Original Article

Pancreatic Histological Changes in Adult Female Albino Rats Treated with Orlistat and the Possible Protective Role of B- carotene

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ABSTRACT

Background: Orlistat is a pancreatic lipase inhibitor licensed for obesity treatment. It is considered to be a safe drug for long term use but many adverse effects were observed such as pancreatitis. B-carotene is a precursor of vit. A which has antioxidant, anti-inflammatory and immune-enhancing effects.

Objective: This study aims to clarify the pancreatic structural changes after Orlistat administration and the possible protective role of B- carotene in adult female albino rats.

Materials and Methods: Fifty adult female albino rats were divided into four groups: Group I (10rats) was control. Group II (10rats) was administered B-carotene at a dose of 0.52 mg/kg/day, orally for 5 weeks. Group III(20rats) was administered a therapeutic dose of Orlistat (32 mg/kg/day) dissolved in 1 ml distilled water orally for 5 weeks then 10 rats were scarified(subgroup III a) and the others 10 rats left without intervention for another 5 weeks(subgroup III b). Group IV (10 rats) was administered B-carotene as group II one hour before the administration of orlistat as group III a for 5 weeks. At the end of the study, each rat was weighed, blood samples were obtained for estimation of blood glucose level and pancreas were dissected and prepared for histological study.

Results: Orlistat treated rats showed a highly significant decrease in body weight and a highly significant increase in blood glucose level. There were signs of B cells inhibition in anti-insulin immunostaining. Destructive changes of acinar cells with marked reduction of secretory granules and strong positive reaction for iNOS reactions were observed. These changes were confirmed by electron microscopic examination. These changes were reversible in withdrawal group. B- carotene coadministration showed amelioration of the histological changes.

Conclusion: B- carotene supplementation has ameliorating effect on pancreas against the deleterious effects of orlistat**.**

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Key Words: B- carotene; iNOS; orlistat; pancreas.

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INTRODUCTION

Obesity and overweight are important public health problems throughout the world, affecting both developed and developing countries. It is strongly linked to major leading causes of mortality such as hypertension, dyslipidemia, diabetes and cardiovascular diseases[1]. Various guidelines recommend drug therapy as a second line in the treatment of obesity when weight loss targets were not reached by life style modification[2].

Orlistat, an antiobesity drug, is a specific long acting reversible lipase inhibitor that acts locally in the lumen of alimentary tract by binding covalently to the serine residue of the active site of gastric and pancreatic lipase enzymes. Thus, digestion and absorption of fat are inhibited leading to oily stool, oily spotting and flatulence[3].

Pancreatitis is an inflammatory disease affecting both exocrine and endocrine pancreas, where it runs as self-limiting course in most cases, in others, it may lead to severe form characterized by extensive necrosis leading to high mortality rate of about 25%[4]. It has been reported that there was a relation between the use of Orlistat and development of pancreatitis in some clinical cases with no evidence of biliary disease or alcohol abuse[5], but few studies had investigated the histological changes of pancreatic tissue after Orlistat intake[6].

B-carotene is a vit A precursor and it is the most plentiful form of carotenoids. It acts as an antioxidant and can be obtained from orange, yellow and green leafy vegetables and fruits[7]. It improves antioxidant activity, ameliorates oxidative stress and decreases apoptosis[8]. Beside its antioxidant activities, it is believed that it has detoxifying characters; also, it is an anti-inflammatory, anti-infectious and immune booster agent[9]. Humans and animals can't synthesize carotenoids de novo, and they are dependent on their diet as a source of these compounds[10]. Previous studies demonstrated the potent protective effect of carotenoids as an antioxidant in chronic diseases, particularly inflammatory joint disease, malignancy, degenerative eye disease and Diabetes Mellitus[11,12,13].

Taken together, the interest of females to lose weight by taking the easy access of antiobesity drugs over the counter has been found to be higher than the males[14], and the lack of histological studies on the protective effect of ß-carotene on orlistat induced pancreatitis. The current work aimed to study the pancreatic microscopic changes in adult female

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albino rats treated with orlistat and the possible protective effect of ß-carotene.

MATERIALS AND METHODS

Drugs

- **Orlistat:** It is available in the form of capsules with a trade name, Xenical 120 mg, introduced by Roche pharmaceuticals, Germany. The content of each capsule was evacuated and then the human therapeutic dose of orlistat (360 mg/day) was converted to animal dose according to Nair and Jacob[15].
- **B-Carotene:** It is available as capsules with a trade name ,Beta carotene forte, introduced by Arab company for gelatin and pharmaceutical products for (MEPACO-MEDIFOOD). Each capsule contains natural B- carotene 15 mg (equivalent to natural vit. A 25000 IU).

Animals

Fifty adult female albino rats, weighing 150-200 gm were employed in the present study. They were housed in four stainless steel cages in clean well-ventilated room. They were allowed free access to laboratory rat chow diet and water ad-libitum. Strict care and hygiene were maintained to keep them in healthy environment all the time. The general conditions and behavior of the animals were noticed. All animals' protocols were approved and observed via the Animal Care Committee of the Research Laboratory of Experimental Animals at the College of Medicine, Menoufia University, Egypt.

Randomly, the rats were divided into four main groups as follows:

- **Group I (control group):** included 10 rats, they received 1ml distilled water daily by oral gavage for 5 weeks.
- **Group II (B- carotene treated group):** included 10 rats, they received B- carotene at a dose of 0.52 mg/kg/ d by oral gavage for 5 weeks[16].
- **Group III (Orlistat treated group):** included 20 rats, they received a therapeutic dose of Orlistat 32 mg/kg/d dissolved in 1 ml distilled water which is equivalent to human dose according to Nair and Jacob[15]., by oral gavage for 5 weeks[17] then 10 rats were scarified (subgroup IIIa) and the others 10 rats were left without intervention for another 5 weeks as a withdrawal group (subgroup IIIb).
- **Group IV (Orlistat and B- carotene treated group):** included 10 rats, they received B-carotene as groups II one hour before the administration of orlistat as subgroup III a for 5 weeks.

All over the experiment, the animals were noticed for food habits and physical activities. At the end of each detected period, the animals were weighted and sacrificed by i.p. injection of 50 mg / kg pentobarbital sodium. Blood samples were obtained from tail vein and then, collected into heparin coated tubes and centrifuged for 1 min. plasma samples were stored at -20 for biochemical study. The pancreas of each rat was dissected out and divided into 2 parts. One part was fixed in 10% buffered formalin overnight for histological and immunohistochemical study. The other part was cut into small pieces and rapidly fixed in 1% phosphate buffered glutaraldehyde, then processed for electron Microscopic study.

I-Biochemical study

Blood glucose level

Plasma glucose levels were estimated by utilizing a commercial glucose Colorimetric Assay Kit (Cayman ChemicalCompany, Ann Arbor, MI, USA). It was performed in the central laboratory, faculty of medicine, Menoufia University.

II- Histological study

Tissue samples fixed in 10 % buffered formalin were processed to get the ordinary paraffin blocks. Sections of 5-6 µm thick were cut and stained with Hematoxylin & Eosin stain (Hx&E) to show the histological structure and Mallory trichrome stain to detect collagen fibers[18,19].

III- Immunohistochemical study

Inducible nitric oxide synthase immunostaining (iNOS):

Paraffin sections were incubated with inducible Nitric oxide synthase (iNOS) rabbit polyclonal antibody (Product GTX15323; dilution 1:100, Gene Tex, USA). Negative control sections were processed by replacing the primary antibody with buffer alone. Human liver tissue was used as a positive control[20]. The positive reaction was cytoplasmic brown color

Anti-Insulin immunostaining

Paraffin sections were incubated with anti-insulin antibody; insulin Ab-6 (INS04 + INS05) Mouse Monoclonal Antibody (Thermo Fisher Scientific, Fremont, CA, USA). Negative control sections were processed by replacing the primary antibody with buffer alone. Pancreas was used as a positive control[21].

IV-Electron microscopic study

Parallel small (1×1 mm) sized pieces of pancreatic tissue were fixed at 4°C phosphate buffered gluteraldehyde solution (pH 7.4) for 4 h. rinsed three times with phosphate buffer (two changes) and post fixed in 1% aqueous buffered osmium tetroxide at room temperature for 2h. After that, the specimens were dehydrated in ascending grades of alcohol, and embedded at the apex of inverted polythene beam capsule filled with liquid resin. The sections were cut using ultramicrotome into semithin (0.5 µm thickness) and ultrathin sections. The semithin sections were stained with toluidine blue for detection of secretory granules. Ultrathin

sections (80–90 nm) were stained with uranyl acetate and lead citrate[22] to be examined by Transmission Electron Microscope (TEM, JEOL 100 CX) at faculty of medicine, Tanta University.

V-Morphometric and Statistical analysis

By using image analyzer system (Image J 1.47v national institute of health, USA) we measured the intensity of the brown color of anti-insulin immune expression at 400×magnification of 5 non-overlapping fields from randomly selected light microscopic slides stained with anti-insulin monoclonal antibody per group. The number of zymogen granules was counted at five fields of randomly selected electron microscopic slides per group.

The collected data were analyzed and compared by student's t-test. The p-value was utilized to test the significance of changes in each parameter in comparison with the control group. The data were expressed as mean ± SD and analyzed utilizing statistical package for the Social Science Software (SPSS)(version 17.0 for windows; SPSS Inc., Chicago, Illinois, USA). *P value* was highly significant if $P<0.001$, but $P<0.05$ was significant and was non-significant if $P>0.05$.

RESULTS

I- General observation

Group I (control) and group II (B- carotene treated)

All animals of these groups showed good general condition and normal behavior, activity and appetite.

Orlistat treated subgroup (Subgroup IIIa)

Throughout the experiment, the animals treated with Orlistat became less active. Diarrhea accompanied by a decrease in body weight was noticed.

Withdrawal subgroup (Subgroup IIIb)

Animals of this group showed improvement in their activity. Also they showed apparent decrease in diarrhea.

Orlistat and B-carotene treated group (Group IV)

The animals of this group showed good general condition and normal activity. Diarrhea accompanied by a decrease in body weight was also noticed.

II- Change in the body weight

There was non-significant change in the mean body weight of B- carotene treated group (group II) as compared to control group ($p1>0.05$), while there was a highly significant decrease in the mean body weight of Orlistat treated and protected groups (subgroup III a and group IV respectively) as compared to control group ($p2$, $p4<0.001$) and significant decrease in the mean body weight of the withdrawal subgroup (subgroup III b) as compared to control group ($p3<0.05$).

Moreover, there was highly significant increase in the mean body weight of the withdrawal subgroup (subgroup III b) as compared to Orlistat treated subgroup (subgroup III a) ($p5 <0.001$) and non-significant change in the mean body weight of protected group (group IV) when compared with Orlistat treated group ($p6>0.05$) and highly significant decrease when compared with withdrawal group ($p7 <0.001$) (Table 1, Histogram 1).

III- Biochemical result

Blood glucose level

B-carotene treated and withdrawal groups (group II & subgroup IIIb) revealed non-significant change in mean blood glucose level when compared with control group ($p1$, $p3> 0.05$). While, there was a highly significant increase in mean blood glucose level in the Orlistat treated and protected groups (subgroup IIIa & groupIV) in comparison with control group ($p2$, $p4<0.001$). Moreover, withdrawal group (subgroup IIIb) and protected group (group IV) exhibited a highly significant decrease in mean blood glucose level in comparison with Orlistat treated (subgroup IIIa) ($p5$, $p6<0.001$) while protected group (group IV) exhibited a highly significant increase in mean blood glucose level in comparison with withdrawal subgroup (subgroup IIIb) ($p7<0.001$) (Table2, Histogram2).

IV- Light microscopic results

Hematoxylin & Eosin stained sections of pancreas of Group I (control) and group II (B- carotene treated): were the same and revealed different sized lobules of closely packed serous acini which lined by pyramidal cells. The cells had apical cytoplasmic acidophilia and basal basophilia with rounded vesicular nuclei. Intra lobular duct lined by cubical epithelium with rounded nuclei was observed. Islet of Langerhans showed central cells with acidophilic vacuolated cytoplasm and rounded vesicular nuclei (Figures 1,2,3).

Sections of pancreas of subgroup III a (orlistat treated subgroup) exhibited disturbance in the normal pancreatic architecture. The pancreatic lobules were separated by wide spaces. Some acini were distorted and acinar cells displayed faint basal basophilia (Figures 4,5). And intracytoplasmic vacuoles, also, some nuclei were deeply stained and pyknotic (Figure 5). Dilated ducts with retained secretion were observed (Figure 4). Many dilated congested blood vessels with inflammatory cellular infilterate were seen. The islet cells appeared with deeply acidophilic cytoplasm and dark pyknotic nuclei (Figure 6).

Sections of pancreas of subgroup III b (withdrawal subgroup) displayed some improvement in the form of restoration of normal structure of the pancreas and most of the acinar cells and their nuclei appeared nearly similar to that of control group. But some acini still revealing disappearance of basal basophilia (Figure 7) and others still revealing vacuolated cytoplasm and pyknotic nuclei. (Figure 8). There were wide spaces inbetween acini (Figure 7).

Group IV (Orlistat and B-carotene treated group) showed good improvement in structure of pancreatic tissue as revealed by restoration of the normal pancreatic architecture with nearly normal acinar cells.The ducts and their linning epithelium appeared nearly normal (Figure 9). The islets of langerhans appeared normal but some cells with small pyknotic nuclei in the center of the islet were observed (Figure 10).

Semi thin sections of groups I& II (control & B-carotene treated groups) revealed abundant apical zymogene granules in acinar cytoplasm (Figures 11,12). While subgroup III a (orlistat treated subgroup) exhibited apparent decrease in zymogene granulesin some acini and abscense of these granules in others (Figure 13). Subgroup III b (withdrawal subgroup) revealed restoration of zymogene granules in some acinar cells (Figure 14). morever, group IV (orlistat and B-carotene treated group) revealed abundant zymogene granules in acinar cytoplasm (Figure 15).

Mallory trichrome stainedSections of pancreas of groups I & II (control and B- carotene treated) revealed minimal amount of collagen fibers in the connective tissue septae and around pancreatic acini and duct (Figures 16,17). While subgroupIII a (Orlistat treated subgroup) exhibited large amount of collagen fibers between pancreatic lobules, around congested blood vessels and duct (Figure 18). Subgroup III b (withdrawal subgroup) revealed decrease in the amount of collagen fibers inbetween acini and surrounding ducts and blood vessels (Figure 19). Group IV (orlistat and B-carotene treated group) displayed minimal amount of collagen fibers in between pancreatic acini and around duct (Figure 20).

Regarding iNOS marker expression , pancreatic tissues obtained from groups I & II (contral and B- carotene treated groups) were similar and displayed negative cytoplasmic immune reactivity for iNOS (Figures 21,22). Subgroup III a (Orlistat treated subgroup) revealed strong positive immune reactivity in the acinar cytoplasm and cytoplasm of B- cells of islet of langerhans (Figure 23). While subgroupIII b(withdrawal subgroup) displayed weak positive cytoplasmic immune reactivity in acinar cells and negative reaction in cytoplasm of islets cells (Figure 24). Group IV (orlistat and B-carotene treated group) showed negative cytoplasmic immune reaction (Figure 25).

Regarding anti-insulin marker expression pancreatic tissues obtained from groups I&II (contral and B- carotene treated groups) were similar and revealed strong positive cytoplasmic immune reactivity in β -cells of islets of Langerhans (Figures 26,27). Subgroup III a (Orlistat treated subgroup) revealed apparent decrease in the immunostaining of in β -cells when compared with control group (Figure 28). Morever, subgroup III b (withdrawal subgroup) displayed strong positive cytoplasmic immune reaction for insulin in β -cells of islets of Langerhans (Figure 29). Group IV (orlistat and B-carotene treated group) showed moderate positive cytoplasmic immune reaction for insulin in β -cells of islets of Langerhans (Figure 30).

V- Transmission electron microscopic results

Ultra-thin pancreatic sections of Group I &II (control & B-carotene treated groups) revealed the acinar cells with euchromatic rounded basal nuclei with prominent nucleolus, multiple packed cisternae of rough endoplasmic reticulum (rER) occupying the base of the acinar cells. Many spherical homogenous electron dense zymogene granules occupied the apical part. The luminal surface was supplied by microvilli (Figures 31,33). The centroacinar cells had rounded euchromatic nuclei, lysosomes , mitochondria and junctional complexes between adjacent cells (Figure 32).

An electron microscopic examination of subgroupIII a (orlistat treated subgroup) revealed that most of the acini exhibited irregular shaped nucleus with dilated perinuclear space and dilated rER. Zymogen granules showed apparent decrease in number with variable sizes and densities (Figure 34). Dilated duct with retained secretion and irregularly shaped nuclei of the linning epithelium were noticed (Figure 35).

Examination of pancreatic specimens from sub group III b(withdrawal subgroup) displayed irregular shaped nuclei in some acinar cells, packed cisternae of rER, and apparent decrease in the number of zymogene granules (Figure 36).

While Group IV (Orlistat and B-carotene treated group) revealed that most of the acinar cells and their nuclei and organelles were nearly similar to those in the control group (Figure 37).

VI- Morphometric results

There was non-significant decrease in the mean intensity of the brown color of anti-insulin immune expression of β- cells of islets of Langerhans in B- carotene treated group (group II) and withdrawal subgroup (subgroup III b) ($p1$, $p3>0.05$) as compared to control group. There was highly significant decrease in the mean intensity of the brown color of anti-insulin immune expression in Orlistat treated subgroup (subgroup III a) and protected group ($p2$, $p4<0.001$) as compared to control group.

While, there was highly significant increase in withdrawal subgroup and protected group as compared to the treated group ($p5$, $p6<0.001$).The protected group showed significant decrease of the brown color of anti-insulin immune expression as compared to the withdrawal group ($p7<0.05$) (Table 3, Histogram 3).

Data in (Table 4) revealed non-significant increase in the mean number of secretory granules in B- carotene treated group (group II) ($p1>0.05$) as compared to control group. There was highly significant decrease in the mean number of secretory granules in Orlistat treated subgroup (subgroup III a) ($p2<0.001$) as compared to control group. There was significant decrease in the mean number of

secretory granules in the withdrawal subgroup (subgroup III b) ($p3<0.05$) as compared to control group. Also, there was highly significant increase as compared to the treated group ($p5<0.001$). The protected group (group IV) showed non- significant decrease in the mean number of secretory granules as compared to control group ($p4>0.05$), highly significant increase as compared to the treated group ($p6 <0.001$) and significant increase as compared to the withdrawal group ($p7<0.05$) (Histogram 4).

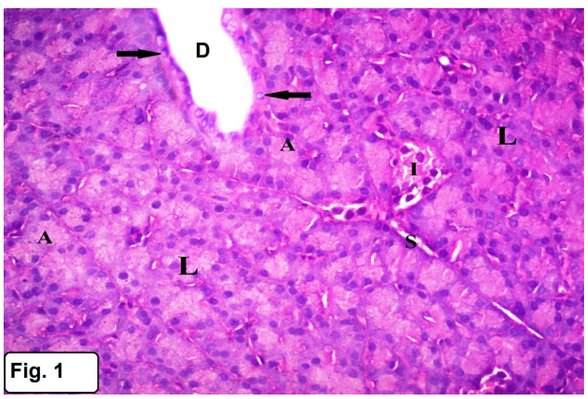


Fig. 1: A photomicrograph of a pancreatic section of control group (group I) showing pancreatic lobules (L)with closely packed pancreatic acini (A)and separated by thin septa (S). Intralobular duct(D) lined by cubical epithelium with rounded nuclei (arrows) is observed. Notice, Islet of Langerhans (I) with multiple nuclei. Hx. &E. ×400

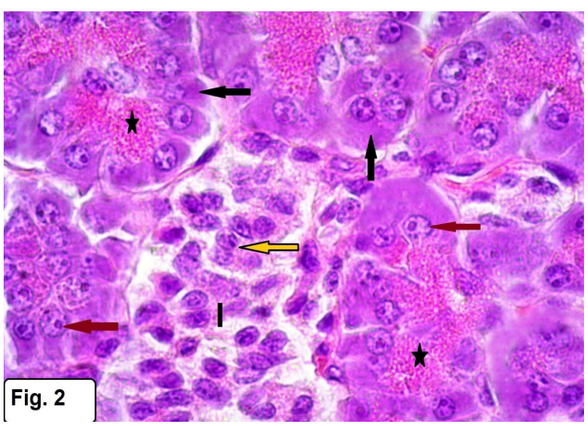


Fig. 2: A photomicrograph of a pancreatic section of control group (group I) showing pancreatic acini lined with pyramidal cells with apical acidophilic granules (*) and basal basophilia (black arrows) and having rounded vesicular nuclei with prominent nucleoli (red arrows). Islet of Langerhans (I) showing central cells (yellow arrow) with acidophilic cytoplasm and rounded vesicular nuclei.Hx. & E.×1000

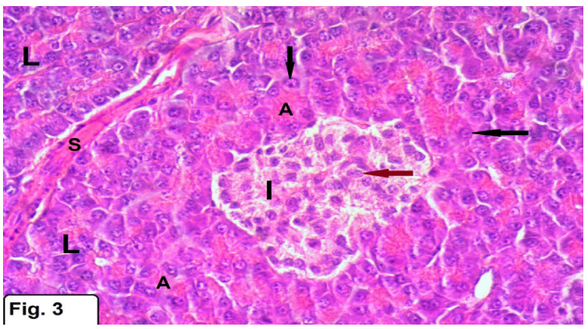


Fig. 3: Aphotomicrograph of a pancreatic section of B-Carotene treated group (group II) showing two pancreatic lobules (L) separated by thin septa (S) of connective tissue and contained closely packed pancreatic acini (A) with basal rounded vesicular nuclei (black arrows). Islet of Langerhans (I) appears normal with central cells (red arrow) having vesicular nuclei.Hx.&E. X400

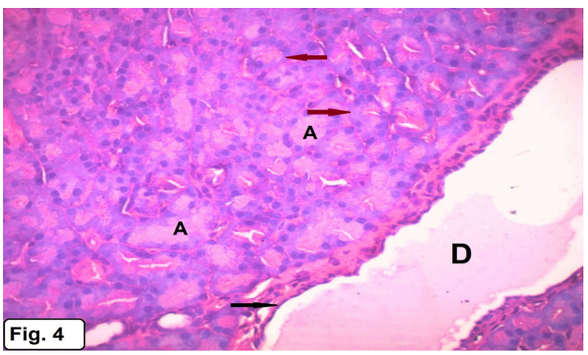


Fig. 4: A photomicrograph of a pancreatic section of orlistat treated subgroup (subgroup IIIa) showing cystic dilatation of the interlobular pancreatic duct (D) and flattening of its lining epithelium in some parts (black arrow) with retained secretion. The pancreatic acinar cells(A) appear with basal faint basophilic cytoplasm (red arrow). Hx.&E. X400

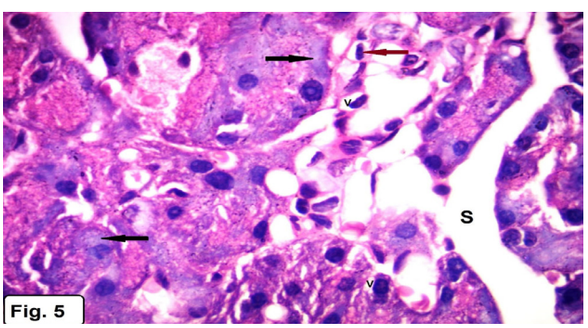


Fig. 5: A photomicrographs of a pancreatic section of orlistat treated subgroup (subgroup IIIa) showing pancreatic lobules separated by wide spaces (S). The acini are distorted with basal faint basophilic cytoplasm(black arrows). Some acinar cells appear with cytoplasmic vacuoles (V) and pyknotic nuclei (red arrow).Hx.&E. X1000

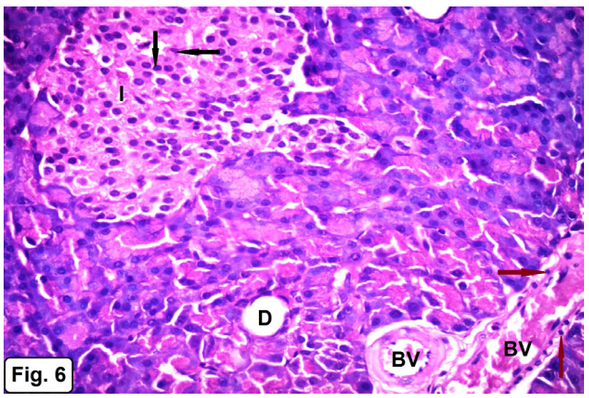


Fig. 6: A photomicrograph of a pancreatic section of orlistat treated subgroup (subgroup IIIa) showing an Islet of Langerhans (I) . The islet cells appear with deeply acidophilic vacuolated cytoplasm and dark pyknotic nuclei (black arrows). congested blood vessels (BV) with inflammatory cellular infilterate (red arrows) and intralobular duct (D) are observed. Hx. & E.×400

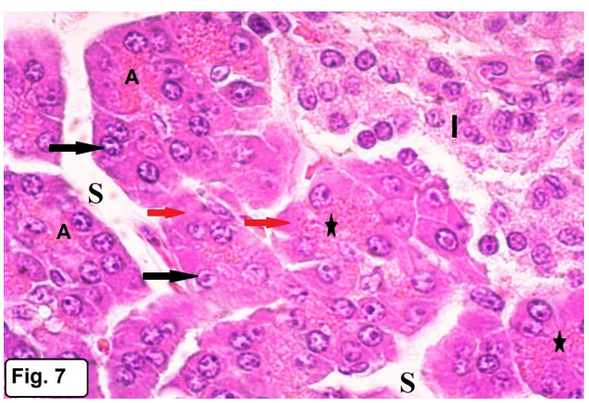


Fig. 7: A photomicrograph of a pancreatic section of withdrawal subgroup (subgroupIIIb) showing pancreatic acini (A) with apical acidophilic granules (*) and basal rounded vesicular nuclei (arrows) , Islet of Langerhans (I) with multiple nuclei appear normal. Notice: disappearance of basal basophilia in most of acini(red arrows) and the presence of wide spaces inbetween them (S). Hx.&E. X1000

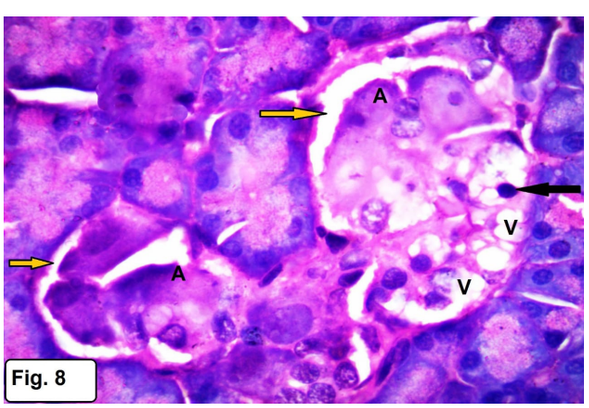


Fig. 8: A photomicrograph of a pancreatic section of withdrawal subgroup (subgroupIIIb) showing distorted degenerated acini (A). The acinar cells showed multiple intra cellular vacuoles (V) and pyknotic nuclei (black arrow).Note, disattachment of the acinar cells from the underlying basement membrane (yellow arrows) Hx. &E. ×1000

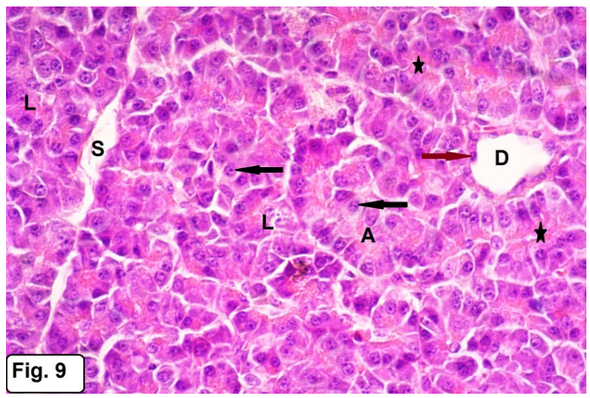


Fig. 9: A photomicrograph of a pancreatic section of rats treated with orlistat and B-Carotene (group IV) showing thin septa (S) separating pancreatic lobules (L) which contain closely packed pancreatic acini (A) with apical acidophilia (stars) and basal rounded vesicular nuclei (black arrows). Intralobular duct (D) lined by cubical epithelium with rounded nuclei (red arrow) is observed. Hx. & E×400

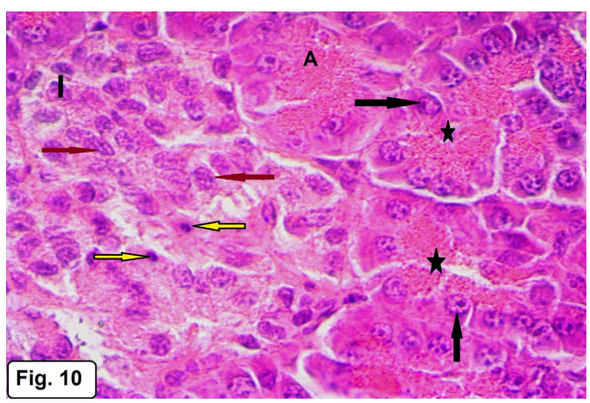


Fig. 10: A photomicrograph of a pancreatic section of orlistat and B-Carotene treated group (group IV) showing pancreatic acini (A) with apical acidophilic granules (*) and basal rounded vesicular nuclei (black arrows). Islet of Langerhans (I) appear with multiple cells having pale nucleus and pale cytoplasm (red arrows) . Cells with small pyknotic nuclei in the center of islet are observed (yellow arrows). Hx. &E. ×1000

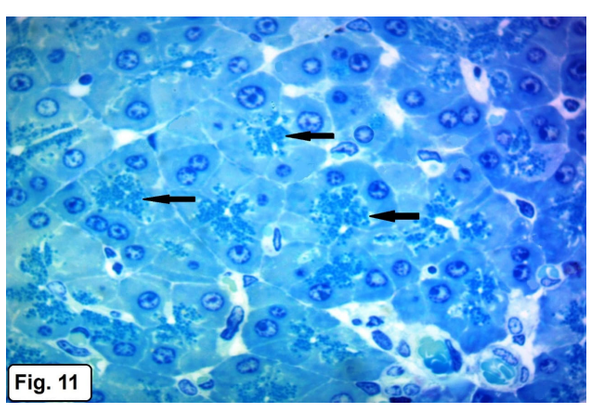


Fig. 11: A photomicrograph of a semithin section of a pancreas of control group (group I) showing abundant apical zymogen granules (blue dots) of the pancreatic acinar cells (arrows). Toluidine blue X400

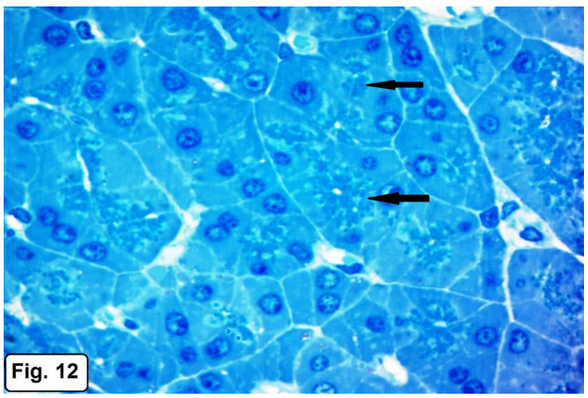


Fig. 12: A photomicrograph of a semithin section of pancreas of B-carotene treated group (group II) showing abundant apical zymogen granules of the acinar cells (arrows). Toluidine blue X400

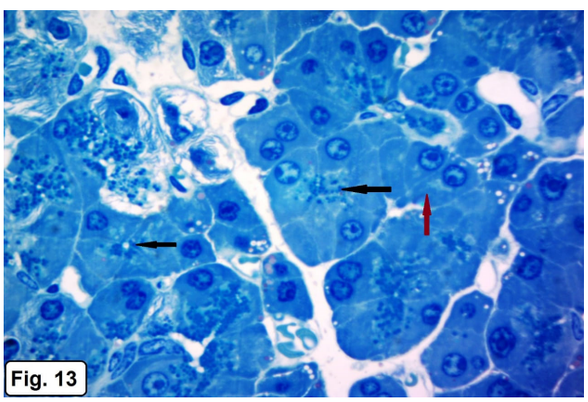


Fig. 13: A photomicrograph of a semithin section of a pancreas of Orlistat treated subgroup (subgroup III a) showing apparent decrease in zymogen granules in some acinar cells (black arrows) and absence of these granules in others (red arrow). Toluidine blue X400

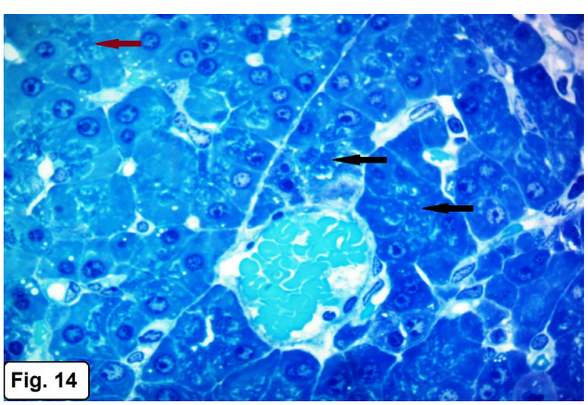


Fig. 14: A photomicrograph of a semithin section of a pancreas of withdrawal subgroup (subgroup IIIb) showing abundant apical zymogen granules in most of the pancreatic acinar cells (black arrows) apparent decrease in zymogen granules in some acinar cells (red arrow). Toluidine blue X400

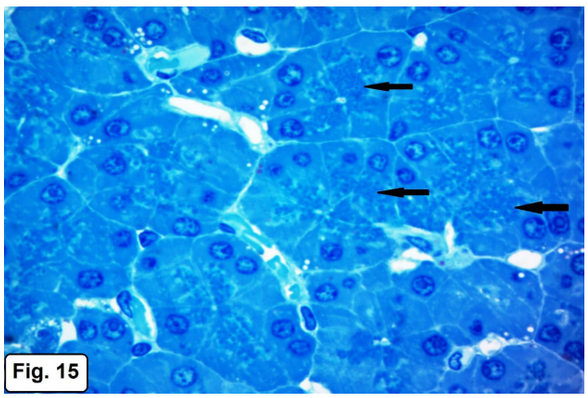


Fig. 15: A photomicrograph of a semithin section of a pancreas of Orlistat and B- carotene treated group (group IV) showing abundant apical zymogen granules in the pancreatic acinar cells (arrows). Toluidine blue X400

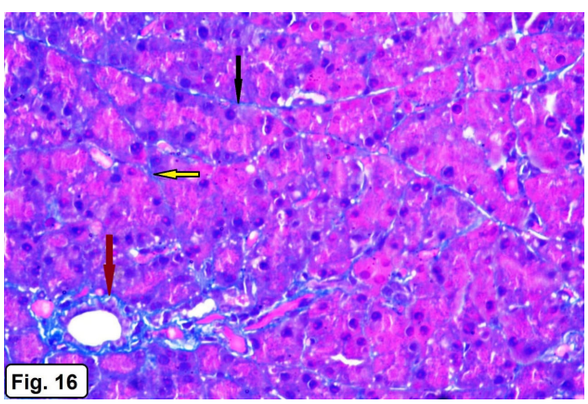


Fig. 16: A photomicrograph of a pancreatic section of control group (group I) showing minimal amount of collagen fibers in the connective tissue septa (black arrow) and around pancreatic duct (red arrow)and acini (yellow arrow). Mallory trichrome X400

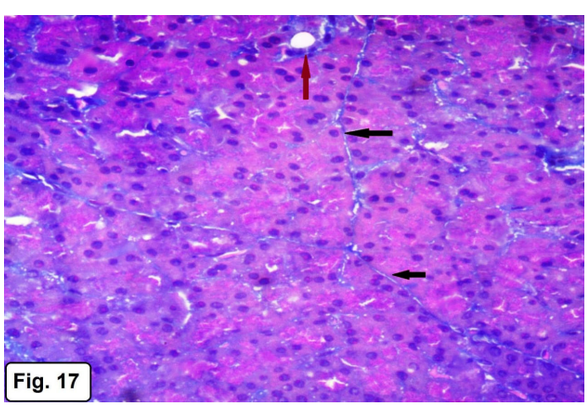


Fig. 17: A photomicrograph of a pancreatic section of B-carotene treated group (group II) showing minimal amount of collagen fibers in the connective tissue septa (black arrows) and around pancreatic duct (red arrow) . Mallory trichrome X400

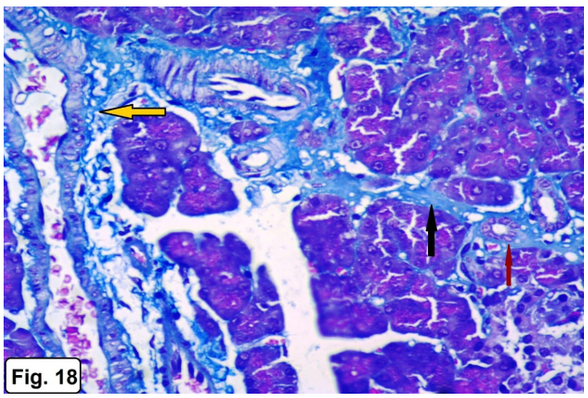


Fig. 18: A photomicrograph of a pancreatic section of Orlistat treated subgroup (subgroup IIIa) showing large amount of collagen fibers between pancreatic lobules (black arrow) , around duct (red arrow) and congested blood vessels (yellow arrow). Mallory trichrome X400

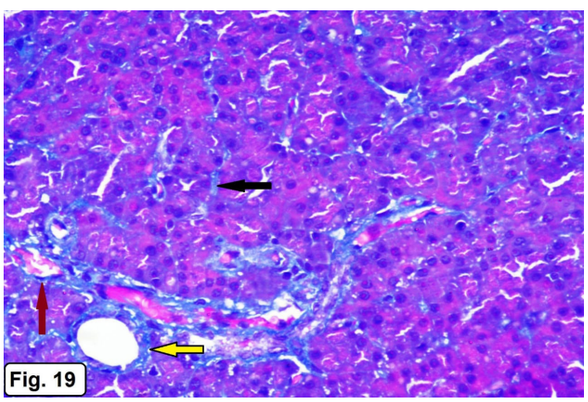


Fig. 19: A photomicrograph of a pancreatic section of withdrawal subgroup (subgroup IIIb) showing moderate amount of collagen fibers around pancreatic acini (black arrow), congested blood vessel (red arrow) and duct(yellow arrow). Mallory trichrome X400

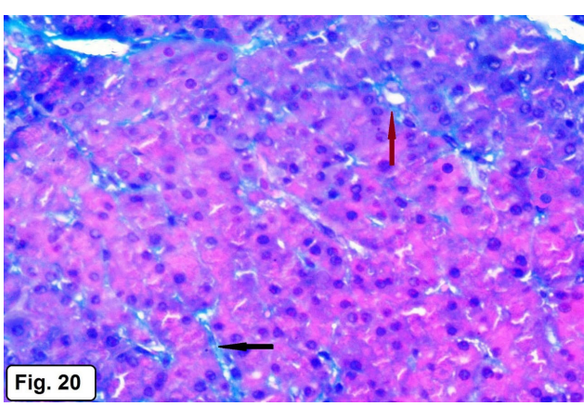


Fig. 20: A photomicrograph of a pancreatic section of rats treated with orlistat and B-Carotene (group IV) showing minimal amount of collagen fibers around pancreatic acini (black arrow) and duct (red arrow). Mallory trichrome X400

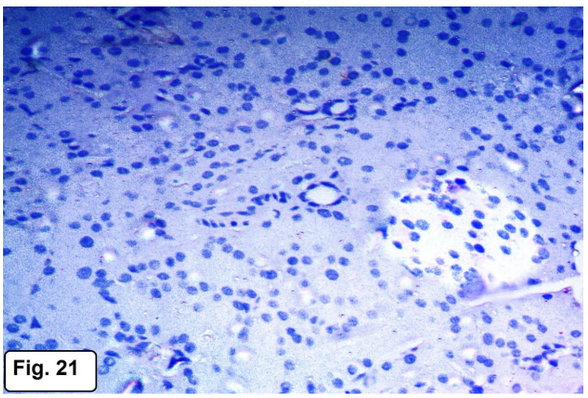


Fig. 21: A photomicrograph of a pancreatic section of control group (group I) showing negative cytoplasmic immune reaction for iNOS. iNOS X400

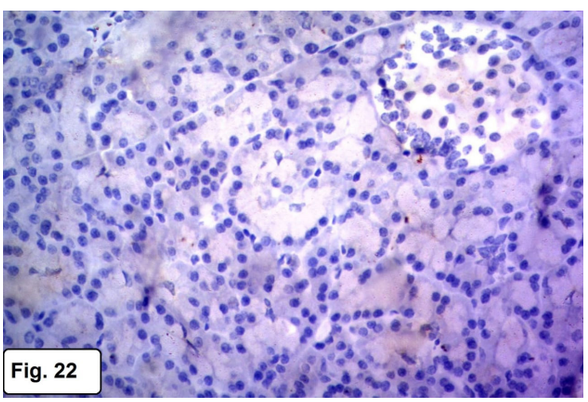


Fig. 22: A photomicrograph of a pancreatic section of B- carotene treated group (group II) showing negative cytoplasmic immune reaction for iNOS. iNOS X400

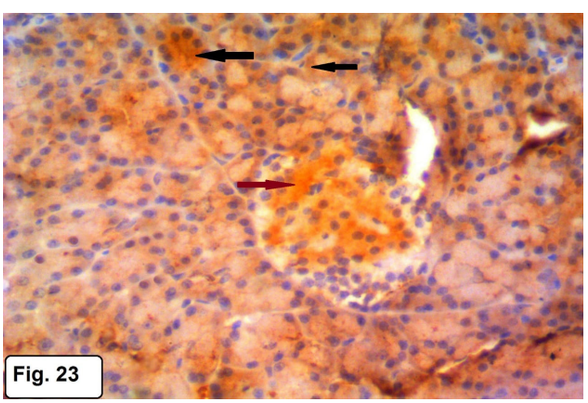


Fig. 23: A photomicrograph of a pancreatic section of Orlistat treated subgroup (subgroup IIIa) showing strong positive immune reaction for iNOS in the cytoplasm of the acinar cells (black arrows) and cytoplasm of islets cells (red arrow). iNOS X400

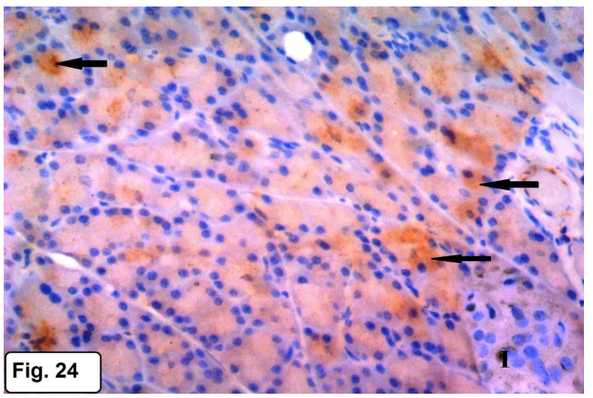


Fig. 24: A photomicrograph of a pancreatic section of withdrawal subgroup (sub group IIIb) showing moderate positive immune reaction for iNOSin the cytoplasm of the acinar cells (black arrows) and negative reaction in cytoplasm of islets cells (I). iNOS X400

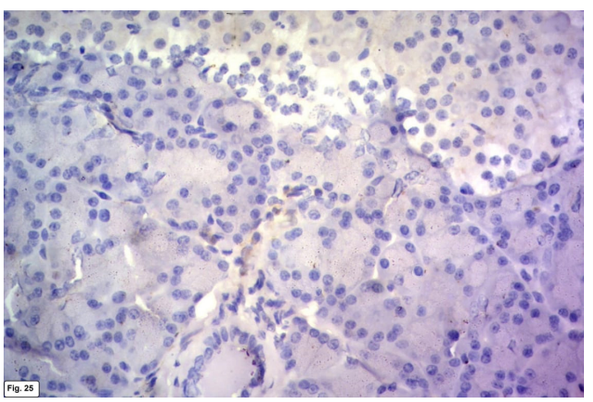


Fig. 25: A photomicrograph of a pancreatic section of Orlistat and B-carotene treated group (group IV) showing negative cytoplasmic immune reaction for iNOS. iNOS X400

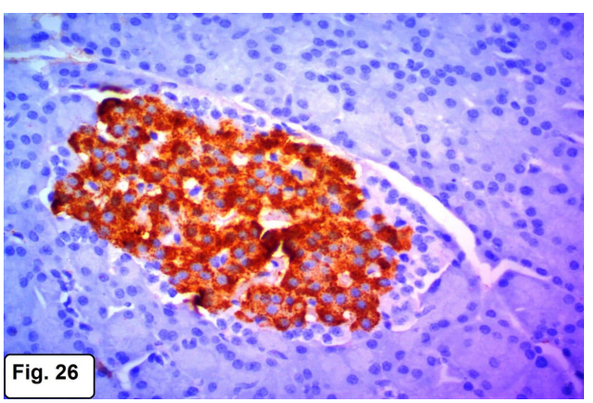


Fig. 26: A photomicrograph of a pancreatic section of control group (group I) showing strong positive cytoplasmic immune reaction for insulin in ß-cells of islets of Langerhans. Anti-insulin Immunostaining x400

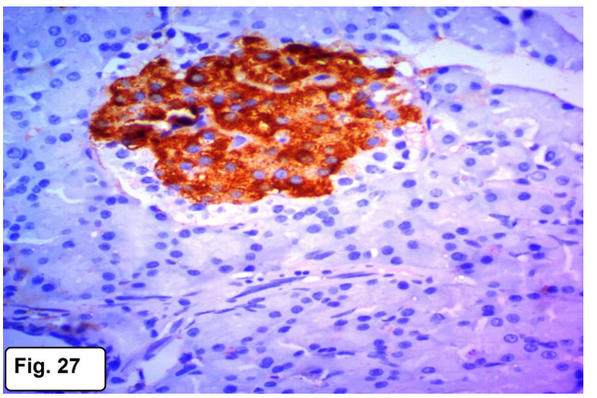


Fig. 27: A photomicrograph of a pancreatic section of B-carotene treated group (group II) showing strong positive cytoplasmic immune reaction for insulin in ß-cells of islets of Langerhans. Anti-insulin Immunostaining x400

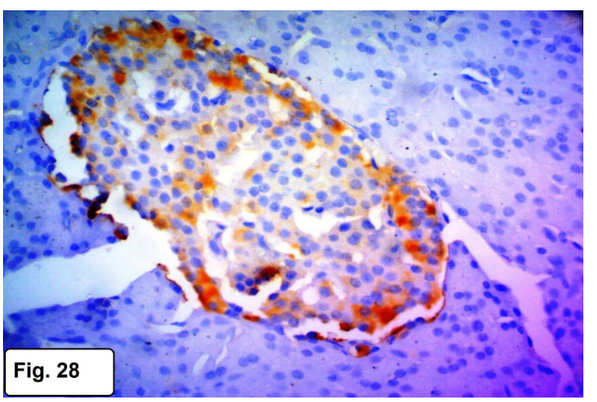


Fig. 28: A photomicrograph of a pancreatic section of Orlistat treated group (subgroup III a) showing apparent decrease in the immunostaining of ß-pancreatic cells. Anti-insulin Immunostaining x400

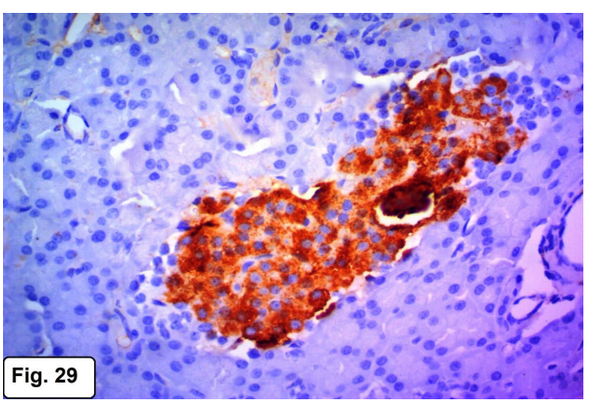


Fig. 29: A photomicrograph of a pancreatic section of withdrawal subgroup (subgroup IIIb) showing strong positive immune reaction for insulin in ß-cells of islets of Langerhans. Anti-insulin Immunostaining x400

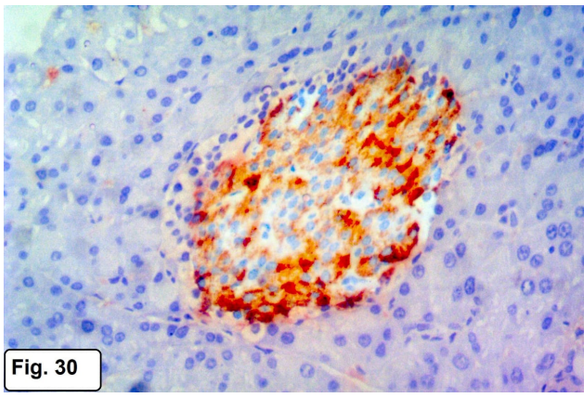


Fig. 30: A photomicrograph of a pancreatic section of Orlistat and B-carotene treated group (group IV) showing moderate positive immune reaction for insulin in ß-cells of islets of Langerhans. Anti-insulin Immunostaining x400

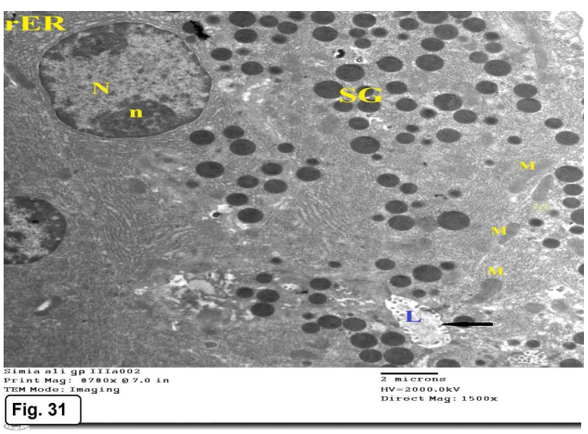


Fig. 31: An electron micrograph of ultrathin section of a pancreas of control group (group I) showing pancreatic acinar cells with basal rounded euchromatic nuclei(N) having prominent nucleolus (n), well developed packed cisternae of rough endoplasmic reticulum (rER), apical electron dense secretory granules(SG), and mitochondria (M). Note, the lumen (L) with few microvilli (arrow). (TEM. ×1500)

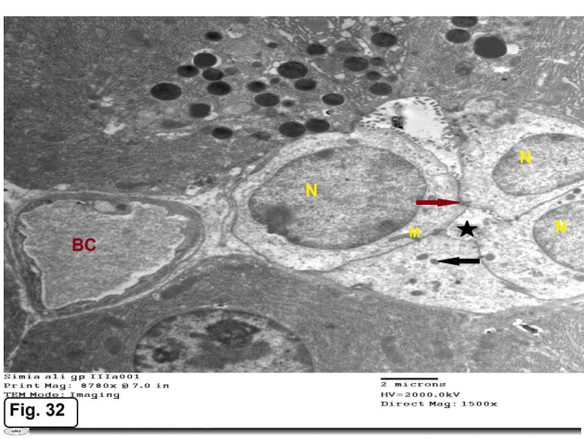


Fig. 32: An electron micrograph of ultrathin section of a pancreas of control group (group I) showing four centroacinar cells around the lumen(*). The centroacinar cells have rounded euchromatic nuclei (N), lysosomes (black arrow) , mitochondria (M) and junctional complexes between adjacent cells (red arrow). Note, the blood capillary (BC). (TEM. ×1500)

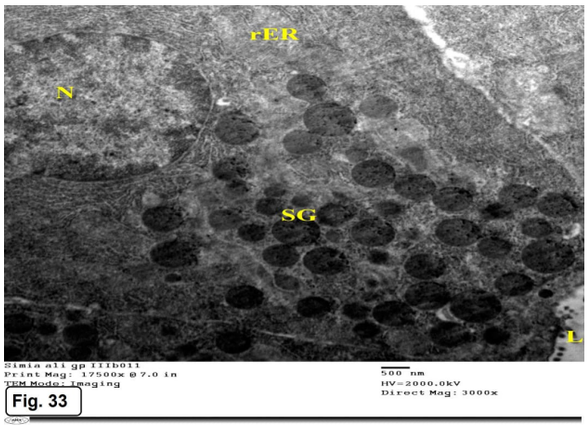


Fig. 33: An electron micrograph of ultrathin section of a pancreas of B-Carotene treated group (group II) showing acinar cell with euchromatic nucleus (N), well developed rough endoplasmic reticulum cisternae (rER), apical electron dense secretory granules (SG) and lumen (L) with few microvilli. (TEM. × 3000)

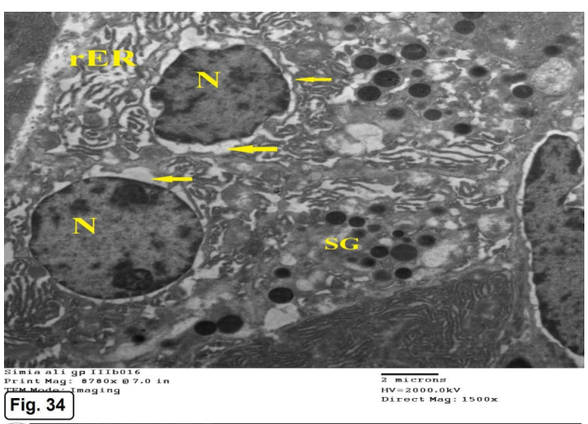


Fig. 34: An electron micrograph of ultrathin section of a pancreas of Orlistat treated group (subgroup III a) showing pancreatic acinar cells with irregularly shaped nucleus (N), dilated perinuclear space (arrows), dilated cisternae of rough endoplasmic reticulum (rER), apparent decrease in the number of electron dense secretory granules (SG) with different sizes and denisties. (TEM. ×1500)

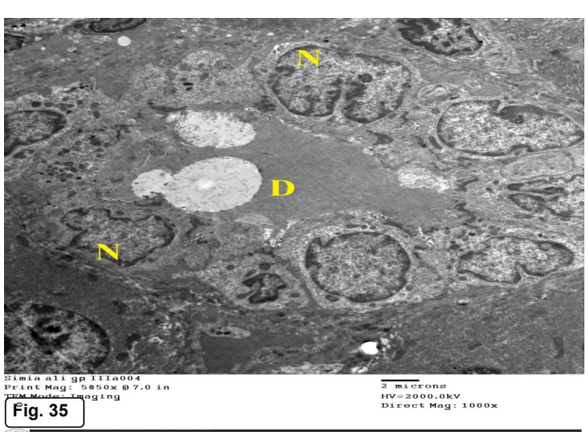


Fig. 35: An electron micrograph of ultrathin section of a pancreas of Orlistat treated subgroup (subgroup IIIa) showing dilated duct with retained secretion (D). Its lining epithelium showed irregular shaped nuclei (N). (TEM.× 1000)

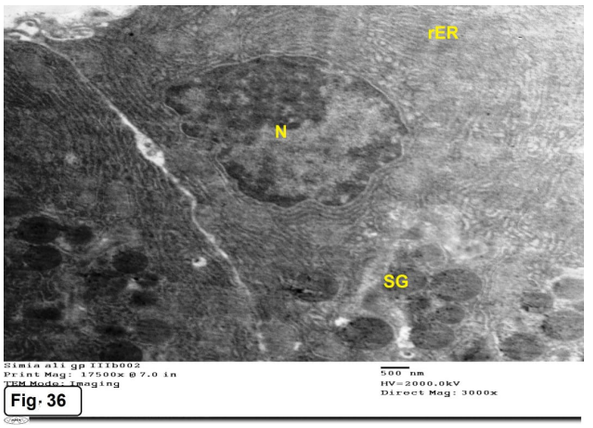


Fig. 36: An electron micrograph of ultrathin section of pancreas of withdrawal subgroup (subgroup IIIb) showing pancreatic acinar cell with irregular shaped nucleus (N), packed cisternae of rough endoplasmic reticulum (rER) and less electron dense granules (SG). (TEM. ×3000)

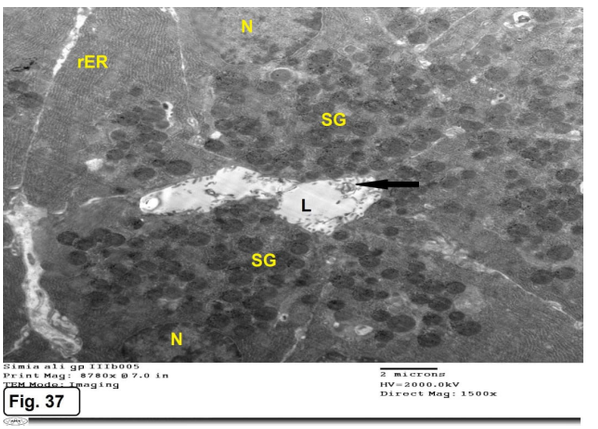


Fig. 37: An electron micrograph of ultrathin section of a pancreas of Orlistat and B- carotene treated group (group IV)showing pancreatic acinar cells surrounding lumen (L) with few short microvilli (arrow). The cells have basal euchromatic nuclei (N), rough endoplasmic reticulum cisternae (rER) and multiple apical electron dense secretory granules(SG). (TEM. X1500)

Table 1: Statistical means of body weights (gm) of various experimental groups

Group	Mean ± SD	Test of significance (T- test)	*P- value*
Group I (Control)	181.5 ± 4.5		
Group II (B-carotene)	183.1 ± 4.5	0.807	P1= 0.433
Subgroup III a (Orlistat)	158.6 ± 4.2	13.795	P2= 0.000
Subgroup III b (withdrawal)	172.7 ± 5.8	3.940	P3= 0.001
Group IV (Protected)	162.3 ± 4.7	10.619	P4=0.000
Subgroup III b versus subgroup III a		6.692	P5=0.000
Group IV versus subgroup III a		1.887	P6= 0.079
Group IV versus subgroup III b		4.615	P7= 0.000

P1, P2, P3, P4: compared to control group
P value > 0.05 = Non significant
P value <0.05= significant
P value <0.001 = highly significant

Table 2: Statistical means of blood glucose level (mg⁄dl) of various experimental groups

Group	Mean ± SD	Test of significance (T- test)	*P- value*
Group I (Control)	113.5 ± 3.6		
Group II (B-carotene)	114.1 ± 3.3	0.389	P1= 0.703
Subgroup III a (Orlistat)	177.5 ± 8.2	29.5	P2= 0.000
Subgroup III b (withdrawal)	114.8 ± 3.8	0.793	P3= 0.440
Group IV (Protected)	154.6 ± 4.5	29.265	P4= 0.000
Subgroup III b versus subgroup III a		28.534	P5= 0.000
Group IV versus subgroup III a		8.566	P6= 0.000
Group IV versus subgroup III b		27.577	P7= 0.000

P1, P2, P3, P4: compared to control group
P value > 0.05 = Non significant
P value <0.05= significant
P value <0.001 = highly significant

Table 3: The means of intensity of insulin immune expression

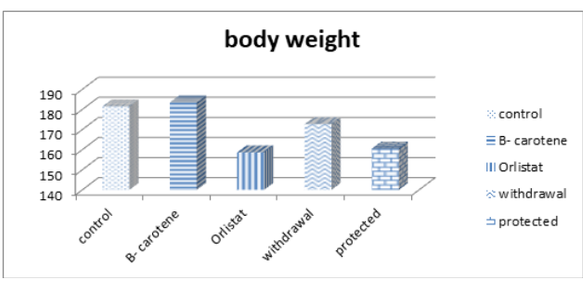
Group	Mean ± SD	Test of significance (T- test)	*P- value*
Group I (Control)	33.2 ± 1		
Group II (B-carotene)	32.3 ± 0.4	1.782	P1 = 0.095
Subgroup III a (Orlistat)	12.8 ± 0.8	14.349	P2= 0.000
Subgroup III b (withdrawal)	32.1 ± 0.7	2.008	P3= 0.063
Group IV (Protected)	28.6 ± 1.5	4.476	P4= 0.000
Subgroup III b versus subgroup III a		5.314	P5= 0.000
Group IV versus subgroup III a		10.315	P6= 0.000
Group IV versus subgroup III b		3.850	P7= 0.002

P1, P2, P3, P4: compared to control group
P value > 0.05 = Non significant
P value <0.05= significant
P value <0.001 = highly significant

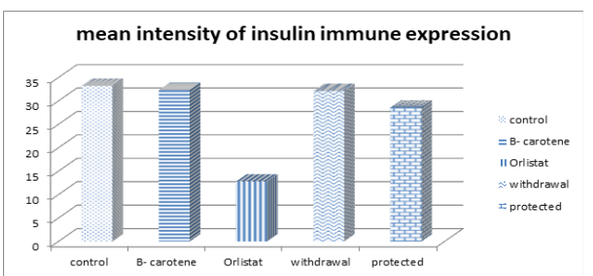
Table 4: The means of number of secretory granules

Group	Mean ± SD	Test of significance (T- test)	*P- value*
Group I (Control)	51.2 ± 6.7		
Group II (B-carotene)	54.6 ± 7.6	0.736	P1 = 0.473
Subgroup III a (Orlistat)	18.6 ± 2.7	6.643	P2= 0.000
Subgroup III b (withdrawal)	36.6 ± 4.9	3.353	P3= 0.004
Group IV (Protected)	50.2 ± 6.3	0.240	P4= 0.813
Subgroup III b versus subgroup III a		5.260	P5= 0.000
Group IV versus subgroup III a		6.752	P6= 0.000
Group IV versus subgroup III b		3.265	P7= 0.005

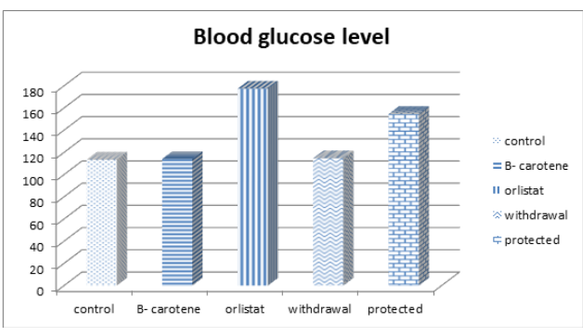
P1, P2, P3, P4: compared to control group
P value > 0.05 = Non significant
P value <0.05= significant
P value <0.001 = highly significant



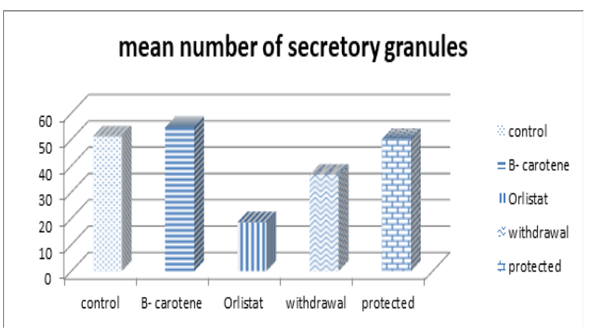
Histogram 1: Statistical means of body weights (gm) of various experimental groups



Histogram 3: The mean intensity of insulin immune expression



Histogram 2: statistical means of blood glucose level (mg/dl) of various experimental groups



Histogram 4: The mean number of secretory granules

DISCUSSION

Increased intake of unhealthy food coupled with a sedentary lifestyle is contributing to the increased prevalence of obesity all over the world[23]. Orlistat has been introduced at the end of 1998 and represented as a magic medicine for obesity without pain of dieting. The drug is licensed for patients with BMI >28 kg/m2 but many adverse effects were observed such as pancreatitis[24]. Therefore this study was undertaken to study the pancreatic microscopic changes induced by Orlistat in adult female albino rats and the possible protective effect of ß-carotene as antioxidant agent.

The present study revealed a significant decrease in body weight of animals treated with Orlistat as compared to the control group. This was in agreement with Galaly *et al*.[25] who attributed this decrease in body weight to orlistat blocking effect to the absorption of fat by inhibiting gastric and pancreatic lipase enzymes leading to the increased excretion of fat in faeces.

This study demonstrated that administration of Orlistat led to a significant increase in blood glucose level together with weak immune reaction to anti insulin monoclonal antibody and positive cytoplasmic reaction to iNOS in islet cells as compared to control group. This was in agreement with Abdelwahab *et al*.[6] in their study on the effect of orlistat on pancreas, Immunohistochemical detection of insulin in rat β- cells of islets of Langerhans using anti-insulin antibody was done and found that administration of orlistat down-regulates insulin production.

The explanation of these changes is that increased expression of islet iNOS causes excessive NO production and contributes to the dysfunction of β -cells and inhibits insulin secretion leading to hyperglycemia[26]. Also, Lipases are involved in insulin secretion; pharmacological inhibition of lipase activity by orlistat impairs insulin secretion and this also explained the decrease in the immunostaining of ß pancreatic cells in the protected group (group IV). Different preparations of ß-cells exhibit lipase activities. Among these is the hormone sensitive lipase (HSL), which, in white adipocytes, is the critical enzyme that hydrolyzes triglycerides to fatty acid. Reduction of lipolysis activity inhibited ß-cells insulin secretion[27].

In this study, examination of pancreatic sections of orlistat-treated group by light microscope showed disorganized architecture of the pancreas. Distorted acini with faint basal basophilia, small pyknotic nuclei, intracytoplasmic vacuolization, decreased zymogen granules and inflammatory cellular infiltrate were detected. Edema and dilatation and congestion of blood vessels were also observed. The islets of Langerhans in Orlistat treated group appeared normal but few cells appeared deeply acidophilic vacuolated cytoplasm and pyknotic nuclei.

Similar changes were observed by Elbakary and Bayomy[28] and Abdelwahab *et al*.[6] in pancreas after orlistat administration.

These findings could be attributed to the accumulation of free radicals and aggrevation of oxidative stress in the body by orlistat. This was supported by the result of inducible nitric oxide synthase (iNOS) immunostaining, which illustrated strong positive reaction of iNOS in orlistat-treated rats. This finding was reported by many other researchers who found that nitric oxide (NO) and other free radicals play roles in oxidative stress aggravation and promote pathogenesis of pancreatitis[29,30].

Inducible NOS (i NOS) generates a huge amount of NO. Excess NO causes both local and systemic vasodilation and may lead to refractory hypotension and these changes initiate anaerobic metabolism. In addition, NO produces peroxynitrite when react with superoxide, peroxynitrite is a powerful cytotoxic agent that may play a critical role in cell damage[31].

Inducible NOS activation produces apoptosis mediators, such as interferon-g. Also production of NO involves in accumulation of the tumour suppressor protein (p53), modulation in the expression of Bcl-2 family members, activation of the caspase cascade, and DNA fragmentation[32]. These effects may explain the cytoplasmic and nuclear structural changes detected in orlistat treated group of this study.

Dilated ducts with retained secretion were observed in orlistat treated group of this study. This result was observed by others as[6] who explained these changes as a sign of tissue injury and cellular dysfunction.

The thickened connective tissue septa observed in Mallory trichrome stained sections of orlistat treated group is due to reactive oxygen species with lipid peroxidation products which can lead to activation of a cascade leading to fibrosis and collagen deposition as reported by Atiq *et al*.[33] in their study of Amiodarone induced liver cirrhosis.

In this study, Electron microscopic results of Orlistat treated group confirmed the light microscopic results. Some nuclei appeared irregular in shape with dilated perinuclear space; it was previously reported by Attiya[13] in her study on streptozotocin induced diabetes. She postulated these changes to condensation and shrinkage of the nuclear material.

Moreover, dilated rough endoplasmic reticulum was observed. This was in agreement with Youssef[34] who detected this in hepatocytes of orlistat treated rats. He reported that the endoplasmic reticulum was especially vulnerable to the free radical attack, because it is considered as a radical producing site. Also, its membranes are rich in polyunsaturated fatty acids which are sensitive to free radical attack.

In the present study, some acinar cells of the treated group showed a significant decrease in the number of zymogen granules with variation in their sizes and densities. Such finding was in agreement with Elbakary and Bayomy[28]. who reported that the number of zymogene granules was

diminished and some of these granules showed peripheral dissolution or even were entirely empty. This may be explained by a defect in the synthesis of submembraneous matrix which leads to a defect in adhesion of the granular content to its surrounding membrane[35].

The present study revealed that most of these findings were reversible after cessation of Orlistat as observed in the results of the blood chemistry analysis and the histopathological study of the pancreatic tissue of animals in the withdrawal group. This is supported by other histological studies[6,28]. And in accordance with case reports of many patients who recovered and left the hospitals[36].

However, the present work proved that co-administration of ß-carotene with orlistat showed noticeable protection of the pancreatic tissue against the hazardous effects of Orlistat on pancreatic sections of rats. This was confirmed by histological, immunohistochemical and electron microscopic results. This was in agreement with Youssef[34]. who attributed that effect to the antioxidant ability of ß-carotene.

The antioxidant effect of carotenoids may be attributed to the presence of long chains of conjugated double bonds which allows chelation of oxygen-free radicals and dissipation of their energy. The chelation of free radicals inhibits the peroxidation of lipids[37].

Hence, it was concluded that orlistat caused marked histological, ultrastructural and immunohistochemical changes in the pancreas of adult female albino rats indicating pancreatitis, however co-administration of B-carotene attenuates these changes indicating its antioxidant effect. So, the use of orlistat alone should be avoided or minimized as possible, however coadministration of B-Carotene with orlistat is recommended in necessary cases to reduce the serious effects of orlistat on pancreas.

CONFLICT OF INTERESTS

There are no Conflicts of Interest.

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الملخص العربى

التغيرات الهستولوجية للبنكرياس في اناث الجرذان البيضاء البالغة المعالجة بعقار الاورليستات والدور الوقائي المحتمل لبيتا- كاروتين

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المقدمة: الاورليستات هو مثبط للانزيمات المحللة للدهون مرخص لعلاج السمنة. يعتبر دواء آمن للاستخدام على المدى الطويل ولكن قد لوحظ له بعض الاثار الجانبية مثل التهاب البنكرياس . بيتا كاروتين هو احد مصادر فيتامين أ والذي له تأثير مضاد للاكسدة والالتهابات كما ان له تاثير تحسيني للمناعة.

الأهداف: الهدف من هذه الدراسة هو توضيح التغيرات التركيبية للبنكرياس بعد اعطاء الأورليستات والدور الوقائي المحتمل لبيتا كاروتين في اناث الجرذان البيضاء البالغة.

لمواد والطرق: تم تقسيم خمسين من اناث الجرذان البيضاء البالغة الى مجموعات: أول ١٠ فئران كانت المجموعة الضابطة.المجموعة الثانية (١٠) تم اعطاء الفئران البيتا كاروتين بجرعة (٠,٥٢ مجم / كجم / يوم)عن طريق الفم لمدة ٥ اسابيع.المجموعة الثالثة (٢٠ فأر) تم اعطائهم جرعة علاجية من الاورليستات(٣٢ مجم/كجم/يوم) مذابة في ١ مل الماء المقطر عن طريق الفم لمدة ٥ أسابيع ثم تم ذبح ١٠ فئرا وترك ١٠ فئران اخرى ل ٥ اسابيع اخرى.المجموعة الرابعة (١٠ فئران) تم اعطائهم البيتا كاروتين بجرعة (٠,٥٢ مجم / كجم / يوم)عن طريق الفم قبل ساعة واحدة من تناول جرعة أورليستات ٣٢ مجم / كجم / يوم لمدة ٥ أسابيع.بعد نهاية المدة تم ذبح الفئران تم الحصول على عينات من الدم وتم تشريح البنكرياس وإعداده لدراسة الهستولوجية والهستوكيميائية المناعية.

النتائج: أظهرت الفئران المعالجة بالأورليستات انخفاضا كبيرا للغاية في وزن الجسم وزيادة كبيرة للغاية في مستوى السكر في الدم. كانت هناك علامات على تثبيط نشاط خلايا بيتافي الدراسة الهستوكيميائية المناعية ضد الانسولين. ولوحظت التغيرات المدمرة لخلايا الحويصلات البنكرياسية مع انخفاض ملحوظ في الحبيبات الإفرازية. تم تأكيد ذلك من خلال الفحص المجهري الالكتروني والدراسة الهستوكيميائية المناعية. كانت هذه التغيرات قابلة للتحسن كما هو موضح ف مجموعة السحب كما اظهر بيتا كاروتين حماية للحويصلات البنكرياسية وجزر لانجرهانز

الاستنتاج: بيتا كاروتين له تأثير تحسيني كبير على البنكرياس ضد الآثار الضارة لأورليستات.

لذا ينصح بتجنب استخدام الاورليستات او التقليل من تعاطيه واذا لزم الأمر ينصح باستخدام البيتا كاروتين معه لتقليل الضرر المحدث على البنكرياس