International Journal of Clinical and Developmental Anatomy
2017; 3(6): 45-56
http://www.sciencepublishinggroup.com/j/ijcda
doi: 10.11648/j.ijcda.20170306.11
ISSN: 2469-7990 (Print); ISSN: 2469-8008 (Online)

Effect of Fluoxetine on the Pancreas of Adult Male Albino Rats and the Possible Protective Role of Omega-3: Light and Electron Microscopic Study

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To cite this article:
Sahar Youssef. Effect of Fluoxetine on the Pancreas of Adult Male Albino Rats and the Possible Protective Role of Omega-3: Light and Electron Microscopic Study. *International Journal of Clinical and Developmental Anatomy*. Vol. 3, No. 6, 2017, pp. 45-56.
doi: 10.11648/j.ijcda.20170306.11

Received: November 23, 2017; **Accepted**: December 12, 2017; **Published**: January 8, 2018

Abstract: Depression is linked with a high risk of type 2diabetes (T2D). The affiliation between depression and diabetes might be correlated to depression itself and, or medications recommended. Significantly, the usage of selective serotonin reuptake inhibitors (SSRIs), the most commonly antidepressants increased the hazard of developing T2D. Nevertheless, the mechanism underlying this suggestion remains vague. Omega-3 had antioxidant and anti-inflammatory activity. So, there is developing evidence that consumption of omega-3 could be expedient. The present study was carried out to investigate the potential effect of omega-3 in the fluoxetine induced alterations in the pancreas of adult male albino rats. Forty adult male albino rats were divided into four groups. Group I served as a control group. Group II received a single daily dose of 300 mg/kg of omega-3. Group III received 24 mg/kg bw/day of fluoxetine hydrochloride. Group IV received omega-3 and fluoxetine as group II and III for 30 days. Light and electron microscopic investigations were carried out. Histological examination using H & E and Masson's Trichrome stain were carried out. The insulin expression in β cells was evaluated using immunohistochemistry. Morphometric results were subjected to statistical analysis. Investigation of group III (Fluoxetine group) showed distorted exocrine pancreas with thick interlobular septa that contained dilated congested blood vessels, cellular infiltration, and fat cells. Marked shrunken of the pancreatic islets was observed. Masson's trichrome stain showed increased collagen fibers deposition. Electron microscopic examination revealed that most of the acinar cells had irregular shaped nuclei with peripheral heterochromatin and wide capillaries. The cytoplasm of β cells had a variety of secretory granules. Most of them had an electron dense core with increased electron lucent halo however, few β granules were empty and coalesced. Omega- 3 supplementation improved the morphology of Langerhans compared to fluoxetine group. Importantly, some pancreatic duct cells revealed a positive reaction against anti-insulin antibodies. The current results demonstrated that fluoxetine harmfully affected the histological structure of the pancreas. Omega-3 diminished effectively some histological, immunohistochemical and electron microscopic changes in a fluoxetine induced pancreatic injury. Omega-3 could stimulates β-cell regeneration from potent islet progenitor cells present in the ductal cells and these might lead to repair of the functional accomplishments of the injured pancreas to a great extent.

Keywords: Fluoxetine, Pancreas, Omega-3, Rats, β Cells

1. Introduction

Fluoxetine (Fluoxetine hydrochloride) is a selective serotonin reuptake inhibitor (SSRI) that is used in the treatment of depression, anxiety disorders and obesity [1]. The most common contrary effects related to fluoxetine are nervousness, insomnia, nausea, and sexual dysfunction [2]. There is a substantial confirmation from animal research and clinical investigations that antidepressant use constituted a major risk factor for impaired glucose homeostasis and type 2 diabetes [3]. SSRI usage is also related to hypercholesterolemia and hypertriglyceridemia [4]. Remarkably, reduction of both appetite and body weight are considerable among fluoxetine side effect [5]. Importantly, SSRI exposure has been reported to decrease beta cell survival and function [6].

The pancreas may be more vulnerable to oxidative stress than other tissues and organs, because pancreatic islet cells displayed particularly weak manifestation of antioxidative enzymes [7]. Collectively, selective serotonin reuptake inhibitor (SSRI) exposure has been exhibited to increase oxidative stress in a number of cell types. Several reports suggested that long term use of SSRIs is concomitant with an increased risk of diabetes, but the mechanism underlying this suggestion is not fully elucidated. Many reports are carried out to discover new remedy that can rise or preserve islet cell mass and function, providing a plane to decrease the threat injury from diabetes mellitus and its complications. The data obtained from using both fluoxetine and melatonin suggested that antidepressants and antioxidants can counter the mood and oxidative disorders associated with diabetes [8]. It is probable to recommend that an antioxidant treatment might modify SSRI-induced beta cell deficits in islets of Langerhans. One such therapy is omega- 3 fatty acids.

Crucially, omega- 3 is vital to human health but it cannot be manufactured within the body. So, it needs to be obtained from food or taken as supplements. Crucially, menhaden fish oil, rich in omega-3, showed useful effect on postpartum depression and decreases the biomarkers related to depression such as corticosterone and pro-inflammatory cytokines [9]. There is growing suggestion that dietary intake of omega-3 could be useful in diabetes prevention by reducing the activity of the proinflammatory processes that stimulate the body, attacking its own insulin producing cells [10]. It is also accomplished of utilizing a strong effect on cell growth, differentiation, and reduction of genes expression involved in lipid [11]. A study carried out on the kidney reported that omega-3 PUFAs protect against ischemic injury [12]. The ability of omega-3 polyunsaturated fatty acids to restore the damage on various organs injuries, including cardiovascular diseases [13] and brain injury are also reported [14].

Therefore, the present study aimed to determine the fluoxetine effect on the exocrine and endocrine pancreatic architecture and to elicit the possible useful role of omega-3 administration through histological, immunohistochemical and electron microscopic approaches.

2. Materials and Methods

2.1. Animals

Forty adult male albino rats were used in the current research. Wistar male albino rats aged three months (140–160 gm.) used in this study purchased from the Central Animal House, Faculty of Medicine, Assiut University. All animal processes were in accordance with the standards guidelines for the care and use of experimental animals by the Committee for the Purpose of Supervision of Experiments on Animals. Moreover, all animals procedure were in agreement to the National Institute of Health (NIH) protocol and they approved by the Institutional Ethics Committee of Assiut University. All rats were reserved under observation before the onset of the experiment to eliminate any infection. The animals were housed in stainless steel cages in suitable temperature (25±1°C), and humidity controlled room with twelve hours, light–dark cycle. Animals were fed with rat chow (standard rat pellet) and they had free access to water.

2.2. Experimental Design

All animals were randomly divided into four groups of ten, (10) rats each:

(1) Group I (Control group): Rats in this group received 1.0 ml tap water once a day orally through a stomach tube [15].

(2) Group II (Omega- 3 group): A single oral daily dose of Omega -3 for a period of thirty days. Omega-3 was purchased from Sedico Pharmaceutical Company (6th of October City, Egypt). Each capsule was evacuated by a syringe carefully and administered to rats at a dose of 300 mg/kg, equivalent to 0.02 ml fish oil/rat once daily, by a gastric tube [16].

(3) Group III (Fluoxetine-treated group): The animals received 24 mg/kg bw/day of fluoxetine hydrochloride (Flutin, Eipico Company, Egypt). Fluoxetine was dissolved in 1.0 ml of tap water once a day through a stomach tube [15].

(4) Group IV (Fluoxetine and Omega-3 group): The animals in this group received fluoxetine in the same manner as group III and omega -3 as group II**.** The present study was carried out for thirty days.

2.3. Light Microscopic Study

The pancreases were dissected out and they divided into two parts. One part was processed for light microscopic examination and the other for electron microscopic examination. Pancreatic specimens for light microscopic examination were fixed in 10% neutral buffered formalin for 24 h. The pancreatic tissues were embedded in paraffin blocks. For histological study, 5 µm thick sections were successively cut and stained with H & E for examination of the general pancreatic structure [17]. Masson's trichrome stain was used to demonstrate the collagen fibers [18].

2.4. Immunohistochemical Study

Expression of insulin was detected in formalin-fixed paraffin-embedded sections. The Immunohistochemical staining was demonstrated using the avidin biotin detection system (USA). 5 µm thick sections were mounted on positively charged slides and subjected to the immunohistochemical technique. Sections were deparaffinized in xylene and rehydrated in alcohol. Pancreatic sections were immersed in 0.3% hydrogen peroxide for thirty minutes to block endogenous peroxidase activity. The pancreatic sections were incubated with polyclonal guinea pig anti-insulin antibody (Dako, USA) at dilution of 1:100 for one hour. The reaction was visualized using 3, 3-diaminobenzidine tetrahydrochloride. All the

sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted by DPX. The specificity of insulin immunoreactivity was established by excluding the primary antibodies from some pancreatic sections. Brown cytoplasmic staining was an indicator of a positive reaction [19]. The examination and photography were done at the Mycology and Biotechnology Unit, Al- Azhar University, Cairo, Egypt.

2.5. Morphometric Study

2.5.1. Mean Area% of Collagen Fiber Deposition

The image analyser was first calibrated automatically to convert the measurement units (pixels) produced by the image analyser program into actual micrometer units. Using the measuring field menu the area% and standard measuring frame were selected from the parameters. In each selected field, the area percentage of the collagen content in the septa between the acini, ducts and around blood vessels using Masson's trichrome stained sections was evaluated. In each randomly chosen field, the section of the pancreas was enclosed inside the standard measuring frame and then the area of collagen fibres were masked by a blue binary colour to be measured. These measurements were done using an objective lens of magnification 10, total magnification X100. Ten readings were obtained in each specimen and the mean values were achieved.

2.5.2. Number of β-Cells in the Islet

The number of β-cells in the islet was evaluated. The β-cells of the pancreas were counted in the anti-insulin immunostained sections under 40 power fields. The number of β-cells was assessed by counting the nuclei of all positive β cells inside one islet in the field. A total number of thirty islets for each group were counted. Three pancreatic sections were examined from each animal in the different studied groups. Morphometric measurements were done by using (Leica Qwin 500c) image analyzer computer system (England) at the Regional Mycology and Biotechnology Center, Al-Azhar University, Cairo, Egypt.

2.6. Statistical Analysis

Results obtained from the image analyzer were subjected to statistical analysis. The morphometric measurements were expressed as mean ±SD. Comparison between the different experimental groups was statistically done using one way analysis of variance (ANOVA). The results were considered statistically significant when the p-values were <0.05.

2.7. Transmission Electron Microscopy Study

Pancreatic specimens for the electron microscope were immediately cut into cubes, 1 μm diameter, and the specimens were fixed overnight in 2.5% phosphate-buffered glutaraldehyde (pH 7.3) at 4°C. The postfixation in 1% buffered osmium tetroxide for one to two hours. Dehydration was performed in ascending grades of ethyl alcohol and clearance was in propylene oxide. Embedding was performed in fresh Epon capsules. Semithin sections, 0.5–1 μm were prepared using LKB ultramicrotome. The sections were stained with 1% toluidine blue and they were examined using a light microscope. Ultrathin sections, 50–80 nm from certain areas of the trimmed blocks were set and collected on copper grids. The ultrathin sections were contrasted with uranyl acetate for ten minutes and lead citrate for five minutes. Finally, the examination by a transmission electron microscope and JEOL 100 CX, (Japan) photographed at 80 kV at the Assiut University, Electron Microscopy Unit was achieved [20].

3. Results

3.1. Histological, Immunohistochemical & Electron Microscopic Study

Group 1 (Control group)

Light microscopic examination of the pancreas of the control adult male albino rats stained with H & E revealed that pancreatic lobules were separated by thin interlobular septa. They were formed of closely packed acini. The exocrine pancreas consists of the pyramidal acinar cells. Acinar cells had apical acidophilic cytoplasm packed with secretory granules and basal oval nuclei (Figure 1A). The presence of islets of Langerhans with a regular contour was obvious. Apparently, most of the islets of Langerhans were large and they well defined in between the exocrine parenchyma. Islets of Langerhans appeared as pale oval areas consist of groups of cells separated by blood capillaries (Figure 1A). It contained lightly stained acidophilic cells organized in branching and anastomosing cords. The β cells that occupied most of the islet with a large rounded vesicular nucleus were seen (Figure 1A).

Masson's Trichrome stained sections of the control pancreas showed delicate collagen fibers around the pancreatic acini, islet of Langerhans, pancreatic ducts and blood vessels (Figure 1B).

Immunohistochemical stained sections of the control pancreas showed strong positive immunoreactivity for the β-cells anti-insulin antibodies. The immunohistochemical stain appeared as brown granules occupying the cytoplasm of the β-cells (Figure 1C).

Electron microscopic examination of the pancreas of the control group showed that the acinar cells had basal rounded euchromatic nuclei. Their cytoplasm contained numerous secretory granules of high-electron density. The well-developed parallel array of rough endoplasmic reticulum was observed (Figure 2A). The β cells of the islets of Langerhans had euchromatic slightly rounded or oval nuclei and numerous secretory granules consisting of an electron-dense core surrounded by an electron-lucent halo (Figure 2B).

Group II (Omega-3 group)

Light microscopic examination of the pancreas of the omega-3 group of adult male albino rats stained with H & E revealed that the exocrine and endocrine pancreas showed more or less similar to that of the control group. The exocrine

pancreatic acini appeared to be consistent in shape. The acini were consists of numerous pyramidal acinar cells with rounded vesicular basally located nuclei (Figure 1D). The islets of Langerhans appeared well demarcated and they were consists of compactly arranged cellular cords separated by small blood sinusoids. The cells revealed large rounded central vesicular nuclei (Figure 1D).

Masson's Trichrome stained sections of the omega-3 group showed that the pancreas displayed slight collagen fibers around the pancreatic acini, islet of Langerhans, pancreatic blood vessels and ducts (Figure 1E).

Immunohistochemical stained sections of the omega-3 group showed positive immunostaining with deep brown color in β cells of most pancreatic islets (Figure 1F) similar to the control (group 1) (Figure 1C).

Electron microscopic examination of the pancreas of the omega-3 group showed that the acinar cells with large apical rounded electron-dense zymogen granules of variable size. The nuclei were euchromatic and the rough endoplasmic reticulum were regularly arranged and numerous (Figure 2C). Clearly, the β-cells contained granules with a dense electron core bounded by a clear halo (Figure 2D).

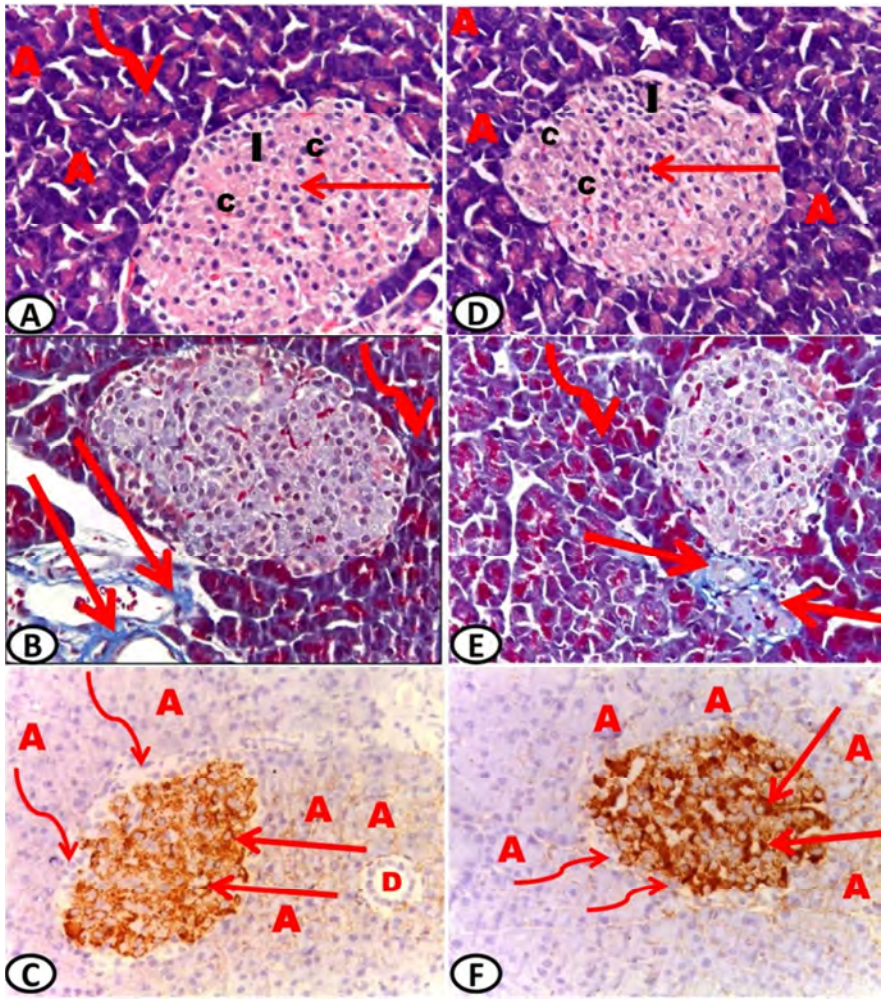

Figure 1. *Photomicrographs of sections of adult male albino rat pancreatic tissue.*

(A) Control group showed normal pancreatic acini (A) with basal basophilia, apical acidophilia and basal rounded nuclei. The lumen of acini is noticed (curved arrow). The presence of large well defined islets of Langerhans (I) can be seen. The islets is composed of cords of endocrine cells (straight arrow) separated by small blood capillaries (C). H & E, x400.

(B) Control group showed delicate collagen fibers around blood vessels (straight arrows) and between acini (curved arrow). Masson's trichrome, x400.

(C) Control group showed strong positive insulin immunoreactivity in most of its ß cells (straight arrows). Notice, negative immunoreactivity in the cells at periphery of islets (curved arrows) and in the acini (A). Minimal tracing of insulin expressing cells in the epithelium of duct (D) can be seen. Insulin immunohistochemical, x400.

(D) Omega-3 group showed normal architecture of exocrine pancreas formed of closely packed acini (A). Acinar cells with apical cytoplasm packed with acidophilic cytoplasmic granules and basal nuclei. Islets of Langerhans (I) appeared as pale oval area consisting of groups of cells (arrow) intermingled with blood capillaries (c). H & E, x 400.

(E) Omega-3 group showed minimal collagen fibers deposition around blood vessels (straight arrows) and around acini (curved arrow). Masson's trichrome, x400.

(F) Omega-3 group showed strong positive cytoplasmic immunoreactivity of insulin ß cells (straight arrows). Note, no insulin immunoreactivity at the periphery of islets (curved arrows) and in the acini (A). Insulin immunohistochemical, x400

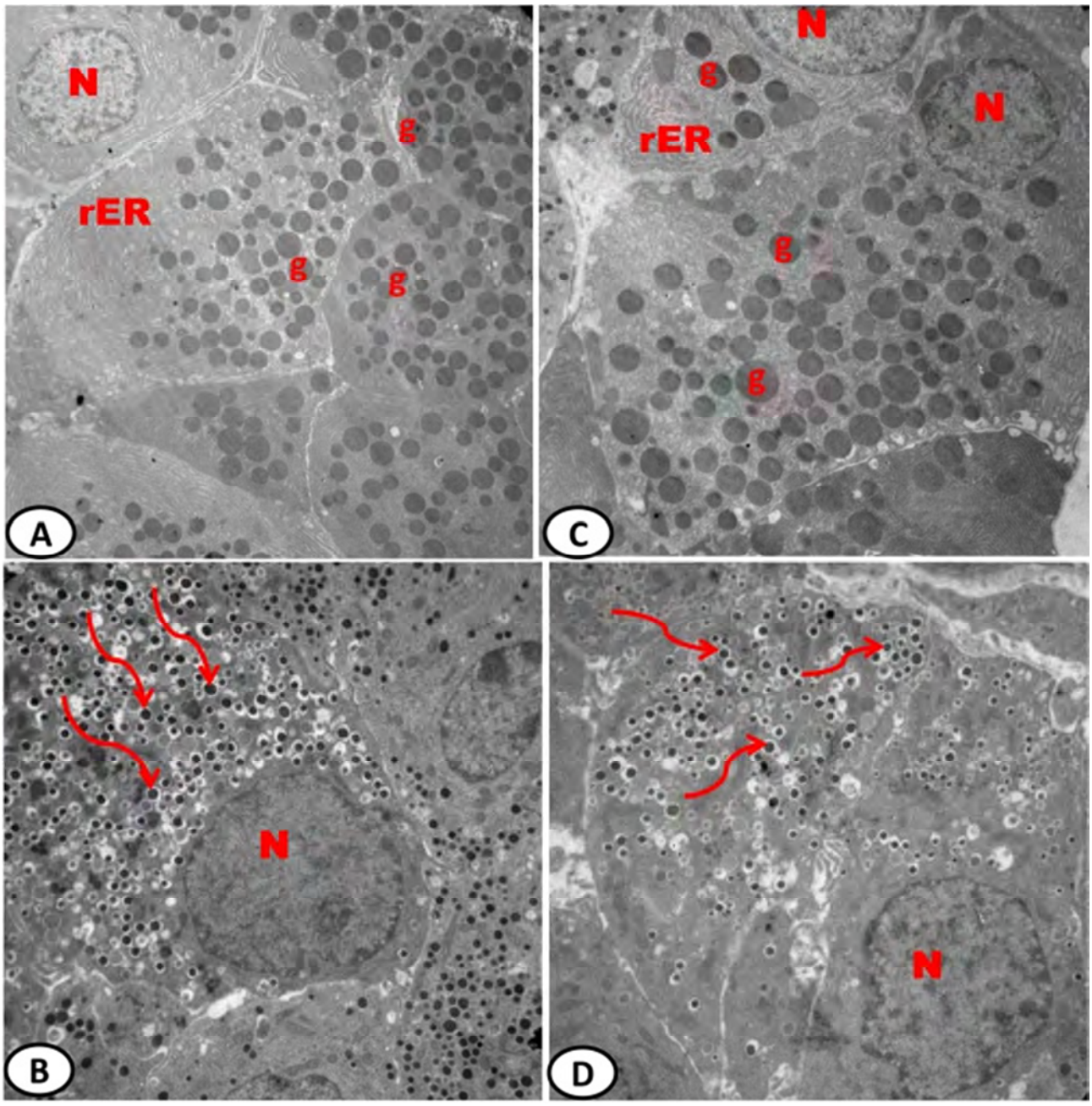

Figure 2. *A transmission electron photomicrographs of ultrathin sections of adult male albino rat's pancreas.*

(A) Control group showed normal pancreatic acinar cell with euchromatic nucleus (N) and well developed rough endoplasmic reticulum (rER). The presence of apical electron dense granules (g) was observed. EM x 2900.
(B) Control group showed normal part of an islet of Langerhans with ß cells that have euchromatic rounded nuclei (N) and profuse secretory granules (curved arrows). The ß granules formed of an electron-dense core surrounded by an electron-lucent halo. EM x 5800.
(C) Omega-3 group showed acinar cell that have rounded nuclei (N). Note, the cytoplasm contains numerous secretory granules (g) of high-electron density. The presence of well-developed rough endoplasmic reticulum (rER) can be seen. EM x 4800.
(D) Omega-3 group showed part of an islet of Langerhans with ß cells that have euchromatic oval nuclei (N). The cytoplasm of β cell contained granules with a dense electron core surrounded by a clear halo (curved arrows). EM x 5800.

Group III (Fluoxetine group)

Light microscopic examination of the pancreas of fluoxetine of adult male albino rats showed that the pancreatic acini were obviously distorted and they lost their normal consistent appearance. The pancreatic lobules separated by thick interlobular septa, which contained dilated irregular ducts, congested blood vessels and cellular infiltration (Figure 3A & B). Numerous fat cells were also observed (Figure 3A). The acinar cells had a few vacuoles (Figure 3C). Most of the islets of Langerhans were distorted, and shrunken (Figure 3C & D). Most of islet cells were lost and reduction in the number of islet cells was obvious. Few islet cells were small in size with small deeply stained nuclei (Figure 3D).

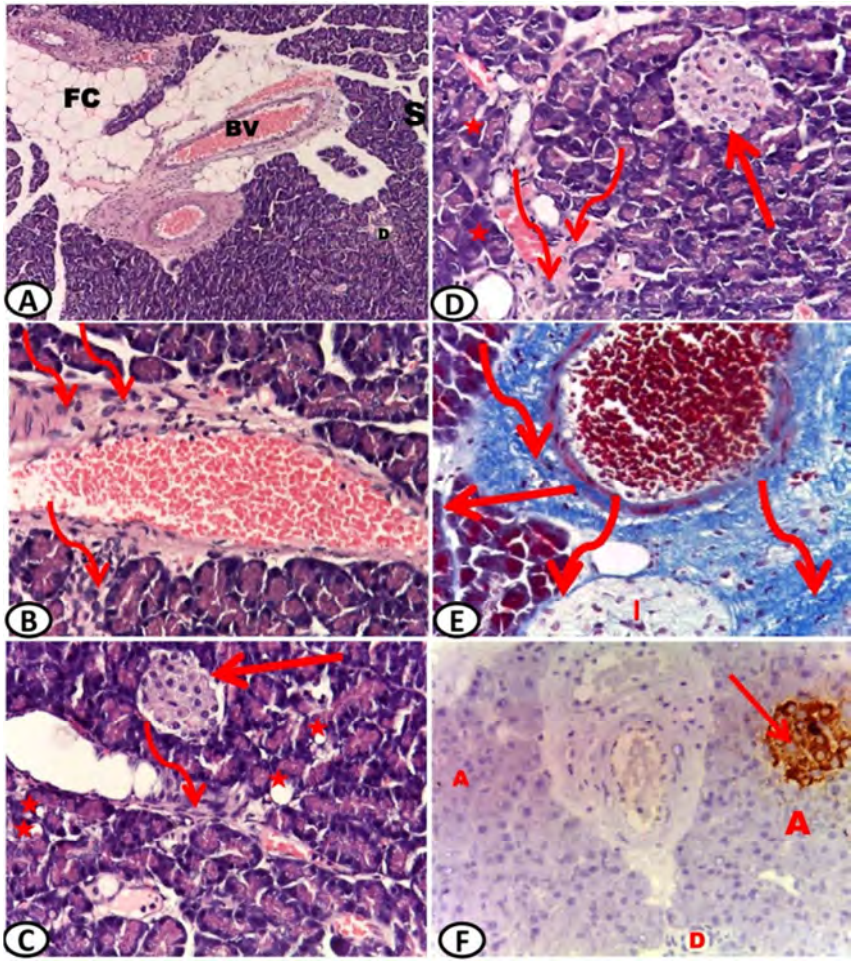

Figure 3. *Photomicrographs of sections of rat pancreas of the fluoxetine group (group III).*

(A) Pancreatic lobules separated by thick interlobular septa (S) and severe dilated congested blood vessels (BV). Notice, fat cells (FC) in the septa between lobules and pancreatic duct (D) can be seen. H & E, x 100.
(B) Wide spaces between pancreatic lobules contain dilated congested blood vessels can be noticed. Note, inflammatory cellular infiltration (curved arrows) around congested blood vessels and in between acini can be observed. H & E, x400.
(C) Few damaged pancreatic acini leaving empty spaces and some acinar cells are vacuolated (stars). Markedly shrunken and distorted islet of Langerhans (straight arrow) is obvious. The presence of inflammatory cellular infiltration can be seen (curved arrow). H & E, x400.
(D) Another small islets of Langerhans (straight arrow) with loss of its normal cell cord arrangement. Note, islets contained few cells with pyknotic nuclei. Cellular infiltrations (curved arrows) and degenerated few acini (stars) can be observed. H & E, x400.
(E) Dense collagen fibers around blood vessels, islets (I) of Langerhans (curved arrows) and thick collagen fibers around acini (straight arrow) can be seen. Masson's trichrome, x400.
(F) β-cells positive insulin immunoreaction (arrow) in small islets. Pancreatic acini (A) and duct (D) showed negative insulin immunoreaction. Insulin immunohistochemical, x400.

Masson's Trichrome stained sections of the fluoxetine group showed a dense collagen fiber around the acini. An obvious increase in the periductal collagen fibers, thickening and hypertrophy of the congested blood vessels surrounded by condensed collagen fibers were noticed. (Figure 3E).

Immunohistochemical stained sections of the fluoxetine group showed markedly decreased in number of β insulin positive cells (Figure 3F).

Electron microscopic examination of the fluoxetine group revealed that most of the acinar cells had irregular shaped nuclei with peripheral heterochromatin (Figure 4A). The presence of wide capillaries was noticed (Figure 4A). Numerous plasma cells with degenerative changes were observed. The degenerative changes were in the form of dilatation of rough endoplasmic reticulum (Figure 4B). In β cells, some type of β cells had large irregular condensed heterochromatic nuclei (Figure 4D). The blood capillaries showed marked dilation and thick bundles of collagen fibers around dilated blood vessels (Figure 4C & E). Apparently, the β-cells showed the presence of degenerative changes. The cytoplasm of β cells had a variety of secretory granules (Figure 4D, E & F). Most of these granules had an electron-dense core and an electron-lucent halo (Figure 4F). Other granules had small electron dense with an increased electron lucent halo (Figure 4F). Few secretory ß granules appeared empty coalesced (Figure 4F).

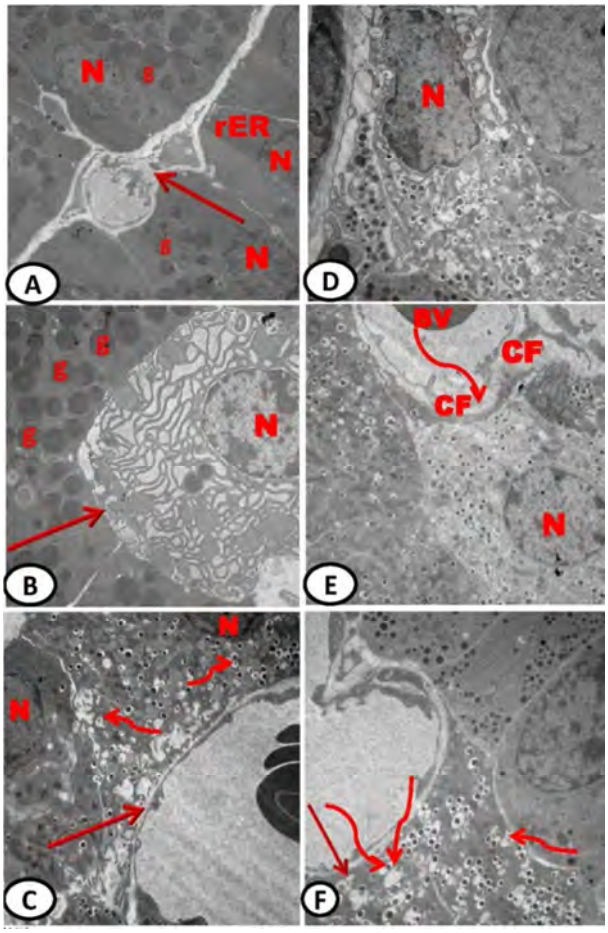

Figure 4. *A transmission electron photomicrographs of ultrathin sections of the pancreas of fluoxetine group (group III).*

(A) Pancreatic acinar cells with irregular shaped nuclei (N). Notice the presence of wide capillaries (arrow). Secretory granules (g) and rough endoplasmic reticulum (rER) can be seen. EM x4800.
(B) Interstitial plasma cell infiltrating the acinar cell (arrow). Plasma cell with cartwheel arrangement of rounded nucleus (N) with peripheral heterochromatin and markedly dilated cisternae of rough endoplasmic reticulum within its cytoplasm and mitochondria. The secretory granules (g) of acinar cell were observed. EMx7200.
(C) Part of the ß cells with dark electron dense nuclei (N), ß granules with increased electron lucent halo (curved arrows) in disrupted cytoplasm are detected. Collagen fibers around dilated blood vessels (straight arrow) are observed. EM x 5800.
(D) The presence of degenerative changes in ß cells in the form of irregular shaped dark electron dense nucleus (N) can be noticed. EM x3600.
(E) The ß cells with thick bundles of dense collagen fibers (CF)(curved arrow) around dilated blood vessels (BV) can be observed. Note, rounded nucleus (N) with peripheral heterochromatin. EM x 5800.
(F) The cytoplasm of the ß cells has a variety of secretory granules. Notice, the increase in halo spaces around most of ß cells granules (curved arrows). Few secretory ß granules appeared empty coalesced (straight arrow). EM x 5800.

Group IV (Fluoxetine & Omega-3)

Light microscopic examination of the pancreas of adult male albino rats that received both fluoxetine and omega-3 revealed that pancreatic acinar cells had an apical acidophilic cytoplasm filled with many secretory granules and basal oval nuclei. Few acinar cells with cytoplasmic vacuoles were noticed (Figure 5A). Partial regeneration of pancreatic islets was detected. Most of islets exhibited an obvious increase in their size. The presence of cellular connections between some ducts and the islets cells were observed. Congested blood vessels were still noticed (Figure 5A).

Masson's trichrome stained sections of the fluoxetine and omega-3 group showed moderate collagen fibers between acini and few collagen fibers around duct and blood vessels (Figure 5B).

Immunohistochemical stained sections of the fluoxetine and omega-3 group showed positive immunoreactions of β-cells for anti-insulin antibodies were obviously increased in numbers (Figure 5C) as compared to the group III (Figure 3 F). Immunopositive small groups of endocrine-like cells related to the wall of dilated duct was also detected (Figure 5D).

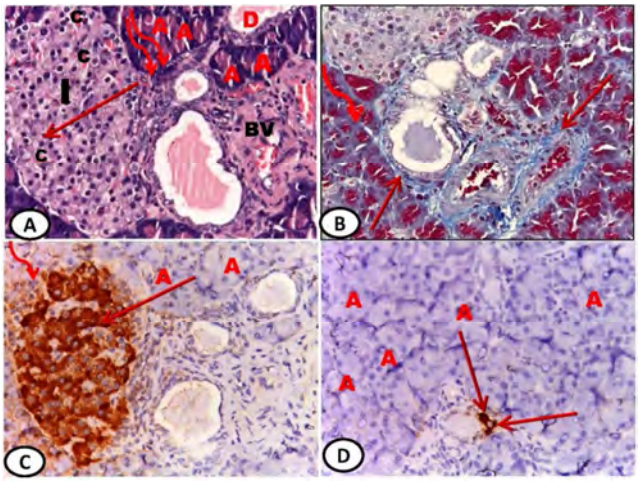

Figure 5. *Photomicrographs of sections of the pancreas of the omega- 3 and fluoxetine group (group IV).*

(A) Restored lobular architecture. The size of islets of Langerhans (I) and total number of islet cells was apparently increased. Slightly congested capillaries (C) can also be seen in some locations of islet. Note, cellular connection between the duct (D) and islet cells can be seen (double curved arrow). Hx & E, X400.
(B) Moderate collagen fibers between acini (curved arrow) and few collagen fibres around duct and blood vessels (straight arrows). Masson's trichrome, X400.
(C) Increased number of β-cells positive insulin exhibiting brown cytoplamic immunostaining (straight arrow). Note, no immunoreactivity was detected peripherally (curved arrow) and in the acini (A). Ant-insulin immunoreactivity, X400.
(D) Group of endocrine like cells (arrows) related to a dilated duct expressing brown cytoplasmic insulin immunoreactivity can be seen. No insulin immunoreactivity in the acini (A). Anti-insulin immunoreactivity, X400.

Electron microscopic examination of the pancreas of the fluoxetine and omega-3 group revealed that acinar cells had many electron-dense secretory granules. Acinar cell was euchromatic with numerous mitochondria and slightly dilation of rough endoplasmic reticulum (Figure 6A). The cells of the islets of Langerhans had euchromatic nuclei with slightly peripheral of heterochromatin. The ß cells appeared nearly normal with profuse secretory granules. Blood capillaries showed moderate dilation with a nearby moderate collagen fibers deposit (Figure 6 B).

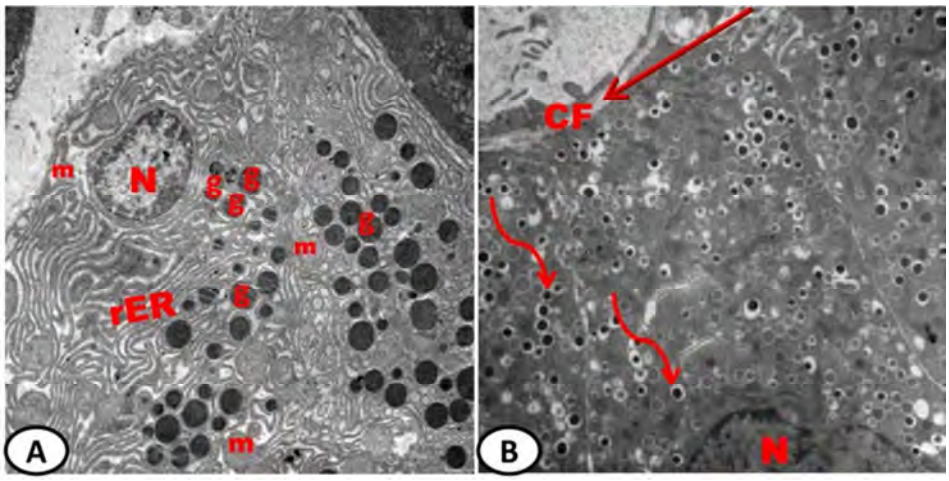

Figure 6. *A transmission electron photomicrographs of ultrathin section of a rat's pancreas of fluoxetine and omega- 3 group (group IV).*

(A) Acinar cells have euchromatic nucleus (N). Notice, the presence of slightly proliferation of dilatation of rough endoplasmic reticulum (rER), numerous mitochondria (m), and plentiful secretory granules (g) of high density in the apical cytoplasm can also be seen. EM x 4800.
(B) Part of ß cells of the islets of Langerhans having euchromatic nucleus (N) with slightly peripheral of heterochromatin and profuse secretory granules (g). The ß cells appeared nearly normal (curved arrows), the presence of slightly collagen fibers (CF) around blood vessels (BV) (straight arrow) can be observed. EM x 7200.

3.2. Morphometric Results

3.2.1. Mean Area Percentage of Collagen Fiber Deposition in the Pancreatic Tissue

Histomorphometric analysis showed marked increase in the mean area percentage of collagen deposition in the pancreatic tissue in group of fluoxetine treated group (group III), (Table 1 and Figure 7) which was statistically significant compared with the mean value of the control, (group I)**.** Group II showed the mean area percentage of collagen fibers revealed nearly normal value which was statistically insignificant compared with group I (P>0.05). On the other hand, group IV which was statistically significant as compared with the fluoxetine group (Table 1 and Figure 7).

Table 1. *Mean area percentage of collagen fibers of all experimental groups.*

Parameter / *Groups*	*Mean± SD (%)*	*P value*
Control group (group I)	8.52±0.26	P > 0.05 (no significant value between group I & group II)
Omega- 3 treated group (group II)	8.43±0.37	
Fluoxetine treated group (group III)	28.34±2.54**	**P<0.001 (significant value between group III & group I)
Fluoxetine & omega -3 treated group (group IV)	9.12±0.22*	*P<0.001 (significant value between group IV & group III)

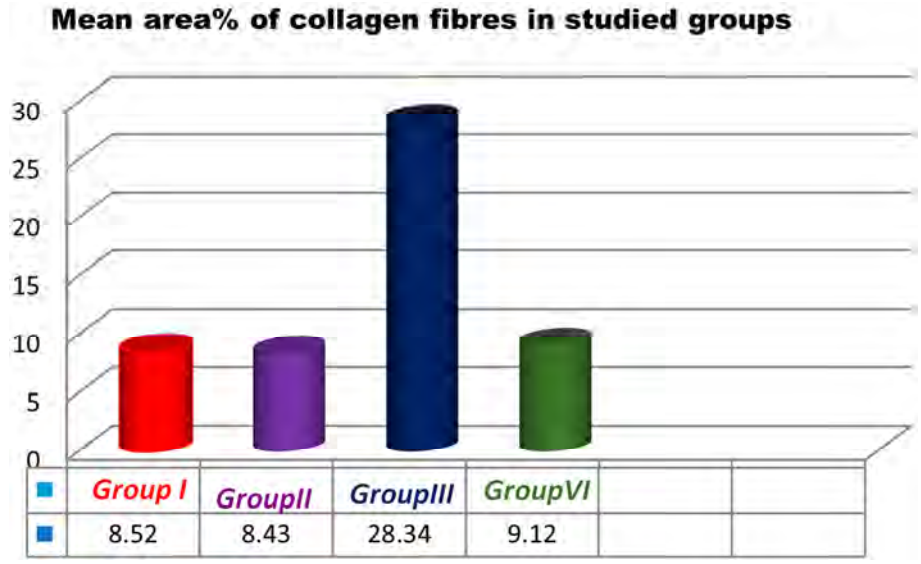

Figure 7. *Showing the mean value of area percentage of collagen fibers deposition of all experimental groups.*

3.2.2. β-Cells/Islet of All Experimental Groups

The number of insulin positive β-cells per islet was analyzed. There was no significant difference in the mean number of anti-insulin positive β-cells of the omega-3 group compared to the control group. Vitally, a significant decrease in the mean number of immunopositive β-cells in the fluoxetine group compared to the control group (Table 2 and Figure 8). Remarkably, there was a significant increase in the mean number of immunoreactive β-cells of fluoxetine and omega- 3 group (group IV) compared to the fluoxetine group (Table 2 and Figure 8).

Table 2. *Insulin positive cells (β-cells/islet) of all experimental groups.*

Parameter / Groups	(β-cells/islet) Mean± SD	P value
Control group (group I)	87.8±7. 7	P > 0.05 (no significant value between control group & omega -3 group)
Omega- 3 treated group (group II)	88.2±6.2	
Fluoxetine treated group (group III)	28.2±20.3**	**P<0.001 (between fluoxetine group & control group)
Fluoxetine & omega- 3 treated group (group IV)	89.2±19.4*	*P<0.001 (between fluoxetine group & fluoxetine+omega-3 group)

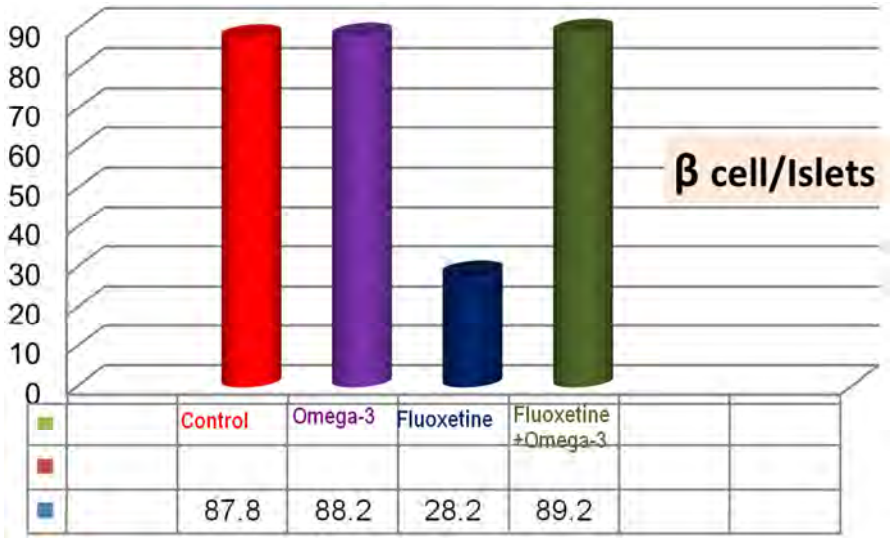

Figure 8. *Showing mean value of insulin positive cells (β-cells/islet) of all experimental groups.*

4. Discussion

The present study revealed that fluoxetine induced histological, immunohistochemical and ultrastructural changes in the adult rat of the pancreas. The current research revealed vascular changes in the form of congested blood vessels and proliferation of duct in the exocrine part of pancreas in the group III. In accordance with these results, it has been reported that the use of fluoxetine was associated with the apparent changes in the ducts could be probably due to the mitochondria. The mitochondrial damage led to ATP reduction with successive failure of biosynthesis and membrane pumps. Consequently, the cells had no energy for the method of transport of secretions resulting in duct dilatation [21]. Moreover, previous in vitro study stated that fluoxetine treatment was able to damage the function of isolated hepatic mitochondria [22].

In the current study, mononuclear cellular infiltration was detected between the exocrine acini and close to islets of Langerhans in the fluoxetine treated group. These current observations were in agreement with the results obtained by other investigators. Previous studies have suggested that during pancreatitis, inflammatory cells activate the beginning of signaling pathways regulating gene expression of inflammatory mediators, or consequences in the increased creating of cytokines, which leads to the expansion of local pancreatic inflammation [23].

In the existent study, fat cells were detected in the pancreas of the fluoxetine-received group. Importantly, a significant increase in the proportion of fluoxetine exposed animals with indication of steatohepatitis and hepatic microvesicular lipid accumulation compared to controls were reported [24]. Indeed, a strong correlation between obesity, hepatic lipid accumulation, hepatic inflammation and insulin resistance in humans and animal model was demonstrated [25]. Fat cells were also detected in the exocrine pancreas during aging [26].

Masson trichome stained sections in the fluoxetine treated group demonstrated the excessive interacinar collagen fibers. Moreover, a highly significant increase in the deposition of collagen fibres around the congested blood vessels and dilated ducts were also observed. This finding coincides with other study. It was suggested that examination of the fluoxetine-treated group revealed deposition of collagen fibres in the interstitum of rat parotid gland [27].

The present study revealed shrinkage and distortion of pancreatic islets, with marked degenerative changes in its cells in the fluoxetine treated group. Recent investigation

proved that fetal and neonatal exposure to fluoxetine results in endocrine and metabolic changes that are consistent with type 2 diabetes (T2D) and its comorbidities, specifically obesity, fatty liver and dyslipidemia [24]. Recent investigation suggested that fluoxetine, impaired E-cadherin-mediated cell adhesion and alters calcium homeostasis in pancreatic beta cells [28]. Others explained the action of fluoxetine on pancreatic beta cells by inhibition of mitochondrial bioenergetics, oxidative stress and induction of apoptosis [29].

The histological results were in agreement with the morphometric results which revealed a significant reduction in number of β-cells/islet in the fluoxetine group compared to the control. The current results were consistent with previous studies. The present results could be partially explained by considering the mode of action of SSRI to rise the production of reactive oxygen species. Vitally, increase the production of ROS lead to damage to mitochondrial, cytoplasmic proteins, lipids and nucleic acids [30]. Recent investigators suggested that the future direction should be focused on loss of normal beta-cell function, rather than total cell mass, a major driver for impaired insulin secretion [31].

Electron microscopic results in the current study of the fluoxetine group come hand by hand with the histological and immunohistochemical results. Crucially, in the present study, the degenerative changes in plasma cells were detected. The degeneration was in the form of dilation of rough endoplasmic reticulum. Dilation of rough endoplasmic reticulum might be due to some defects in the progress of secretory material. Other researchers were in agreement with current data, they found the interstium of parotid gland in the group received fluoxetine rich in dendritic and plasma cells. Most acini of parotid gland contained huge dilated rough endoplasmic reticulum with retained secretion [27]. Further insight into the rough endoplasmic reticulum dilatation in plasma cells was provided by other researchers who suggested that this might reveal an increase in their immunological activity [32]. Importantly, the acinar cell impairment has been investigated in several experimental models. It is thought to be as a result of production of free radicals that they could aggravate cell damage causing lesions of the cell membrane and cytoskeleton that led to impair function of intercellular proteins and decreasing the level of antioxidant [33].

In the current work, examination of pancreas in the fluoxetine and omega-3 group revealed that the improvement in the pancreatic tissue. The pancreas regained nearly its normal general architecture. The acini appeared normal with a moderate amount of apical zymogen granules and pancreatic lobules were separated by thin interlobular septa. Previous report from other investigators recommended that omega-3 had a role in reducing of acute pancreatic inflammation [34]. The present work could be proved by other researchers who explained that the anti-inflammatory and antioxidant properties of omega-3 act through mechanisms. Interestingly, one of these mechanisms was declining the production of leukotriene B4, the pro-inflammatory mediators that act by modifying the secretory functions and cell immunity of the acini leading to cellular impairment [35].

Interestingly, in the present study, the administration of omega-3 lead to an apparent improvement in the pancreatic islet as the number of islet cells was increased. The regeneration of pancreatic islets was observed. Obviously, most of the islets were enlarged in size, with nearly a normal architecture. The nuclei were rounded and vesicular whereas the cytoplasm of beta cells was stunted with dense granules. These data are in accordance with the findings detected in Field and Co-collaborator recommended that the administration of omega -3could change the rate of insulin degradation and possibly affect insulin sensitivity, causing in normoglycemia [36]. Other investigators suggested that Omega-3 decreased all plasma lipids by reducing their lipogenesis and increasing their β -oxidation and catabolic rate [37]. While other authors added that the essential fatty acids present in ω-3 are important in the production of prostaglandins, local chemical regulators through which all hormones such as insulin and glucagon exert their actions. Importantly, the increased intake of ω-3 fatty acids was associated with preservation of integrity of pancreatic beta cells through stoppage of the occurrence of autoantibodies in the blood that signal the immune system to attack insulin producing cells [38].

In the current work, the administration of ω-3 showed delicate deposition of collagen fiber, signifying the potential protective role of ω-3 against fluoxetine. Previous researchers are in agreement with the current findings. They suggested that pancreatic fibrosis was reduced after ω-3 PUFA administration in pancreatitis [39]. The current finding was explained by other researchers. They outlined the essentiality of omega-3 fatty acids for protection against fibrosis and proved that omega-3 fatty acids lowers the activation of pancreatic stellate cells [40].

Importantly, in the current study, the presence of small groups of endocrine-like cells related to the dilated ducts was noticed in the fluoxetine and omega-3 group. The current result might suggest a ductal origin for the regenerated islets. Remarkably, some of them showed immunoreactive to the anti- insulin antibody. Other researchers suggested that the endocrine cells of the rat pancreatic islets of Langerhans, including insulin-producing β cells, turn over every forty to fifty days by the apoptosis and by the proliferation and the differentiation of new islet cells from progenitor epithelial cells placed in the pancreatic ductal cells [41]. The current data were recommended by others, they supported that pancreatic duct cells might serve as a basis of regeneration in the adult rats after partial pancreatectomy [42].

There was a remarkable finding in the present work of the fluoxetine and omega-3 group, the presence of cellular connections between some ducts and the islets cells. Indeed, the pancreatic duct epithelium contributes as a pool for precursors for islet and acinar tissues after birth and into adulthood [43].

From these evidences, the hypothesis in the current work

that omega- 3 could act as the right stimulus for beginning of islet neogenesis from potent islet precursor cells such as duct cells. Therefore, omega-3 could restore β-cells by stimulating the diverse sources of β-cells precursors in adult rat through proliferation and transdifferentiation process. Similarly, other researchers reported that platelet rich plasma (PRP) might put the pancreas into an environment similar to the postnatal developmental one where new lobules were formed [44]. This explanation was in contrast to other investigators who proposed that the adult pancreatic β-cells are formed by self-duplication, rather than stem-cell differentiation. Kopp and co-worker recommended that the derivation of endocrine cells from the ducts occurred only in early postnatal life. The researchers suggested that no endocrine or acinar cell neogenesis occurred in adult mice either physiologically or after pancreatic duct ligation [45].

Importantly, limited electron microscopic studies on omega-3 and fluoxetine group. The present transmission electron microscopic study of this group showed that most of the nuclei of acinar cells were euchromatic and marked improvement of beta cells. Most of beta cells appeared with nearly normal architecture. Omega-3 fatty acids might diminish the hazard of development of diabetes by inhibiting hyperglycemia and pancreatic insulitis. Indeed, previous researchers reported that the antioxidant activity of omega -3 was completed by a reduction in nitric oxide synthase leading to a decrease in oxidative stress responsible for diabetic complications [46]. Recent investigation supported the suggestion regarding the anti-diabetic effect of omega-3 fatty acids and pioglitazone combination mediated via fibroblast growth factor 21 [47].

5. Conclusion and Recommendations

Fluoxetine harmfully affected the histological structure of pancreas in adult male rats. The current study could add a new recommendation of the defensive role of omega -3 against fluoxetine induced pancreatic damage. Therefore, it is recommended to achieve further studies by investigating the role of oxidative changes to recognize the exact mechanisms.

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