healthy conditions. All ethical guidelines for animal handling were followed by the animal facility. The experimental protocol was approved by the Institutional Animal Care Committee of Cairo University, Cairo, Egypt.

**Experimental Design:**

An experimental study was done from July 2019 to October 2019. After one week of housing, 62 adult male albino rats were randomly divided into six groups:

- **Group I (control group; n=12):** The rats were further divided equally into 4 subgroups: subgroup Ia: The rats were left without intervention. Subgroup Ib: The rats were administered intraperitoneally with 2.5 ml of l-arginine solution/kg body weight of sodium citrate buffer (vehicle of L-arginine). Subgroup Ic: The rats were injected intraperitoneally a single dose of 1 ml PBS (vehicle of ghrelin). Subgroup Id: The rats were injected intravenous with a single dose of 0.5 ml PBS (vehicle of exosomes).
- **Group II (affected group; n=10):** Rats were injected intraperitoneally by l-arginine at a dose of 500 mg/100g body weight that is 2.5 ml of l-arginine solution/100g body weight five times at 3-day intervals.
- **Group III (recovery group; n=10):** Rats were treated as group II and left for 12 weeks without treatment.
- **Group IV (ghrelin group; n=10):** Rats were treated as group II, then 8 weeks after induction of chronic pancreatitis, Ghrelin was administered intraperitoneal with a single dose of ghrelin (20 µg/kg) for successive three days.
- **Group V (exosomes group; n=10):** Rats were treated as group II, then 8 weeks after induction of chronic pancreatitis rats were injected intravenous with a single dose of 100 µg BMSC-EX diluted in 0.5 ml PBS.

- **Group VI (ghrelin and exosomes group; n=10):** Rats were treated as group II, then rats were injected intraperitoneally with ghrelin and intravenous with MSC-Exos in the same doses as group IV and V, 8 weeks after induction of chronic pancreatitis.

**Sampling:**

Rats were sacrificed under ether inhalation anesthesia after 8 weeks after induction of chronic pancreatitis in group II. Rats of groups I, III, IV, V and VI were sacrificed after 12 weeks after induction of chronic pancreatitis. Specimens of the pancreas were fixed in 10% buffered formalin for 5 days. They were then processed and embedded in paraffin. Serial sections of 5–7 µm thickness were cut for ordinary , special staining and immunostaining.

**Induction of chronic pancreatitis**

CP was induced by administration of l-arginine intraperitoneally at a dose 2.5 ml of l-arginine solution/100 g body weight five times at three days intervals. The dose was adjusted according to the individual rat's weight.

**Preparation of MSCs-derived exosomes and exosomes labeling with PKH-26**

Exosomes derived from mesenchymal stem cells were isolated from the supernatant of MSCs. First, rat bone marrow-derived MSCs (BM-MSCs) were prepared in the stem cell and molecular biology unit, central lab, Cairo University. MSCs were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 0.5% human serum albumin (HSA) (Sigma-Aldrich, St. Louis, MO, USA). More than 99% of viability of the cells cultured was detected by trypan blue exclusion. Cells were plated at 4000 cells/$cm^2$ for 7 days then trypsinized, counted, and replated in expansion medium at a density of 2,000 cells/$cm^2$ for another 7 days, stored at –80 ◦C, then centrifuged at 2,000 g for 20 min to remove debris, and then additional centrifuged at 100,000 g for 1 hour at 4 ◦C (Beckman Coulter, Fullerton, CA, USA) then washed in serum free medium 199. Exosomes were stored at -80 ◦C for this experiment. PKH26 (Sigma-Aldrich, St. Louis, MO, USA) was used to detect exosome in pancreas. The exosome pellet was diluted with PKH-26 kit solution to 1 ml, and 2 ml of fluorochrome was added to suspension and incubated at 38.5 °C for 10 min. After that, 7 mL of serum-free HG-DMEM was added to the suspension, then ultracentrifuged at 100,000Xg for 1 h at 4° C (11).

**Histological and Immunohistochemical Studies**

Paraffin sections of thickness (5-7 µm), mounted on glass slides for H&E stain to examine histological changes in various

groups and Masson trichrome staining for demonstration of collagen fibers deposition (12).

Other sections were mounted on +ve charged slides for immunohistochemical staining:

1. Immunohistochemical staining for detection of iNOS (marker for activation of macrophage). The primary antibody used was the rabbit polyclonal antibody (Thermo Fisher Scientific, U.S.A; cat. no. NM_000625) (PBS with 0.02% sodium azide and 50% glycerol pH 7.3). Site of the reaction was brownish color of cytoplasm (13).

2. Immunohistochemical staining for detection of CD8 (marker for cytotoxic T cell). The primary antibody used was Monoclonal mouse antihuman CD8 antibody (cat. no. C8144/B. Dako. Denmark) (ready-to-use, provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium azide). Site of the reaction was brownish color of cytoplasm (14).

Immunohistochemical study was conducted using the avidin– biotin peroxidase method, followed by diaminobenzidine (DAB) (Dakopatts, Glostrup, Denmark) was added to slides as a chromogen). The slides were washed with distilled water, then sections were counterstained with hematoxylin.

**Morphometric Study and Statistical Analysis**

The mean area percentage of collagen fibers deposition and iNOS and CD8 immunostaining were quantified in five images from five non-overlapping fields of each slide at magnification 400. Rats using Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA). All the data collected from the experiment was recorded and analyzed using IBM SPSS Statistics software for Windows, Version 20 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) with Post Hoc LSD test was used to compare differences among the groups of morphometric results. In each test, the data was expressed as the mean (M) value, standard deviation (SD) and differences were considered to be significant at p value $< 0.01$.

## Results

**MSCs- Exosomes Characterization**:

A transmission electron microscopic examination of purified exosomes demonstrated spherical double-membrane bound shape (60-100) nm (figure 1).

Exosomes labeled with PKH26 fluorescent dye were detected as strong red fluorescence in pancreatic tissues (figure 2).

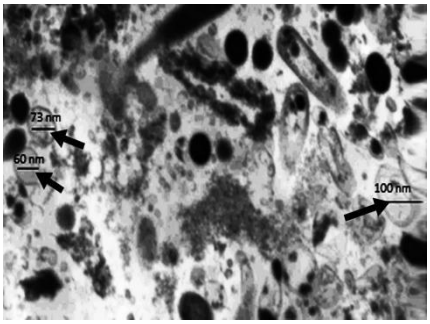



**Fig. (1):** A transmission electron micrograph of exosomes showing their characteristic spheroid double-membrane bound morphology with a diameter of 60-100 nm (black arrow). **(X2000)**

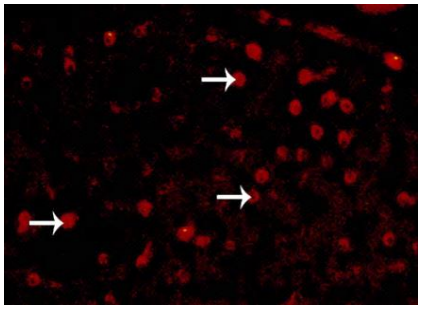


**Fig. (2):** A fluorescent microscope photograph showing homing of exosomes labeled with PKH26 dye in pancreatic tissue (white arrow). **(X 1000)**

**H & E Stain results:**

Examination of all subgroups of the group I (control group) showed similar histological architecture. Group I (control group): pancreatic sections from group I showed thin connective tissue septa dividing the pancreas into lobules of variable sizes and shapes. Within the lobules, rounded or oval serous acini represented the exocrine portion of the pancreas. The endocrine part (the islets of Langerhans) was observed among the exocrine part. The pancreatic acini appeared rounded or oval in shape. They were lined with pyramidal cells arranged around a narrow acinar lumen. The acinar cells contained basal rounded nuclei. The supranuclear portions of the cells were packed with acidophilic zymogen granules, while the basal portions of the cells had basophilic cytoplasm. The islets of Langerhans appeared pale pink oval or rounded areas inside the pancreatic lobules and formed of circular groups of cells (figure 3a1&a2).

Group II (affected group): pancreatic sections from group II showed distorted pancreatic acini with extensive cytoplasmic vacuolization, loss of basal basophilia. Some acini show disappearance of their nuclei. Dilated congested blood vessels & dilated interlobular septa and inflammatory cellular infiltration. The islet of Langerhans with empty spaces between cell cords. Islet cells show apparent decrease in the number with dilated congested blood capillaries (figure 3b).

Group III (recovery group): pancreatic sections from group III showed distorted acini with extensive cytoplasmic vacuolization with dilated congested blood vessels. Empty spaces between cell cords of islets are observed (figure 3c).

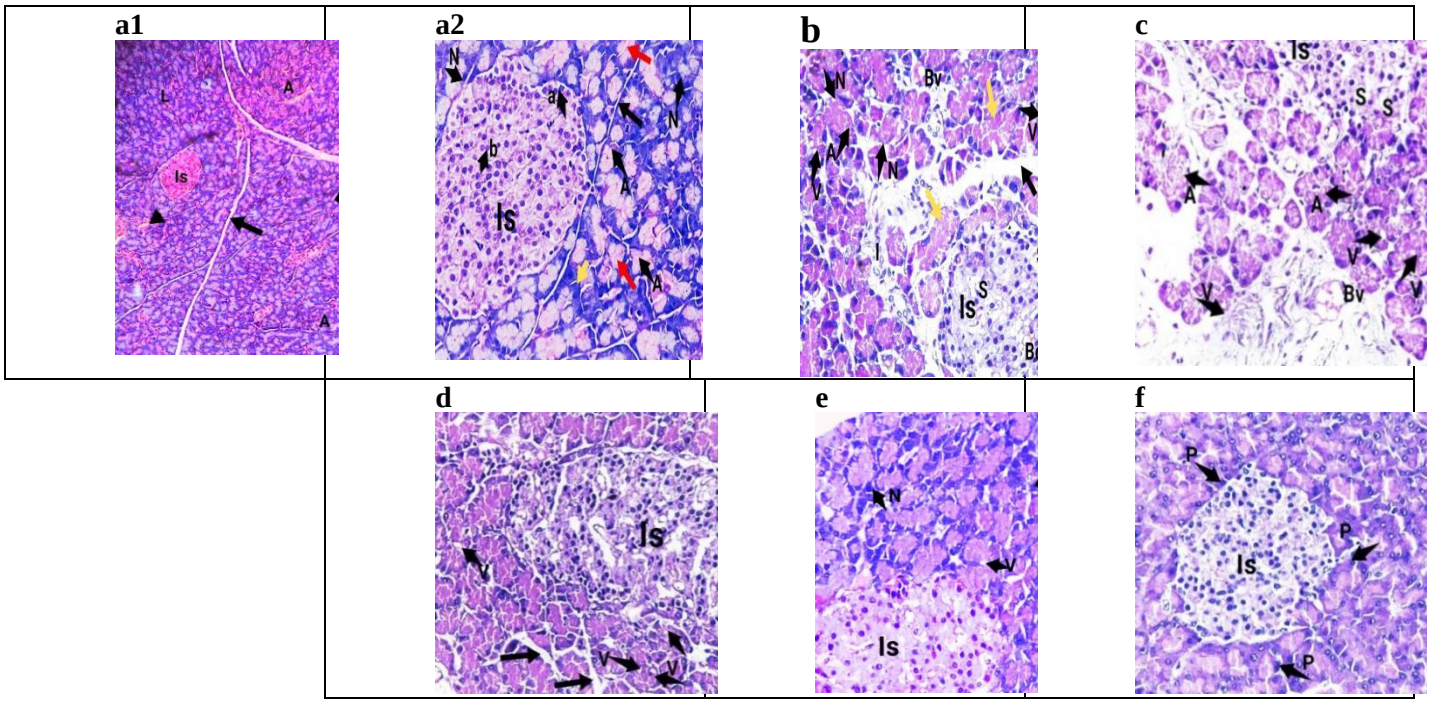



**Fig. (3): (a1)** A photomicrograph of a section in the pancreas of the control group (group I) showing normal histological architecture of the pancreatic tissue. Pancreatic lobules (L) composed of the exocrine component consisting of closely packed secretory acini (A) with central acidophilia and peripheral basophilia and endocrine component islets of Langerhans (Is). Note the interlobular septum (black arrow) and intralobular duct (head arrow). (H&E X100), **(a2)**showing pancreatic lobules separated by thin connective tissue septa (black arrow). Each lobule is formed of closely packed acini (A+arrow) of variable size and shape. The pancreatic acini are lined with large pyramidal cells with basal rounded nuclei (N+arrow). The cytoplasm of acinar cells shows basal basophilia (yellow arrow) and apical acidophilia (red arrow). A pale stained area of an islet of Langerhans (Is) can be seen between the secretory acini. The islet of Langerhans consists of cords of cells. The β-cells (b+arrow) are located mainly in the central zone of the islet with rounded and lighter nuclei. The α-cells (a+arrow) are located peripherally with oval darkly stained nuclei, **(b)** affected group (group II) showing distorted pancreatic acini (A+arrow) with extensive cytoplasmic vacuolization (V+arrow), loss of basal basophilia (yellow arrow). Some acini show disappearance of their nuclei (N+arrow). Dilated congested blood vessels (Bv) & dilated interlobular septae (black arrow) and inflammatory cellular infiltration (I) also are noticed. The islet of Langerhans (Is) with empty spaces (S) between cell cords. Islet cells show apparent decrease in the number with dilated congested blood capillaries (Bc), **(c)** recovery group (group III) showing distorted acini (A+arrow) with extensive cytoplasmic vacuolization (V+arrow). Empty spaces (S) between cell cords of islets (Is) are observed. Notice: the dilated congested blood vessels (Bv), **(d)** ghrelin group (group IV) showing acinar cells vacuolization (V+arrow), dilated septa (black arrow) and increase number and size of islet cells (Is), **(e)** exosomes group (group V) showing some acinar cells vacuolization (V+arrow) with deeply stained pyknotic nuclei (N+arrow) and islet cells regeneration with increase in the number and size of islets of Langerhans (Is), **(f)** ghrelin and exosomes group (group VI): showing nearly normal acini and islet of Langerhans. However, some acinar cells deeply stained pyknotic nuclei (P+arrow). **(H&E X400)**

Group IV (ghrelin group): pancreatic sections from group IV showed acinar cells vacuolization, dilated septa and increase number and size of islet cells (figure 3d).

Group V (exosomes group): pancreatic sections from group V showed some acinar cells vacuolization with deeply stained pyknotic nuclei and islet cells regeneration with increase in the number and size of islets of Langerhans (figure 3e).

Group VI (ghrelin and exosomes group): pancreatic sections from group VI showed nearly normal acini and islet of Langerhans. However, some acinar cells deeply stained pyknotic nuclei (figure 3f).

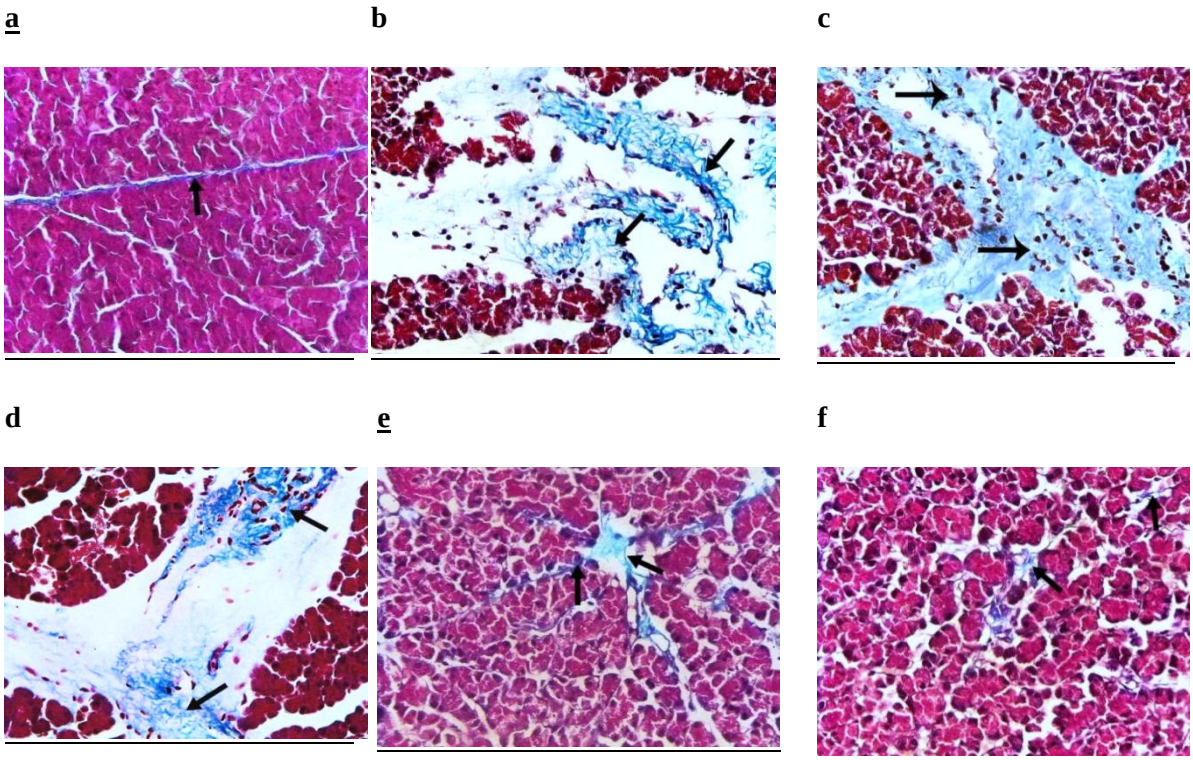



**Fig. (4): (a)** A Photomicrograph of a section in the pancreas of the control group (group I) showing minimal interlobular deposition of collagen fibers (black arrow), **(b)** affected group (group II) showing marked interlobular deposition of collagen fibers (black arrow), **(c)** recovery group (group III) showing intense periacinar and interlobular deposition of collagen fibers (black arrow), **(d)** ghrelin group (group IV) showing periacinar and interlobular deposition of collagen fibers (black arrow), **(e)** exosomes group (group V) showing few periacinar and interlobular deposition of collagen fibers (black arrow), **(f)** ghrelin and exosomes group (group VI) showing minimal periacinar and interlobular deposition of collagen fibers (black arrow). **(Masson trichrome staining X400).**

**Masson trichrome staining results:**

- Group I (control group): showed minimal interlobular deposition of collagen fibers (figure 4a).
- Group II (affected group): showed showing marked interlobular deposition of collagen fibers (figure 4b).
- Group III (recovery group): showed showing intense periacinar and interlobular deposition of collagen fibers (figure 4c).
- Group IV (ghrelin group): showed periacinar and interlobular deposition of collagen fibers (figure 4d).
- Group V (exosomes group): showed few periacinar and interlobular deposition of collagen fibers (figure 4e).
- Group VI (ghrelin and exosomes group): showed minimal periacinar and interlobular deposition of collagen fibers (figure 4f).

***iNOS staining results:***

Group I (control group): showed pancreatic acini with negative immunostaining reaction for inducible nitric oxide synthase (figure 5a).

Group II (affected group): showed pancreatic acini with strong positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase in the form of dark brown granules (figure 5b).

Group III (recovery group): showed pancreatic acini with intense positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase in the form of dark brown granules (figure 5c).

Group IV (ghrelin group): showed pancreatic acini with positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase in the form of brown granules (figure 5d).

Group V (exosomes group): showed pancreatic acini with few positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase in the form of lightly stained brown granules (figure 5e).

Group VI (ghrelin and exosomes group): showed pancreatic acini with minimal positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase in the form of lightly stained brown granules (figure 5f).

**<u>CD8</u>** ***staining results:***

- Group I (control group): show pancreatic acini and islet with negative

immunostaining reaction for CD8 (figure 6a).

- Group II (affected group): show pancreatic acini and islet with markedly positive immunostaining reaction for CD8 (figure 6b).
- GroupIII (recovery group): show pancreatic acini and islet with intense positive immunostaining reaction for CD8 (figure 6c).
- Group IV (ghrelin group): show pancreatic acini and islet with positive immunostaining reaction for CD8 (figure 6d).
- Group V (exosomes group): show pancreatic acini and islet with few positive immunostaining reaction for CD8 (figure 6e).

Group VI (ghrelin and exosomes group): show pancreatic acini and islet with minimal positive immunostaining reaction for CD8 (figure 6f).

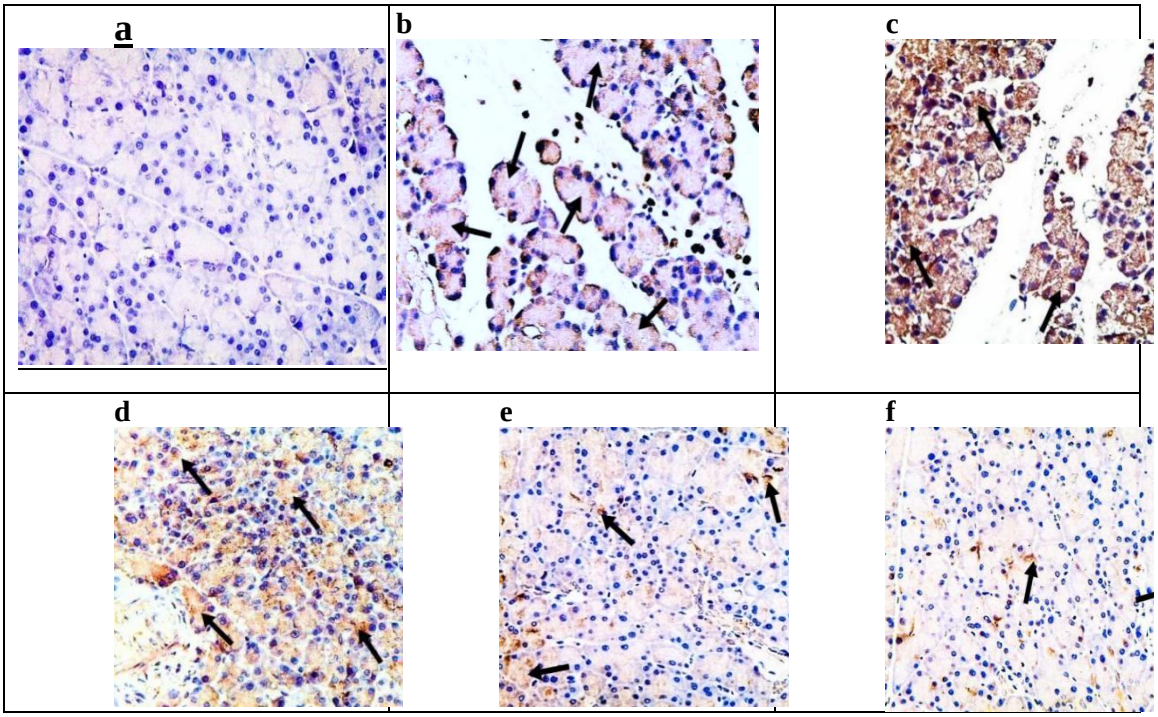



**Fig. (5): (a)** A Photomicrograph of a section in the pancreas of the control group (group I) showing pancreatic acini with negative immunostaining reaction for inducible nitric oxide synthase, **(b)** affected group (group II) showing most pancreatic acini with strong positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase in the form of dark brown granules (black arrow), **(c)** recovery group (group III) showing pancreatic acini with intense positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase in the form of dark brown granules (black arrow), **(d)** ghrelin group (group IV) showing pancreatic acini with positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase in the form of brown granules (black arrow), **(e)**

exosomes group (group V) showing pancreatic acini with few positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase in the form of lightly stained brown granules (black arrow), **(f)** exosomes and ghrelin (group VI) showing pancreatic acini with minimal positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase in the form of lightly stained brown granules (black arrow). **(Anti iNOS, X400)**

## Morphometric and Statistical Results

The mean area percentage ± SD of collagen fibers deposition, iNOS and CD8 immunostaining are represented in Tables 1,2,3 and (Histograms 1&2&3). The mean area percentage of of collagen fibers deposition, iNOS and CD8 were significantly increased ($P < 0.01$) in groups II and III compared with group I. The mean area percentage of collagen fibers deposition, iNOS and CD8 immunostaining were significantly decreased ($P < 0.01$) in groups IV, V and VI compared with group II.

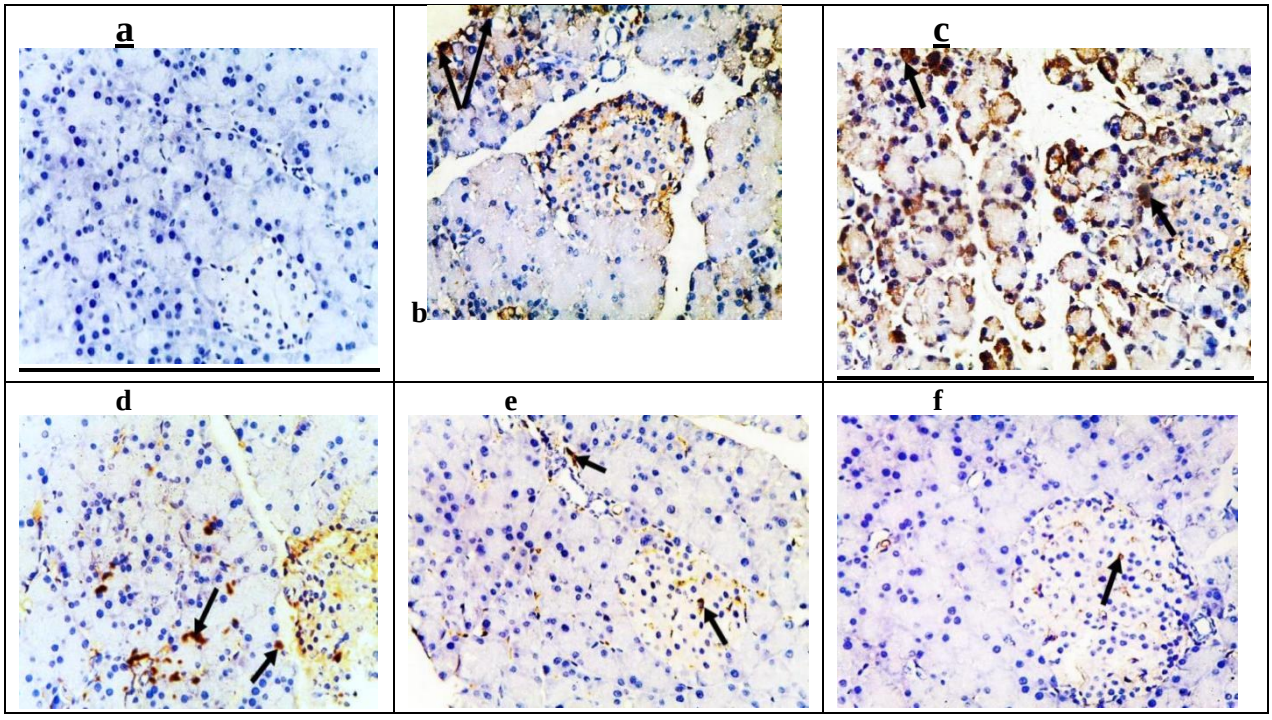
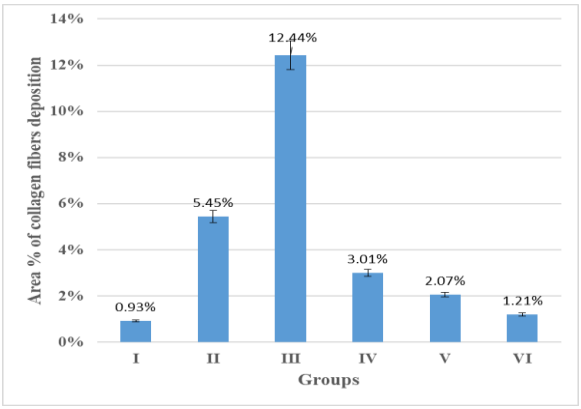
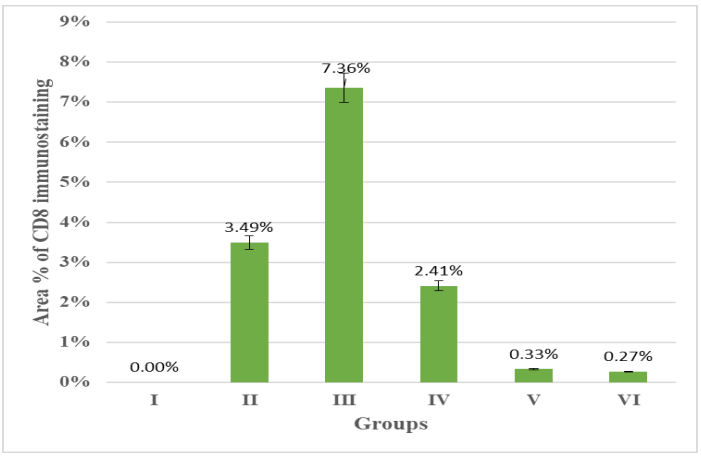


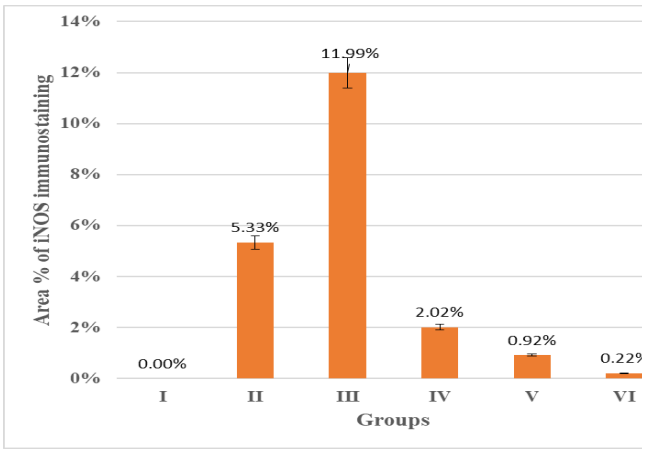

**Fig. (6):** A Photomicrograph of a section in the pancreas of the control group (group I) showing pancreatic acini and islet with negative immunostaining reaction for CD8, affected group (group II) showing pancreatic acini and islet with markedly positive immunostaining reaction for CD8 (black arrow), recovery group (group III) showing pancreatic acini and islet with intense positive immunostaining reaction for CD8 (black arrow), ghrelin group (group IV) showing pancreatic acini and islet with positive immunostaining reaction for CD8 (black arrow), exosomes group (group V) showing pancreatic acini and islet with few positive immunostaining reaction for CD8 (black arrow), exosomes and ghrelin (group VI) showing pancreatic acini and islet with minimal positive immunostaining reaction for CD8 (black arrow). **(CD8, X400).**




**Histogram (1):** Showing the mean area % of collagen fibers deposition of all groups




**Histogram (3):** Showing the mean area % of CD8 immunostaining in all groups




**Histogram (2):** Showing the mean area % of iNOS immunostaining in all groups

**Table (1),** Showing the mean area % and SD of collagen fibers deposition in all groups with comparison between groups by Post Hoc LSD test. **Table (2),** Showing the mean area % and SD of iNOS immunostaining in all groups with comparison between all groups by Post Hoc LSD test. **Table (3),** Showing the mean area % and SD of CD8 immunostaining in all groups with comparison between all groups by Post Hoc LSD test.

**Table (1):** Showing the mean area % and SD of collagen fibers deposition in all groups with comparison between groups by **Post Hoc LSD** test.

| | **Group I** | **Group II** | **Group III** | **Group IV** | **Group V** | **Group VI** |
|---|---|---|---|---|---|---|
| **Mean area %** | 0.93% | 5.45% | 12.44% | 3.01% | 2.07% | 1.21% |
| **SD** | 0.0871 | 1.0191** | 1.1153** | 0.3060* | 0.1148* | 0.1390* |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Significance at P < 0.01** | 2,3,4,5 | 1,3, 4,5,6 | 1,2,4,5,6 | 1,2,3,5,6 | 1,2,3,4,6 | 2,3,4,5,6 |

**(more significant compared to group I ), *(less significant compared to group II)

Group II has a significant value in collagen fibers deposition compared to group (I), Group III has more significant value compared to group (I, II), Group IV has less significant value compared to group (II), Group V has less significant value compared to group (II, III), Group VI has less significant value compared to group (II, III) and no significant value compared to group (I).

**Table (2):** Showing the mean area % and SD of iNOS immunostaining in all groups with comparison between all groups by **Post Hoc LSD** test.

| | **Group I** | **Group II** | **Group III** | **Group IV** | **Group V** | **Group VI** |
|---|---|---|---|---|---|---|
| **Mean area %** | 0.00% | 5.33% | 11.99% | 2.02% | 0.92% | 0.22% |
| **SD** | 0 | 0.7104** | 0.8338** | 0.0869* | 0.11643* | 0.0531* |
| **Significance at P < 0.01** | 2,3,4,5 | 1,3, 4,5,6 | 1,2,4,5,6 | 1,2,3,5,6 | 1,2,3,4,6 | 2,3,4,5,6 |

**(more significant compared to group I ), *(less significant compared to group II)

Group II has a significant value in iNOS immunostaining compared to group (I), Group III has more significant value compared to group (I, II), Group IV has less significant value compared to group (II), Group V has less significant value compared to group (II, III), Group VI has less significant compared to group (II, III) and no significant value compared to group (I).

**Table (3):** Showing the mean area % and SD of CD8 immunostaining in all groups with comparison between all groups by **Post Hoc LSD** test.

| | **Group I** | **Group II** | **Group III** | **Group IV** | **Group V** | **Group VI** |
|---|---|---|---|---|---|---|
| **Mean area %** | 0.00% | 3.49% | 7.36% | 2.41% | 0.33% | 0.27% |
| **SD** | 0 | 0.4747** | 0.3380** | 0.4415* | 0.0508* | 0.0149* |
| **Significance at P < 0.01** | 2,3,4 | 1,3, 4,5,6 | 1,2,4,5,6 | 1,2,3,5,6 | 2,3,4 | 2,3,4 |

**(more significant compared to group I ), *(less significant compared to group II)

Group II has a significant value in CD8 immunostaining compared to group (I), Group III has more significant value compared to group (I, II), Group IV has less significant value compared to group (II), Group V has less significant value compared to group (II, III), Group VI has less significant compared to group (II, III) and no significant value compared to group (I).

## Discussion

Chronic pancreatitis is a disease characterized by inflammation, fibrosis and destruction of endocrine and exocrine tissues. Chronic pancreatitis is a progressive disease. There is no physiological treatment is available to reverse its course (15).

L-arginine is inexpensive and showed all features of chronic pancreatitis that appear in human including pancreatic inflammation, fibrosis, atrophy and exocrine insufficiency (8).

The mechanism of chronic pancreatitis induction by l-arginine was explained by (16,17) who stated that l-arginine is an agonist for G-protein-coupled receptor for the family C, which includes G-PRC6A, (G-Protein-coupled receptor family C group 6 member A) and CaSR (calcium-sensing receptor). These receptors presented in the pancreas cause mutations lead to pancreatitis. Administration of l-arginine cause an imbalance of amino acids that lead to pancreatitis. Conversion of l-arginine to l-ornithine represent important step in the induction of pancreatitis. Oxygen free radicals generated from l-arginine causes pancreatitis lead to activation of PSCs which cause acinar necrosis and activation of cytokine which lead to activation of PSCs (17).

Group II showed that the pancreatic tissue was characterized by distorted acini with cytoplasmic vacuolization and infiltration of inflammatory cells, vacuolation between islet of Langerhans with dilated congested capillaries. This study showed a significant increase ($P < 0.01$) in collagen fibers deposition, iNOS and CD8 immunostaining compared to control group. These results agreed with some investigators (16,18,19) and explained by others (17,20) who stated that in chronic pancreatitis, mitochondrial dysfunction is an important downstream factor for impaired autophagy leads to alterations in membrane integrity by protein denaturation and lipid peroxidation, deregulated lipid metabolism and endoplasmic reticulum stress. Reactive Oxygen Species (ROS) increased due to mitochondrial dysfunction which has cytotoxic effect on tissues.

Statistically increased Masson trichrome staining was due to presence of stellate cells in the pancreas and its major role in the formation of pancreatic fibrosis. PSCs activation result in increasing production of ECM and α-SMA and components including fibronectin and collagen[1]. There is balance between newly synthesized ECM and its degradation is involved in regeneration process of the pancreas. α-SMA is an excellent marker of activated PSCs (17). Chronic pancreatitis increased number of T cells. $CD8^+$ T cell consider first-line defense against damaging epithelial events in chronic pancreatitis during the progression (21).

After 12 weeks, group III (recovery group) showed distorted acini with extensive cytoplasmic vacuolization. Empty spaces between islets, dilated congested blood vessels is observed. There was more significant increase ($P < 0.01$) in collagen fibers deposition, *iNOS* and CD8 immunostaining compared to group II, and this explained by (22,23) who showed that pathological condition of impaired pancreatic recovery in which the damage tissue cause high deposition of ECM proteins and persistent inflammation. There is more than 90% of acinar cell destruction, periductal fibrosis, histological alterations, tubular complexes and adipose tissue were detected.

Group IV (ghrelin group) in the present study showed acinar cells vacuolization. Increase number and size of islet cells, dilated septa. There was a significant decrease ($P < 0.01$) in collagen fibers deposition, iNOS and CD8 immunostaining expression compared to group II, III. These results were well-matched and confirming the findings of previous study mentioned by (24) who stated that administration of ghrelin increased pancreatic cell proliferation measured as a rate of pancreatic DNA synthesis and accelerate the increase in pancreatic weight during regeneration of the pancreas. Administration of ghrelin decline inflammatory cell infiltration and acinar cells vacuolization of pancreatic tissue.

Some researchers (25,26) explained that ghrelin accelerated regeneration of the gland, stimulation of pancreatic cell proliferation and decline pancreatic inflammation if given after development of pancreatitis. Ghrelin can protect the pancreatic tissue and prevent the development of the inflammatory process in the pancreas. The protective effect of ghrelin depends on release of IGF-1 and GH. Pancreatic fibrosis was induced by

activation of mTORC1 signaling in gastric cells and subsequently impairs glucose homeostasis through ghrelin suppressing. Exogenous ghrelin administration Inhibit this signaling pathway.

Exosomes contain abundant miRNAs and transmit these contents into the recipient cells. They are the principal therapeutic agents that mediates the paracrine action of MSCs and supports their therapeutic abilities. They can offer similar therapeutic effects of mesenchymal stem cells without the potential side effects associated with cell therapy (7).

Group V (exosome group) of this study showed moderate improvement in the pancreatic histology with little vacuolization of acinar cells and deeply stained pyknotic nuclei. There was significant decrease (P <0.01) in collagen fibers deposition, iNOS and CD8 staining expression compered to group II. These results agreed with (27,28) who reported that MSCs derived exosomes have genetic and protein material that when transferred to recipient cells can initiate the activation of various repair mechanisms. Exosomes have huge potentials in tissue regeneration and injury repair as MSCs. Exosomes improve the efficiency of this treatment and the survival of transplanted pancreatic islets. The crosstalk between beta-cells and endothelial cells and is important for islet transplantation because it is associated with the revascularization process.

Some researchers (29,30) explained that the anti-fibrosis effect of miRNAs in MSC derived exosomes has become a research hotspot for fibrosis by regulating reactive oxygen species, mitochondrial DNA damage and inflammatory activation. Fibroblast proliferation is key to fibrosis. Exosomes suppress fibroblast proliferation.

Group VI (ghrelin and exosomes group) of this study showed nearly normal pancreatic lobular architecture. There are nearly normal acini and islet of Langerhans separated by blood capillaries However, some acinar cells deeply stained pyknotic nuclei. There was significant decrease (P <0.01) in collagen fibers deposition, iNOS and CD8 staining expression compered to group II and insignificant (P <0.01) compared with control group.

These results agreed with (31) who reported that the antioxidant levels are tightly regulated within the cell and can significantly affect stem cell behavior, characteristics, and survival. Stem cell

transplantation at the site of tissue injury experiences oxidative and inflammatory stresses, which can lead to apoptosis. The combination between stem cell and antioxidant improves functional repair.

## Conclusion

This work concluded that both ghrelin and exosomes can treat chronic pancreatitis, but their co-administration can give better results.

**Source of funding:**

This research did not receive any specific grant from funding agencies in the public, commercial, or not for-profit sectors.

**Author contribution:**

Authors contributed equally in the study.

**Conflicts of interest:**

No conflicts of interest.

## Reference

1. Bansod, S.; Khurana, A. and Godugu, C. (2019): Cerulein-induced chronic pancreatitis in Swiss albino mice: An improved short-term model for pharmacological screening. J. Pharmacol. Toxicol. Methods, 96:46-55.
2. Yamada, T.; Inadomi, J. M.; Bhattacharya, R.; Hwang, J. H. and Ko, C. W. (2020): Specific Gastrointestinal Diseases (Pancreatitis): In Yamada's handbook of gastroenterology. 4th edition, Part 2, Chapter 31, Pp. 335- 349. Wiley-Blackwell. Hoboken, NJ.
3. Napolitano, T.; Silvano, S.; Vieira, A.; Balaji, S.; Garrido-Utrilla, A.; Friano, M. E, et al. (2018): Role of ghrelin in pancreatic development and function. Diabetes, Obesity and Metabolism, 20(2):3–10.
4. Tkach, M. and Théry, C. (2016): Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. Cell, 164(6):1226–1232.
5. Colombo, M.; Raposo, G. and Théry, C. (2014): Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu. Rev. Cell Dev. Biol., 30(1):255–289.
6. El andaloussi, S.; Mäger, I.; Breakefield, X. O.; Wood, M. J. A. (2013): Extracellular vesicles: Biology and emerging therapeutic opportunities. Nat. Rev. Drug. Discov., 12(5): 347–357.
7. Zhang, M.; Zang, X.; Wang, M.; Li, Z.; Qiao, M.; Hua, H, et al. (2019): Exosome-based nanocarriers as bio-inspired and versatile vehicles for drug delivery: recent advances and challenge. J. Mater. Chem. B., 7(15): 2421-2433.
8. Eltantaway, E. Khalaf, G.; and Hamam, G. (2015): Effect of experimentally induced chronic pancreatitis on the structure of hepatocytes and the duodenal villi in adult male albino rats. The Egyptian Journal of Histology, 38 (1):1-9.

9. Tümer, C.; Bilgin, H. M.; Obay, B. D.; Diken, H.; Taşdemir, E.; and Şermet, A. (2007): Effect of ghrelin administration on phagocytic activity in acute cold-restraint stress exposed rats. Regulatory Peptides, 138(3):113–117.

10. El-Azab, N. E.; El-Mahalawaya, A. M.; Mostafa, O. and Sabry, D. (2018): Histological and immunohistochemical study of the potential therapeutic impacts of bone marrow mesenchymal stem cells and exosomes for sciatic nerve crush injury model in rats. The Egyptian Journal of Histology,41 (4):160-176.

11. Mohamady, R. and Mohamed, O. (2020): Mesenchymal stem cells derived extracellular vesicles ameliorate cortical cerebellar changes-induced by aspartame in rats, Histological and immunohistochemical study. Egyptian Journal of Histology, 43(2), 380-389.

12. Bancroft, J. D. and Layton, C. (2019): The Hematoxylin and eosin and Carbohydrates. In: Bancroft's Theory and Practice of Histological Techniques E-Book. 8th edition. Chapter10& 12. Pp. Pp.126-139 and 153-176.El Sevier, Philadelphia.

13. Seys, L. J.; Verhamme, F. M.; Schinwald, A.; Hammad, H.; Cunoosamy, D.M, et al. (2015): Role of B cell–activating factor in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine, 192(6):706-718.

14. Mason, D.Y.; Cordell, J. L.; Gaulard, P.; Tse, A. G. D. and Brown, M. H. (1992): Immunohistological detection of human cytotoxic/suppressor T cells using antibodies to a CD8 peptide sequence .J. Clin. Pathol., 45:1084-1088.

15. Kwon, C. I.; Cho, J. H.; Choi, S. H.; Ko, K. H; Tirkes, T.; Gromski, M. A, et al. (2019): Recent advances in the diagnosis and management of chronic pancreatitis. Korean J. Intern. Med., 34(2):242-260.

16. Moustafa, E. M.; Moawed, F. S. M. and Abdel-Hamid, G. R. (2020): Icariin Promote Stem Cells Regeneration and Repair Acinar Cells in L-arginine / Radiation -Inducing Chronic Pancreatitis in Rats. Dose response, 18(4):1-13.

17. Sharma, S.; Rana, S.V.; Rana, S.; Bhasin, D. K.; Nada, R. and Malhotra, S. (2017): Severe Chronic Pancreatitis due to Recurrent Acute Injury: NonInvasive Chronic Pancreatitis Model of Rat. J. Pancreas, 18(2):107-120.

18. Nadella, S.; Ciofoaia, V.; Cao, H.; Kallakury, B.; Tucker, R. D. and Smith, J. P. (2020): Cholecystokinin Receptor Antagonist Therapy Decreases Inflammation and Fibrosis in Chronic Pancreatitis. Dig. Dis. Sci., 65(5):1376-1384.

19. Soliman, G. F.; Ibrahim, W. and Abdallah, H. (2019): Therapeutic Effect of L-Carnitine on Acute Pancreatitis Induced by L-Arginine in Rats: Possible Role of Beclin Gene and Inducible Nitric Oxide Synthase. Med. J. Cairo Univ., 87(3):1793-1803.

20. Biczo, G.; Vegh, E. T.; Shalbueva, N.; Mareninova, O. A.; Elperin, J.; Lotshaw, E, et al. (2018): Mitochondrial dysfunction, through impaired autophagy, leads to endoplasmic reticulum stress, deregulated lipid metabolism, and pancreatitis in animal models. Gastroenterology, 154 (3):689–703.

21. Manohar, M. Verma, A. K. and Venkateshaiah, S. U. and Mishra A. (2017): Immunological Responses Involved in Promoting Acute and Chronic Pancreatitis. J. Clin. Immunol. Res., 1(1):1-8.

22. Lugea, A.; Nan, L.; French, S. W.; Bezerra, J. A.; Gukovskaya, A. S. and Pandol, S. J. (2006): Pancreas recovery following cerulein-induced pancreatitis is impaired in plasminogen-deficient mice. J. of Gastroenterology, 131(3):885-899.

23. Hegyi, P.; Takács, T.; Tiszlavicz, L.; Czakó, L.; Lonovics, J. (2000): Recovery of exocrine pancreas six months following pancreatitis induction with L-arginine in streptozotocin-diabetic rats. J. Physiol., 94:51−55.

24. Warzecha, Z.; Ceranowicz, P. and Dembinski, A.; Cieszkowski, J.; Kusnierz-Cabala, B.; Tomaszewska, R, et al. (2010): Therapeutic effect of ghrelin in the course of cerulein-induced acute pancreatitis in rats. Journal of Physiology and Pharmacology, an Official Journal of the Polish Physiological Society, 61(4):419-427.

25. Bonior, J.; Warzecha, Z.; Ceranowicz, P.; Gajdosz, R.; Pierzchalski, P.; Kot, M., et al. (2017): Capsaicin-sensitive sensory nerves are necessary for the protective effect of ghrelin in cerulein-induced acute pancreatitis in rats. International journal of molecular sciences, 18(7): 1402-1430.

26. Akki, R.; Raghay, K. and Errami, M. (2021): Potentiality of ghrelin as antioxidant and protective agent. Redox rep., 26(1):71–79.

27. Pang, H.; Luo, S. and Xiao, Y. (2020): Emerging Roles of Exosomes in T1DM. Front. Immunol., 11:1-12.

28. Gnecchi, M.; Danieli, P.; Malpasso, G. and Ciuffreda M. C. (2016): Paracrine Mechanisms of Mesenchymal Stem Cells in Tissue Repair. Methods Mol. Biol., 1416(8):123-146.

29. Jia, Y. C.; Ding, Y. X.; Mei, W. T.; Wang, Y. T.; Zheng, Z.; Qu, Y. X, et al. (2021): Extracellular vesicles and pancreatitis: mechanisms, status, and perspectives. International journal of biological sciences, 17(2): 549–561.

30. Huang, Y. and Yang, L. (2021): Mesenchymal stem cell-derived extracellular vesicles in therapy against fibrotic diseases. Stem Cell Res. Ther., 12(1): 435-447.

31. Salem, M. Y.; El-Azab, N. E; Helal, O. K.; Gabr, M. H. and Abd El-Halim, H. E. (2015):Does selenium improve the stem cell therapeutic effect on isoproterenol-induced

myocardial infarction in rats. Histological and immunohistochemical study. The Egyptian Journal of Histology, 38(4):679-691.

**To cite this article:** Walaa S. Attaia, Mohammed M. Yousef, Nahla E. El-Azab, Nessrein E. Salem. Possible Therapeutic Effect of Ghrelin and Exosomes Derived from Mesenchymal Stem Cells on Induced Chronic Pancreatitis of Adult Albino Rats (Histological and Immunohistochemical Study). BMFJ 2022; 39 (academic issue):246-265