Protective effect of Octreotide on histological and ultrastructural changes in the exocrine pancreas of albino rats induced by L-asparaginase (Elspar)

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Abstract: Hypothesis: this study aimed to assess the effect of L- asparaginase (ASNase) on rat pancreatic acinar cells and then investigated the preventive effects of the drug (Octreotide) one of the synthetic somatostatin analogue as a prophylactic drug against ASNase –induced pancreatic injury in rats. **Material and methods**: There were 7 groups of rats, control group (group1) received intraperitoneal (i.p) normal saline, experimental groups (II, IV, VI) received i.p. of ASnase at different doses 200, 500, and 1000 IU respectively for (5 days total time of experiment). The protective groups (III, V, VII) received octreotide (10µg/Kg/day) subcutaneously 8 hours before the ASNase different doses for 5 days. **Results:** The apparent histopathological changes observed lightly and ultrastructurally in rat pancreatic acinar cells compared to control rats were increase accumulation of apical zymogen granules of variable size and shape in acinar cells, edema, vacuolization which extend to apical portions of cells, congestion and dilatation of blood vessels and further damage in the form of focal areas of necrosis of pancreatic tissue with inflammatory infiltration of mononuclear cells mainly lymphocytes also some degree of interlobular fibrosis was detected with doses 500 and 1000 IU but minimal changes with dose 200IU. Biochemically there is significant increase of pancreatic amylase enzyme above the upper normal level in rats given 500IU ASNase group IVwhich obviously decrease after giving octreotide, no significant change in group II (ASNase 200IU),while in group VI (ASNase 1000IU) a slight non significant decrease was observed compared to control group which was slightly improved in group VII after giving octreotide. There was no significant difference between the control group and experimental groups for the serum lipase enzyme. However, Octreotide injection provided protection against histological damage manifested by disappearance of cytoplasmic vacuolation and restoration of more regular acinar pattern and the pancreatic enzymes remained within normal limits. **Conclusion** Although ASNase by itself did not cause pancreatitis, it did cause increased levels of pancreatic enzymes and histological damage to the pancreas associated with pancreatic injury or pre-pancreatitis in experimental animals. In this study prior treatment with octreotide prevented the development of ASNase-induced pancreatic injury, although previous clinical studies have established the usefulness of octreotide in the management of L-asparaginase induced pancreatitis and pseudocyst, they did not examine prophylactic application.

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1.Introduction

Leukemia is cancer of the blood and develops in the bone marrow. There are different types of leukemia according to the American Cancer Society; acute lymphocytic leukemia (ALL) is the type of leukemia that most commonly affects children with an incidence of 3-5 per 100.000 children in Europe and the US (Hjalgrim et al, 2004).

L-asparaginase has been an element in the treatment for acute lymphoblastic leukaemia (ALL) and non-Hodgkin lymphoma (NHL) since the late 1960s and remains an essential component of their combination chemotherapy protocols (Raja et al,2012)

Based on this, it has also been included in most contemporary, multi-agent regimens for adult ALL (Gokbuget Hoelzer, 2002).

L-Asparaginase as a drug has demonstrated effectiveness in induction and subsequent phases of various chemotherapeutic strategies. The incorporation of L-asparaginase has significantly improved event-free survival in children with ALL and NHL, and failure to complete planned L-asparaginase treatment has been linked to increased relapse rate (Silverman et al, 2001).

L-asparaginase primarily targets malignant lymphoblasts by depletion of the external sources of

asparagines, through hydrolysis of asparagines to aspartic acid and ammonia, given the most malignant cells having that limited asparagines synthetase activity, a resulting lack of asparagines which leads to apoptosis and malignant cell deaths (Duval et al, 2002, Van den Berg, 2011). Furthermore, L-asparaginase exhibits glutaminase activity which may contribute tothe cytotoxicity of this drug (Offman et al, 2011).

Asparaginases are associated with a unique set of side effects, hypersensitivity reactions, due to antiasparaginase antibody production, have been observed in up to 60% of patients at some time during E. coli asparaginase therapy compared with the pegylated enzyme (pegasparaginase) (Avramis et al., 2002).

Apart from immunological reactions toward the foreign macromolecule, healthy tissue was also frequently affected.

Hepatotoxic effects of E.coli asparaginase are well documented in both human and animal systems **(**Canellos et al, 1969) as the mammalian liver is the organ primarily responsible for the homeostatic regulation of asparagine, coagulation abnormalities and thromboembolic complications due to impairment of protein synthesis (Gugliotta et al, 1992), gastrointestinal system **(**Pratt et al, 1971), reversible hypertriglyceredemia (Hoogerbrugge et al, 1997), central nervous system disorders (Leonard and Key, 1986) were also observed.

Asparaginase-associated pancreatitis (AAP) is defined as acute pancreatitis in patients that are receiving L-asparaginase treatment at the time of onset of acute pancreatitis. This pancreatic inflammation probably reflects premature activation of intraacinar pancreatic proenzymes or zymogens within the acinar cells (Gorelick and Thrower, 2009**)**.The activated zymogens, especially the protease trypsin, cause injury to the pancreatic acinar cells, which leads to the production and release of a cascade of cytokines **(**Pandol, 2006) and an inflammatory response, which may include systemic inflammatory response syndrome and multiorgan failure (Gardner et al, 2009; Malmstrom et al, 2012**)];** with the lungs and kidneys most commonly affected. Patients may develop pleural effusions, toxic pneumonia, acute respiratory distress syndrome, and renal failure (Pastor et al, 2003). The pathophysiology behind AAP is unknown (Alvarez &Zimmerman, 2000; Flores-Calderon et al, 2009; Kearney et al, 2009; Vrooman et al, 2010), but is regarded to reflect systemic depletion of asparagine with a subsequent reduction of protein synthesis, especially in organs with high protein turnover, such as the liver and pancreas.

Genetic predispositions are likely to play a role, because AAP occurs after one or a few administrations of L-asparaginase with a high likelihood of recurrence at re-exposure (Kearney et al, 2009). The clinical presentation, imaging methods, biochemical markers and complications in AAP do not differ significantly from acute pancreatitis in other pediatric populations (Alvarez & Zimmerman, 2000; Kearney et al, 2009).

Short term complications of acute pancreatitis include pancreatic necrosis and the formation of pseudocysts (Bai et al, 2011) Although, most pseudocysts have been reported to occur within 4 weeks of acute pancreatitis (Zaheer et al, 2012).

In addition, the necrotic pancreatic lesions can become infected. Such necrosis will typically occur after the first week (Zavyalov et al, 2010**).** Changes in the endocrine and exocrine pancreatic function, predominantly in the form of an impaired glucose metabolism severe diabetic ketoacidoses as well as non-ketotic, hyperosmolar hyperglycaemias, which usually respond to exogenous insulin, have been observed underasparaginase treatment (Falletta et al.,1972).

In the present study, we use a rat model to investigate the effects of ASNase on pancreatic acinar cells by examining histopathological changes in the exocrine pancreas both lightly and ultrastrcturallyand measuring the levels of serum pancreatic enzymes.

There are 2 principal functions for animal model research in acute pancreatitis: These are investigations of the molecular mechanisms underlying the pathobiologic responses of acute pancreatitis and testing of potential therapies before human trials (Falletta et al.,1972).

Three different phases can be distinguished in the development of acute pancreatitis: the first two take place in the pancreas itself, while in the third and final phase extrapancreatic symptoms may occur (Pandol et al, 2007).

In the first phase the acinar cell is damaged and this leads to cell death; this initiates the second phase of local inflammation of the pancreas, which causes the typical local signs and symptoms. Premature conversion of trypsinogen into trypsin, a proteolytic enzyme responsible for activation of a number of digestive enzymes in the gastrointestinal lumen, is considered the starting event in the development of acute pancreatitis (Bhatia et al, 2005)**.**

This activation of trypsinogen is normally mediated by ***enterokinases***, an intestinal brush border enzyme,cleaves a small trypsinogen activation peptide from trypsinogen to generate trypsin. Trypsin also activates trypsinogen, and trypsin efficiently activates all other pancreatic digestive enzymes. This process occurs in the intestinal lumen (Whitcomb and Cohn, 2006).

Trypsin activity is controlled within the pancreatic acinar cell by being synthesized in the

inactive form as trypsinogen. Human trypsinogen can slowly autoactivate to trypsin, which can initiate the zymogen activation cascade. This is prevented by three mechanisms: 1) packaging of zymogens within dense granules separated from some of the lysozomal enzymes cathepsin B.

2**)** synthesis of pancreatic secretory trypsin inhibitor (PSTI), also known as serine protease inhibitor Kazal type1(SPINK1). 3) is trypsin autolysis regulated by the level of Ca^{2+}.

Subsequent to acinar cell injury, the second phase is characterized by attraction and activation of neutrophils and macrophages in the pancreas. This inflammatory process is initiated by the nuclear factor-κB (NF-κB) pathway (Gukovsky, et al 1998**)** which initially leads to the local production of Interleukin (IL)-1β and TNF-α both in the acinar cells and local macrophages.

Systemic complications of acute pancreatitis which are the third phase are caused by a systemic inflammatory response syndrome,once these pro-inflammatory mediators enter the systemic circulation, organ dysfunction may develop.

In addition to activation of digestive enzymes, other factors are involved in the disease pathogenesis such as excess cytosolic calcium (Raraty et al 2000; Frick, 2012 mitochondrial dysfunction such as mitochondrial permeability transition pore or mitochondrial calcium overload (Gerasimenko and Gerasimenko, 2012),, cell deaths pathways (apoptosis and necrosis) Gukovskaya and Pandol, 2005 and overproduction of reactive oxygen species such as superoxide, singlet oxygen, hydroxyl radical, hydrogen peroxide (Rau et al 2000) and nitrogen species such as nitrogen oxide (Chvanov et al 2005).

Octreotide, which was introduced in the early 1980s, hasseveral advantages over somatostatin, such as a much longerhalf-life and the option of subcutaneous administration (Pless et al 1986). Effects of octreotide appear to occur as a result ofbinding to G-protein, which inhibit adenylatecyclase andconsequently decrease cyclic-adenosine monophosphateproduction (Katz and Erstad,1989). It is a powerful inhibitor of exocrine pancreatic secretion and cholecystokinin production, and it also minimizes oxidative stress caused by the systemicinflammatory response syndrome due to toxic mediators in the pancreatic necrotizing process (Greenberg et al 2000). Prevention of pancreatitis and hyperamylasemia has been confirmed (Li et al 2007).

Several studies have evaluated the effect of octreotide on the incidence of clinical pancreatitis after endoscopic retrograde cholangiopancreatography,and postoperative complications, such aspancreatic duct fistula following pancreaticoduodenectomy and pancreatic transplantation (Bonatti et al 2006**).** Although conflicting results have been described by (Binmoeller et al 1992) previous studies have established the usefulness of octreotide formanagement of ASNase-induced pancreatitis and pseudocyst (Garrington et al 1998) but they have not shown a preventive effectin clinical cases or experiments. Therefore, we examinedwhether octreotide administered during ASNase injectionwould protect the acinar cells from the damage caused byASNase.

2.Material and Methods:

A. Materials:

1. Experimental Animals:

Seventy male albino rats weighting 200 to 240 g were obtained from the animal house of King Fahd Research Center (King abdul Aziz Univeristy).

The animals were grouped and housed in cages and maintained under standard laboratory conditions (temperature 25±2º) with dark and light cycle (12h/12h). They were allowed free access to water and were fed a standard laboratory chow.

2. Test Drugs:

1-Escherichia coli ASNase (Elspar) was purchased from Sigma-Aldrich. ASNase was reconstituted with 0.9% sodium chloride solution adjusted to contain 250IU ASNase per milliliter.

2-Octreotide acetate (Sandostatin) was purchased from Novartis Pharmaceuticals (Jeddah).

The dose of Asparaginase chosen was according to the previous study of (Suzuki et al, 2008) who give in the clinical treatment of ALL, E. coli ASNase in the first induction therapy a dose of 200 IU/kg or 6000 IU/m2 given in a series of six or nine intramuscular or intravenous injections in order to deplete the circulating asparagine. In their experimental study in rats, the group that received ASNase 1000 was about fivefold higher than that used clinically, because the half-life of ASNase is shorter in rats than in humans (5–6 hours vs 26 hours) (**Putter, 1970).** Thus, the 1000 IU/kg dose of ASNase used is almost pharmacologically equal to the 200 IU/kg dose that is used clinically.

3. Animal Grouping:

Seventy male albino rats weighting 200 to 240 g were obtained from the animal house of King Fahad research center (King abdul Aziz Univeristy). The rats were divided into the following groups each containing 10 rats: Control **Group I** received normal saline intraperitonealy, **Group II** received a single intraperitoneal injection of ASNase at a dose of 200 IU/kg body weight, **GroupIII** receivedoctreotide (10µg/Kg/day) subcutaneously 8 hours before the 200 IU of ASNase**, Group IV** received intraperitoneal injection of ASNase at a dose of 500 IU/kg body weight, **Group V** receivedoctreotide (10µg/Kg/day) subcutaneously 8 hours before the 500 IU of ASNase**,**

Group VI received intraperitoneal injection of ASNase at dose of 1000 IU/kg body weight, **Group VII** receivedoctreotide (10µg/Kg/day) subcutaneously 8 hours before the 1000 IU of ASNase.

The total duration of the experiment was 5 days. The body weight of each rat from all groups was recorded everyday using a sensitive balance.

B. Methods:

1. Body weight and Pancreatic weights:

The body weight of each rat from all groups was recorded every day using a sensitive balance.

The animals in all groups were weighed before their sacrifice. The pancreas was removed, dried by tissue papers and weighed prior to fixation. Determination of the change in the weight of pancreas was estimated by comparing it to body weight. The ratios of weight in gm/ kg body weight were compared between experimental groups.

2. Biochemical Analysis and Determination of Enzymes:

The biochemical determination of serum amylase and serum lipase was done by collecting blood at the end of experiment from the carotid artery, the blood samples were collected in tubes containing a serum-separating agent. The collected serum was used for measuring the serumamylase level by the modified International Federation of Clinical Chemistry method (**Lorentz,** [52] using serum amylase kit **(Siemens)**, results were expressed in international unit per liter (IU/L) and estimated by biochemistry analyser**. (DIMENSION** X PAND). Serum lipase levels were estimated by the same method using serum lipase kit (Siemens) results were expressed in international unit per liter (IU/L) and estimated by biochemistry analyser**.(DIMENSION** X PAND).

3.Light Microscopy:

Histological examination was done after the rats were killed, the abdomenwas opened and the whole pancreas was quickly dissected, trimmed of fat, blotted and weighed. Random cross-sections of the head, body and tail were cut in two parts one for light and the other for electron microscopes.

Light microscope specimens were fixed in 15% formol saline, dehydrated in ascending grades of alcohol, cleared in xylol and embedded in paraffin. Sections of 5 um thickness were cut,stained with Hematoxylin& eosin stain and observed by light microscopes.

4. Electron Microscopy:

Very small pieces of the pancreatic tissue ($1mm^3$) of second part were first prefixed in 3% glutaraldehyde at room temperature for 2 hours.The specimens were then left over night in the phosphate buffer at 4°C. (pH 7.4) Specimens were then post-fixed in 1% osmium tetroxide in phosphate buffer(pH 7.4) for two hours at 4°C., then rinsed three times in distilled water, dehydrated through a series of graded alcohols, cleared inpopylene oxide and embedded in epoxy-resin embedding media.

Semithin sections, about 3 um in thickness were cut with a glass knife on an Ultratome V microtome. The sections were stained with toluidine blue 1% solution for viewing with a light microscope. Ultrathin sections from selected sections were stained with lead citrate and uranyl acetate and studied under a transmission electron microscope.

5.StatisicalyAnalysis:

Data are presented as means ± standard deviations. Comparison of data between different groups was done using the independent *t*-test. The difference was considered significant when $p < 0.05$.

3. Results

A. Effect on Pancreas Weight Relative to Body Weight:

All experimental rats had no significant change in body weight compared with controls. There was a significant increase ($p < 0.05$) in the pancreas weight relative to body weight (PW: BW) ratio in asparginase treated groups (IV and VI) compared with the control group. Treatment with octreotide in groups V and VII significantly decreased the PW: BW ratio compared with the corresponding asparginase treated groups as shown in **Fig. 1**. and **table 1.**

Table 1. Comparison between body weight (gm), pancreatic weight (gm) and ratios of weight of pancreas (gram) to body weight (kilogram) in all groups (mean ± SD)

Groups	Mean body weight ± SD (Grams)	Mean weight of Pancreas ± SD (Grams)	Weight of pancreas in grams/kg body weight
Group I (Control)	219.4 ± 6.09	0.486± 0.02	2.22
Group II (Asp 200 IU)	201.2 ± 4.87	0.688± 0.05	3.41
Group III (Asp 200 IU+OCT)	197.4 ± 11.4	0.540± 0.16	2.74
Group IV(Asp 500 IU)	219.7 ± 7.23	1.265 ± 0.08*	5.76*
Group V (Asp 500 IU+OCT)	215.0 ± 4.16	0.805 ± 0.31#	3.74#
Group VI (Asp 1000 IU)	223.0 ± 16.6	1.682 ± 0.20*	7.54*
Group VII (Asp 1000 IU+OCT)	225.1 ± 15.4	0.785 ± 0.36#	3.49#

Significance was considered at $p < 0.05$, * significant change compared with control;
significant change compared with corresponding asparginase treated group.

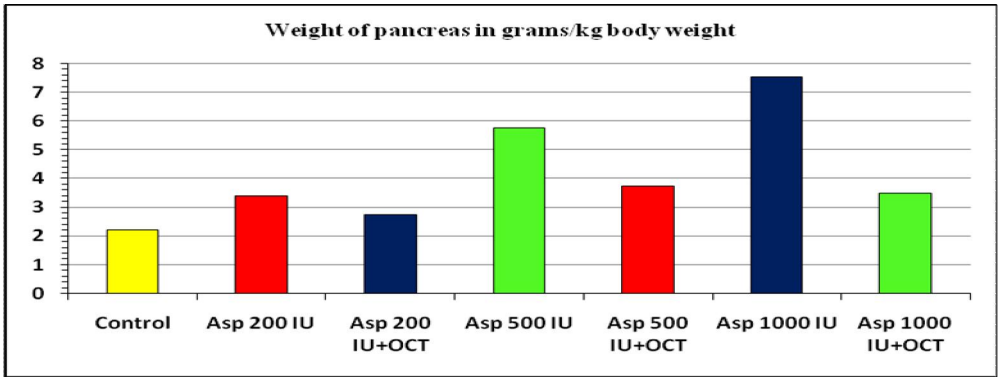

Fig.1The effect of asparginase and octreotide treatment on pancreatic weight relative to body weight all groups

B. Comparison of Serum pancreatic enzymes of ASNases group and ASNases plus octreotide:

Table (2) and figures **(2, 3)** demonstrate the effect of asparaginase and octreotide treatment on serum amylase and lipase of control and experimental rats.

Serum amylase levels showed no significant increase in **group II** (ASNase 200mg/kg/day) compared with the control group ($p > 0.05$). The serum amylase level was not significantly lowered in **group III (ANSase200mg+ Octeotride**) compared with their respective non octeotride treated group (group II).

Administration of asparaginase (500mg/kg/day) in **group IV** produced significant ($p<0.05$) increase in serum amylase with mean of 3098.7 ± 62.18 (U/L) compared to control group after 5 days.

Moreover, **in group V** treatment with octreotide (0.1 µg/Kg/day) subcutaneously 8 hours before the 500 IU of ASNase for 5 days produced a significant ($p <0.05$) decrease in serum amylase with mean of 917.25 ± 80.9 (U/L) compared to group V.

As shown from the table, administration of ASNase 1000 mg/kg/dayin **group VI** resulted in a slight non significant ($p >0.05$) decrease in serum amylase 911.0 ± 62.2 (U/L) compared to control group. Furthermore, non significant ($p >0.05$) increase in serum amylase 1076.7 ± 52.9 (U/L) wasobserved in theoctreotide treated **group VII** compared to control group and **group VI**.There was no significant difference between the control group and experimental groups for the serum lipase.

Table II.Serum amylase and lipase levels in control and experimental animals

Groups	ıylase (IU/L)	Lipase (IU/L)
Group I (Control)	992.00 ± 86.12	33.50 ± 2.08
Group II (ASP 200 IU)	1017.2 ± 83.81	31.75 ± 1.71
Group III (ASP 200+OCT)	866.50 ± 28.58	29.75 ± 1.71
Group IV (ASP 500 IU)	3098.7 ± 62.18	33.75 ± 2.50
Group V (ASP 500+OCT)	917.25 ± 80.9	33.75 ± 0.95
Group VI (ASP 1000 IU)	911.0 ± 62.2	34.50 ± 1.29
Group VII (ASP 1000+OCT)	1076.7 ± 1076.7 ± 52.9	33.00 ± 1.83

Data are expressed as mean ± SD.

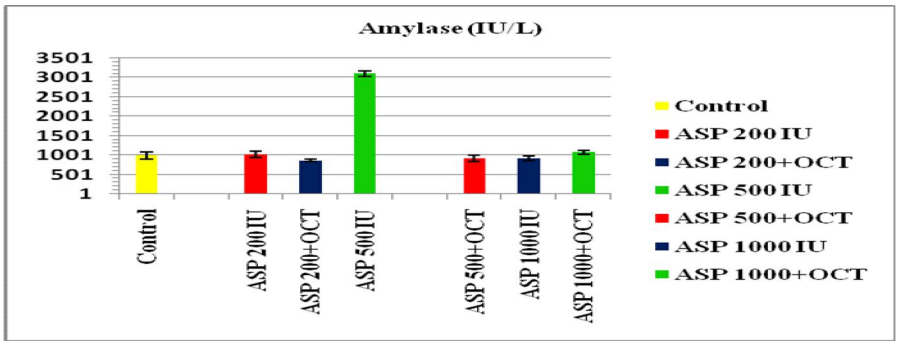

Fig. 2 Serum amylase levels in control and experimental animals

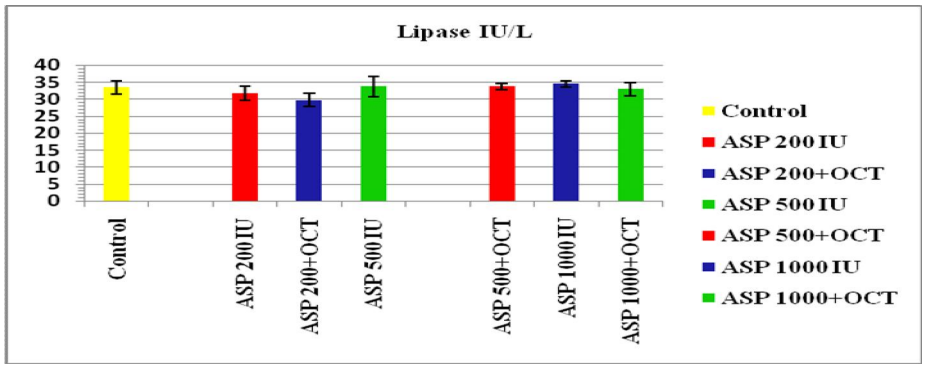

Fig.3 Serum lipase in control and experimental animals

C. Histological results:

1. Light microscopic findings:

Group I (control): Histological examination of H&Estained sections of the exocrine pancreas of this group revealed normal architecture. Pancreatic lobules were separated by thin connective tissue septa containing the interlobular ducts. The lobules contained acini of different sizes and shapes **(Fig. 4A).** Masson's trichrome stained sections of control rats' pancreas showed thin layer of collagenous fibers around blood vessels and formed thin septa around the lobules**(Fig. 4B)**

Group II (ASNase 200 IU): Examination of H&Estained sections of this group revealedmoderate increase in the zymogen granules that was the most prominent finding in this group. The acini appeared larger in size than those of the control group. Widening of interstitial spaces was also detected**.** Pancreatic ducts were slightly dilated and full of eosinophilic condensate of secretions**(Fig. 5A).** Mild increase in the collagen fibers surrounding acini, blood vessels and ducts were seen in Masson's trichrome stained sections **(Fig. 5B).**

Group III (ASNase 200 IU+ Octreotide): the pancreatic tissue showed almost normal histological architecture. The acini showed an increased rate of mitosis. The blood vessels and ducts within the connective tissue septa were apparently normal in size (**Fig. 5C).** Almost normal connective tissue septa around blood vessels and in between acini were seen by Masson's trichrome stain **(Fig. 5D).**

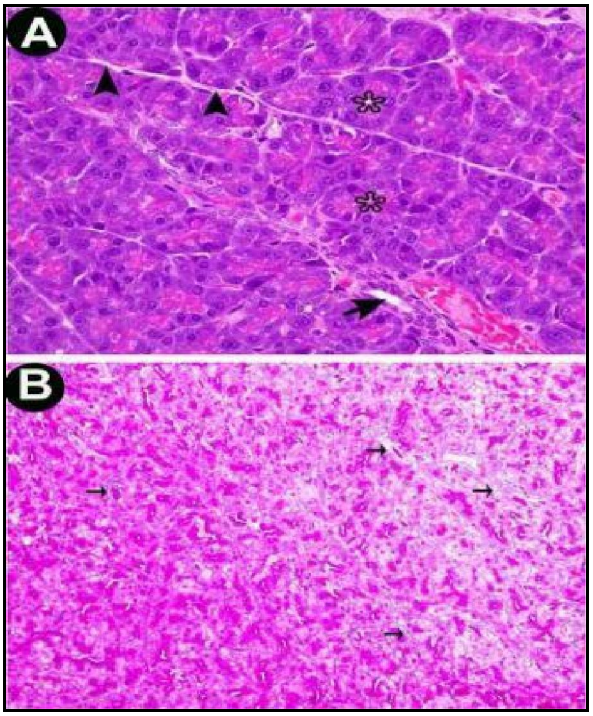

Fig. 4: Photomicrograph of sections in the pancreas of a control rat showing: (A) pancreatic lobules consisting ofacini of different sizes and shapes (star), separated by thin septa (arrowheads) containing interlobular duct (arrow);H&E,Original mag x400 (B)Thin layer of collagenous fibers around blood vessels and forming thin septa around the lobules (arrow);Masson Trichrome, Original mag x200.

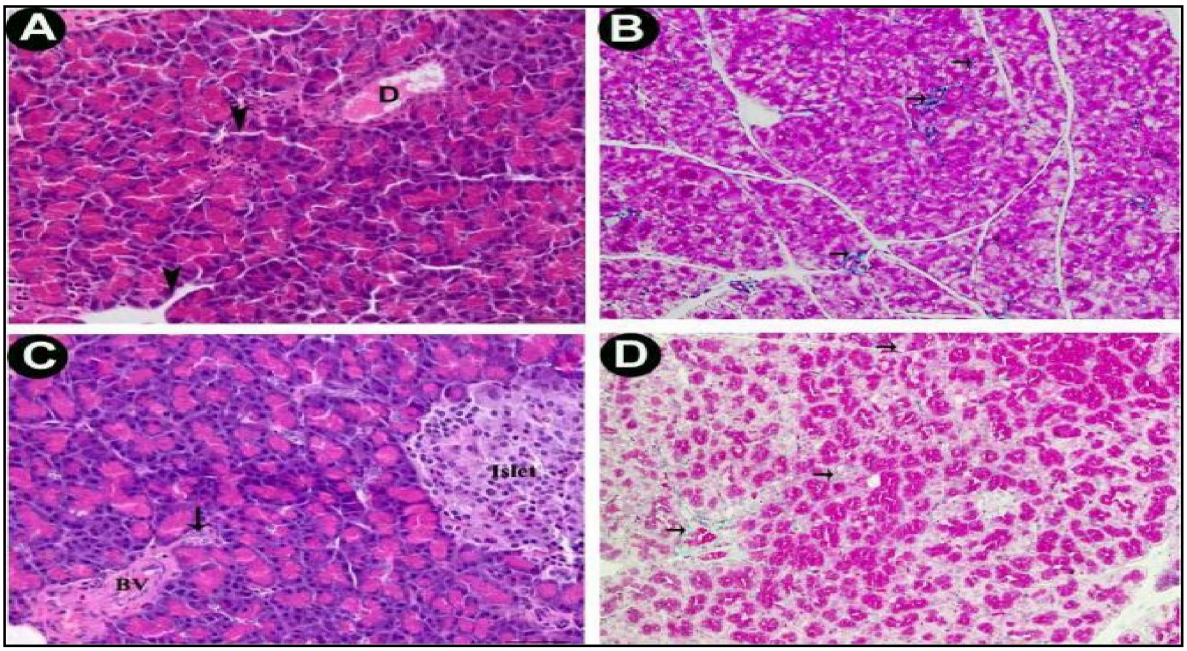

Fig. 5: photomicrograph of sections in the pancreas of ratsfrom group II**(A-B)** and group III**(C-D)** showing: **(A)** moderate increase in the zymogen granules, widening of interstitial spaces (arrowheads) and dilated pancreatic ducts (D); H&E,Original mag x400 **(B)**mild increase in the collagen fibers surrounding acini, blood vessels and ducts; Masson Trichrome, Original mag x200**).(C)**almost normal histological architecture. The blood vessels (BV) and ducts (arrow) within the connective tissue septa are apparently normal in size and architecture; H&E,Original mag x400. **(D)**almost normal connective tissue septa around blood vessels and in between acini (arrows); Masson Trichrome, Original mag x200.

Group IV (ASNase 500 IU): Examination of H&Estained sections of this group revealedmore structural changes in the exocrine pancreas, these changes varied from cellular swelling with vacuolar changes to cellular necrosis. Central vacuolation of the acini which extended to the apical portions of the cells was detected (**Figs. 6A).** Inflammatory infiltration of mononuclear cells mainly lymphocytes were prominent in the interlobular connective tissue septa **(Fig.6**B). Congestion and dilatation of blood vessels appeared with increase in collagen fibers of interlobular connective tissue septa, especially around dilated congested blood vessels and dilated ducts **(Fig.6C)**

Group V (ASNase 500 IU+ Octreotide): Octeotide was probably enough to protect the pancreatic tissue. This protection was manifested by disappearance of cytoplasmic vacuolation and restoration of more regular acinarpatteren. Increased mitotic figures of the acinar cells and increased zymogen granules in slightly dilated acini were frequently observed **(Fig.6D).**Apparent decrease in the connective tissue surrounding blood vessels and ducts was demonstrated in Masson's trichrome stained sections **(Fig.6E)**

Group VI (ASNase 1000 IU): The histological changes were progressed at higher dose of ASNase (1000 IU/kg body weight). In some animals, large areas of disturbance in acinar pattern were found.Pancreatic ducts were also full of secretions **(Fig. 7.A).** Cellular infiltrations were diffusely distributed in between acini and around blood vessels. Widening of interstitial spaces, which might suggest interstitial oedema, were also detected.Some acinar cells appeared vacuolated with destruction of their cytoplasm with various degree of nuclear degeneration **(Figs. 7B).**There was an increase in the collagen fibers normally present in minimal amount in the septa and around blood vessels and ducts **(Fig.7C).**

Group VII (ASNase 1000 IU + Octreotide): A major improvement was observed following the concomitant administration of octreotide with asparginase. A few acinar cells were degenerating as manifested by the presence of cytoplasmic vacuoles **(Fig. 7D).** A decrease in the collagen fibers around blood vessels and ducts were also notedin Masson`s trichrome stained sections **(Fig. 7E).**

2. Ultrastructuralmicroscopic findings:

Group I (Control): Electron microscopic examination of the ultrathin sections of the pancreas in this group revealed acinar cells which were roughly pyramidal in shape. Their nuclei were central to basal in position and rounded in shape with

prominent nucleoli and large supranuclear Golgi complex.The apical half of the cells contained a number of rounded, electron-dense, membrane-bounded zymogen granules. The luminal cell membrane formed microvilli **(Fig.8A).**The lateral cell membranes of acinar cells were separated from one another by deep intercellular canaliculi that frequently approach the basal lamina. The base of the cells rested on the basal lamina**(Fig.8B).** The basal half of the cells was mostly filled with the rough endoplasmic reticulum (rER) cisternae which diverged to accommodate long mitochondria that had numerous cristae and many matrix granules **(Fig.8A-C).**Centroacinar cells have flattened nucleus and sparce organelles **(Fig.8D).**

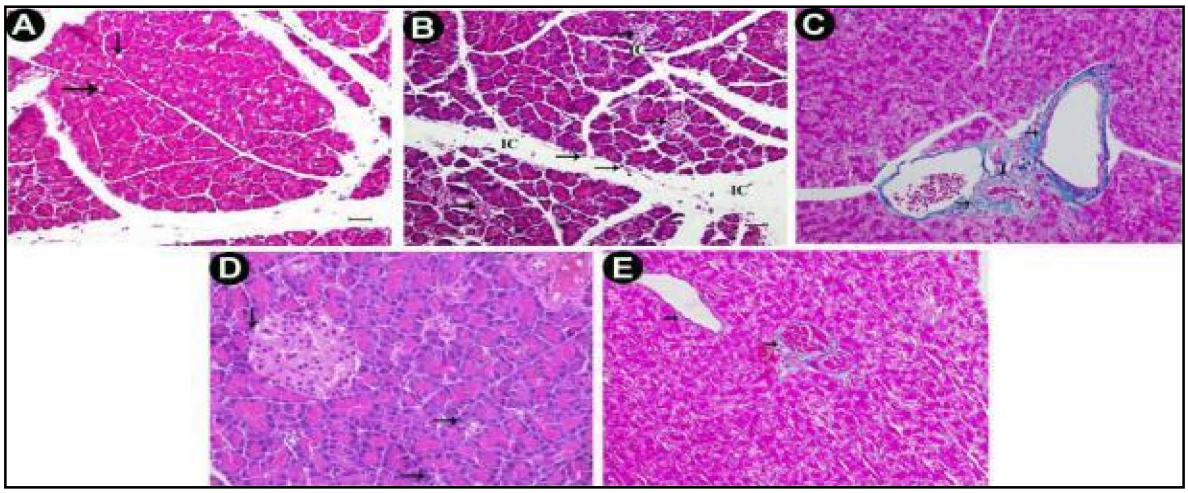

Fig.6: photomicrograph of sections in the pancreas of rats from group IV **(A-C)** and group V **(D-E)** showing: **(A)**vacuolation of the acinar cells (arrows), **(B)** inflammatory infiltration of mononuclear cells (arrows) in the widened interlobular connective tissue septa(IC) (H&E,Original mag x200). **(C)**increase in connective tissue around dilated blood vessels and ducts (arrows);Masson Trichrome, Original mag x200**. (D)** apparently normal structure of pancreatic acini. Note the presence of mitotic figures (arrows); H&E,Original mag x400**.(E)** apparent decrease in the connective tissue surrounding blood vessels and ducts; Masson Trichrome, Original mag x200.

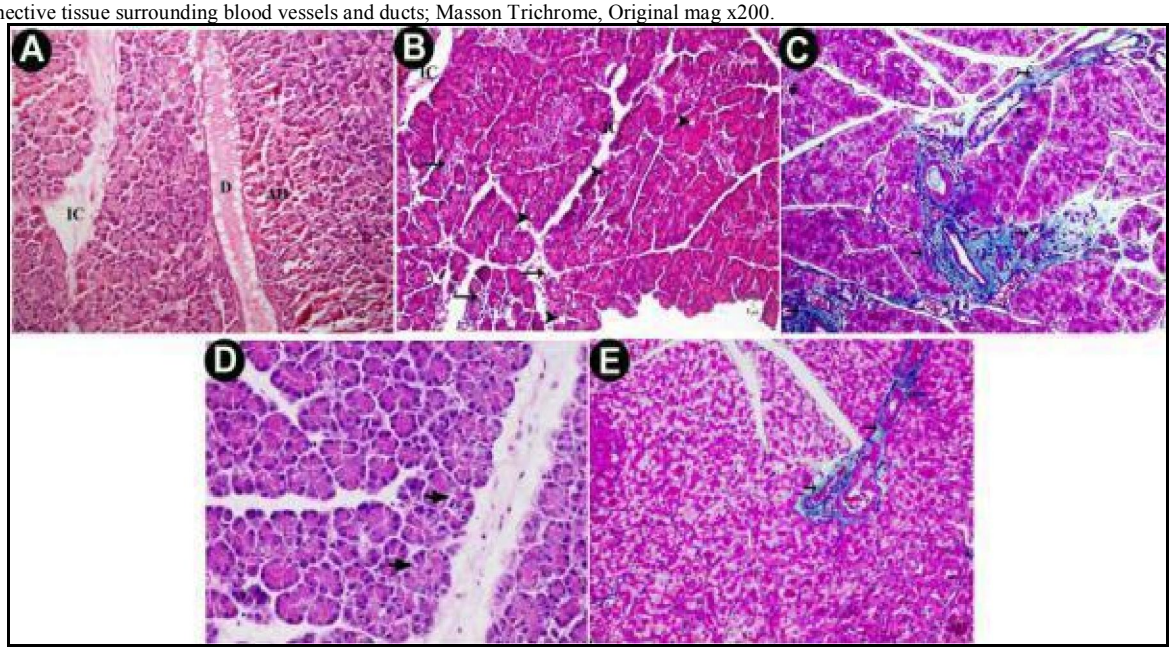

Fig.7: photomicrograph of sections in the pancreas of rats from group VI **(A-C)** and group VII **(D-E)** showing: **(A)** areas of disturbance in acinar pattern (AD), widening of interstitial spaces(IC). A pancreatic duct is full of secretions (D). **(B)** Vacuolated acinar cells (arrowheads) and cellular infiltrations (arrows) in between acini and around blood vessels; H&E, Original mag x200**(C)**an increase in the collagen fibers in the septa and around blood vessels and ducts (arrows); MassonTrichrome, Original mag x200**.(D)**Almost normal acinar architecture. Note the presence of cytoplasmic vacuolations (arrows) in some acini; H&E, Original mag x400. **(E)**a decrease in the collagen fibers around blood vessels and ducts (arrows); MassonTrichrome, Original mag x200.

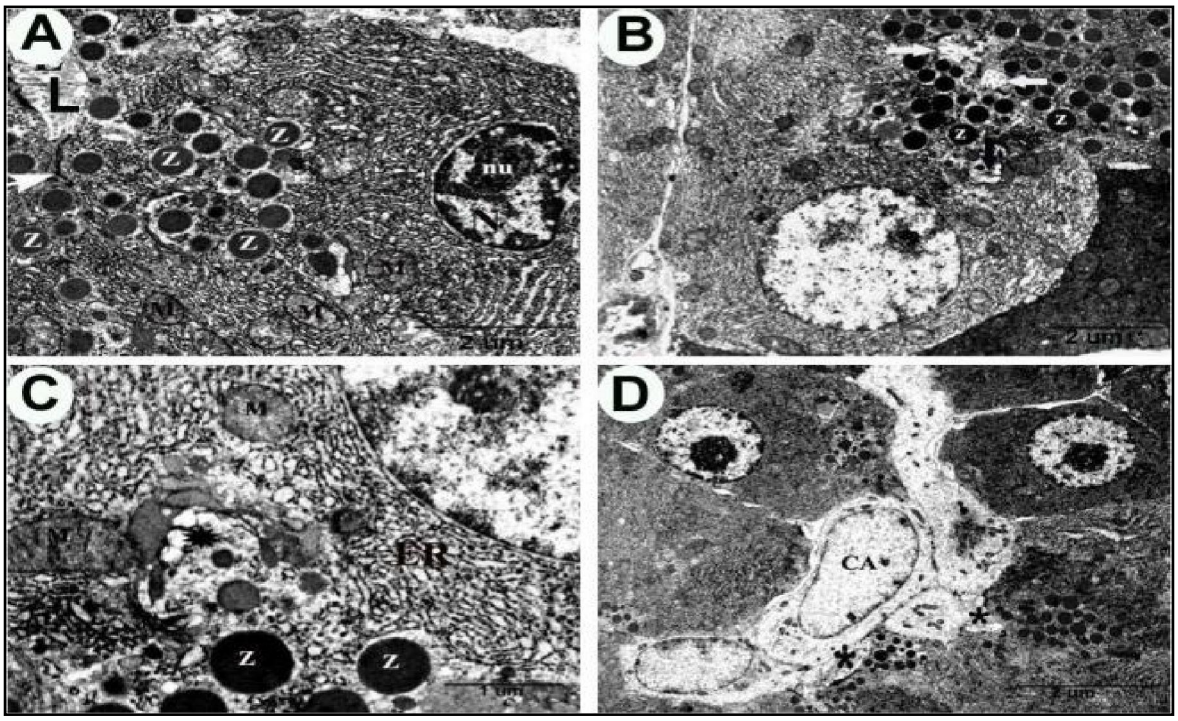

Fig. 8: TEM of ultrathin sections in the pancreas of a control rat showing: **(A)** pancreatic acinar cells having basal rounded nucleus (N) with large prominent nucleolus(nu), zymogen granules (Z), mitochondria (M), junctional complexes are located near the luminal surface where adjoining cells meet (arrow) and the luminal cell membrane formed microvilli (Lu)**. (B)** Intercellular canaliculi (white arrow) into which microvilli project from lateral cell membranes of acinar cells. **(C)** Prominent Golgi cisternae (star) and rER. **(D)** centroacinar cell (CA) with flattened nucleus and sparce organelles. Note the microvilli projecting into the lumen (stars). [A-B-D;bar = 2µm) and (C) bar =1µm]

Group II (ASNase 200 IU): Electron microscopic examination of the ultrathin sections of the pancreas in this group revealed focal areas of pancreatic lobules structurally affected, while the rest of the exocrine gland showed normal ultrastructural appearance.Moderate increase in the zymogen granules of some pancreatic acinar cells was a prominent feature. The Zymogen granules showed variable densities and some of them showed disintegrated envelops. Various numbers of zymogen granules were agglomerated. Some groups of such granules showed different stages of dissolution in the granule membrane **(Fig.9A).** Some acinar cells showed vacuoles of variable sizes that appeared either empty or autophagic vacuole **(Fig.9B).** Increased intraductal secretions were also noticed**.** Infiltrating elements could also be seen intravascular and around the acini**(Fig9 C).**The affected cells showed a dilated Golgi apparatus, fusion of zymogen granules and secondary lysosomes with a variable degree of vacuolation andprominent dilatation of the intercellular canaliculi**(Fig.9D)**

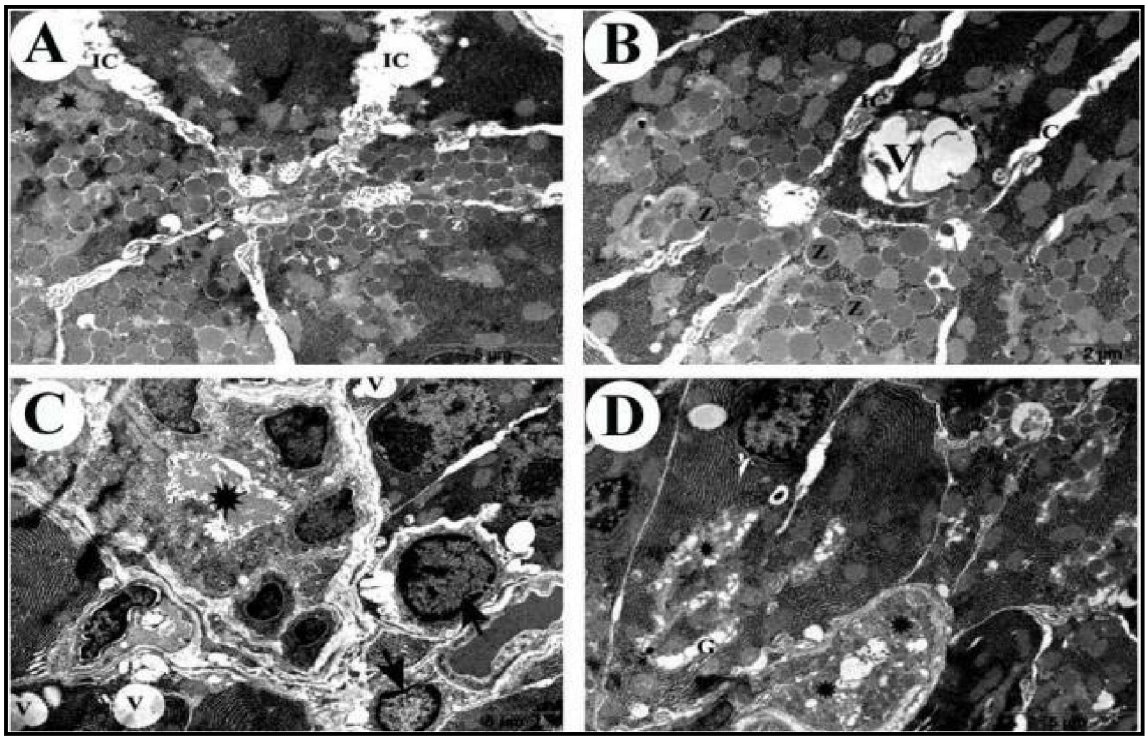

Fig. 9: TEM of ultrathin sections in the pancreas of a rat from group II showing: **(A)** moderate increase in the zymogen granules (Z). The Zymogen granules show variable densities and some of them show disintegrated envelops (arrowheads). Various numbers of zymogen granules are agglomerated (star). Prominent dilatation of the intercellular canaliculi (IC) is also noticed. **(B)** Large autophagic vacuole (V). **(C)** Increased intraductal secretions (star), infiltrating elements (arrows) are seen intravascular and around the acini and some acinar cellsshow vacuoles of variable sizes (V). **(D)** A dilated Golgiapparatus (G), fusion of zymogen granules and secondary lysosomes with a variable degree of vacuolation (star). [A-C-D bar = 5µm and C bar= 2µm]

Group III (ASNase 200 IU + octreotide): Electron microscopic examination of the ultrathin sections of the pancreas in this group revealed acinar cells with no ultrastructural changes compared with controls **(Fig.10).**

Group IV (ASNase 500 IU): Electron microscopic examination of the ultrathin sections of the pancreas in this group showed that acini were packed with zymogen granules in comparison with their controls. Some acinarlumina were dilated and full of secretions**.**Moreover, Acini showed dilatation of rEr and Golgi cisternae. Ballooning degenerating mitochondria with disrupted ill-defined cristae and secondary lysosomes were also demonstrated**.**One hallmark of sublethal injury to acinar cells was the presence of autophagic vacuoles. Pyknotic cellswith fragmented and/or condensed nuclei were observed**(Fig.11A).**As regards zymogen granules, disintegration or variation of electron density and peripheral dissolution were observed in many acinar cells **(Fig.11B).**Several cistermae of rER in some acinar cells lost their polarity and formed concentric whorls **(Fig.11C).**Undamaged and damaged acinar cells were seen next to each other within the same acinus. The damaged cells showed variable degree of degranulation and vacuolation**(Fig.11D).**Myelin figures were also shown in affected cells **(Fig.11E).** Mononuclear cellular infiltrations were demonstrated in the widened interstitial tissue **(Fig.11F).**

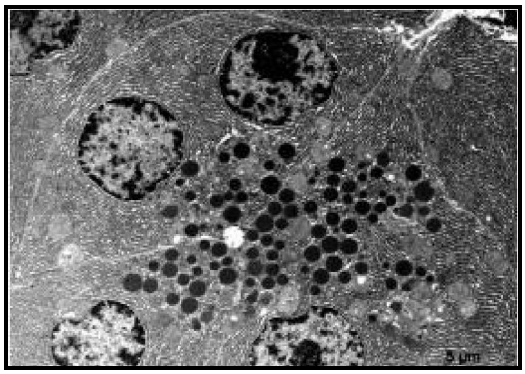

Fig.10: TEM of an ultrathin section in the pancreas of a rat from group III showing acinar cells with no ultrastructural changes compared with controls. [bar= 5µm]

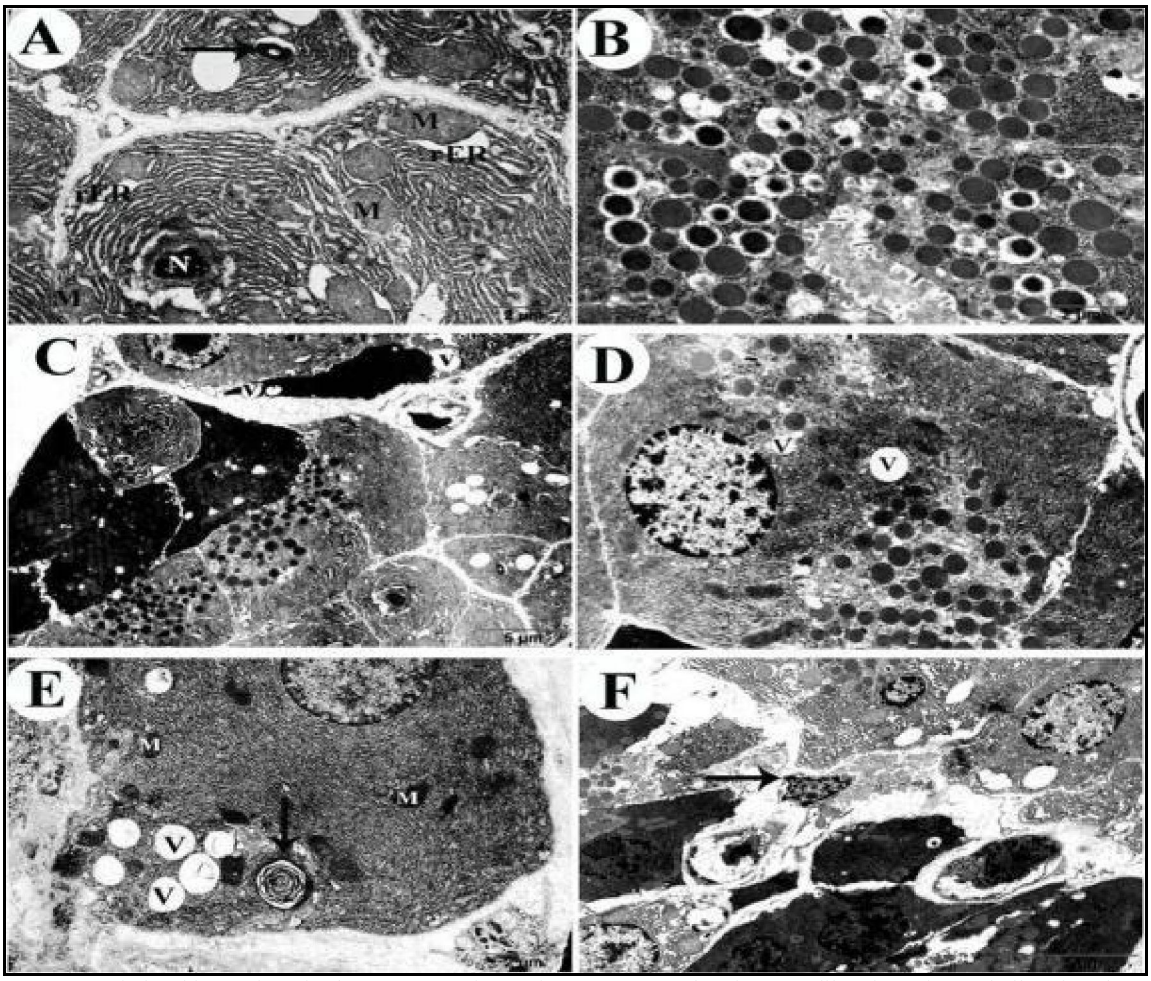

Fig.11:TEM of ultrathin sections in the pancreas of a rat from group IV showing **(A)** dilatation of rER, ballooning degenerating mitochondria with disrupted ill-defined cristae (M), secondary lysosomes (S),nuclear pyknosis (N) and autophagic vacuole (arrow). **(B)** Disintegration or variation of electron density and peripheral dissolution of zymogen granules (Z), acinar lumen is dilated and full of secretions (L). **(C)**cistermae of rER in some acinar cells lost their polarity and formed concentric whorls. **(D)**Undamaged and damaged acinar cells are seen next to each other within the same acinus and damaged cells have darkly stained cytoplasm and multiple vacuolations (V). **(E)** Cytoplasmic vacuoles (V), dark profiles of mitochondria (M) and myelin figures (arrow). **(E)** Mononuclear cellular infiltrations (thin arrow) are demonstrated in the widened interstitial tissue around damaged cells. [A-B-D bar=2µm and C-E bar =5µm]

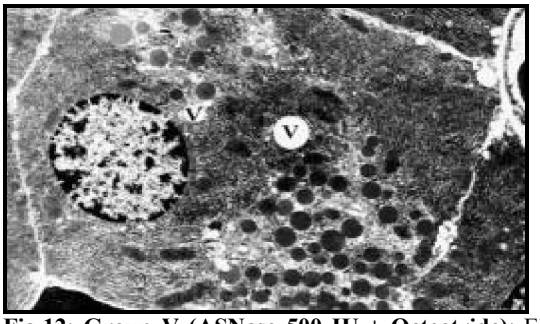

Fig.12: Group V (ASNase 500 IU + Octeotride): Electron microscopic examination of the ultrathin sections of the pancreas in this group showed that most acinar cells appeared nearly normalized. Minute subcellular abnormalities were detected in few acinar cells. Few cytoplasmic vacuoles were detected. [bar= **5** µm]

Group VI (ASNase 1000 IU): Electron microscopic examination of the ultrathin sections of the pancreas in this group revealed partial degranulation of varying degrees or slight cytoplasmic vacuolation seen in individual acinar cells**.** Swollen mitochondria with ill-defined cristae were also detected**.** Some granules showed dissolution in their membranes and sometimes fused with swollen mitochondria ending in formation of irregular cavities**(Fig.13A).**The changes in the zymogen granules were accompanied by alterations in the mitochondria showing irregular profiles. Their matrix space sometimes became more electron-opaque **(Fig.13B).**Acinar cells showed marked dilatation of rER cisternae or their vesiculation (i.e the cisternae were broken up into multiple vesicles) Swollen mitochondria with ill-

defined cristae were also detected **(Fig.13C).** Condensation of nuclear chromatin and shrinkage of the nuclei (pyknosis) could be seen in some affected cells Swollen mitochondria with ill-defined cristae were also detected **(Fig.13D).** Infiltrating elements could also be seen within or more frequently, around the acini**(Fig.13E).**

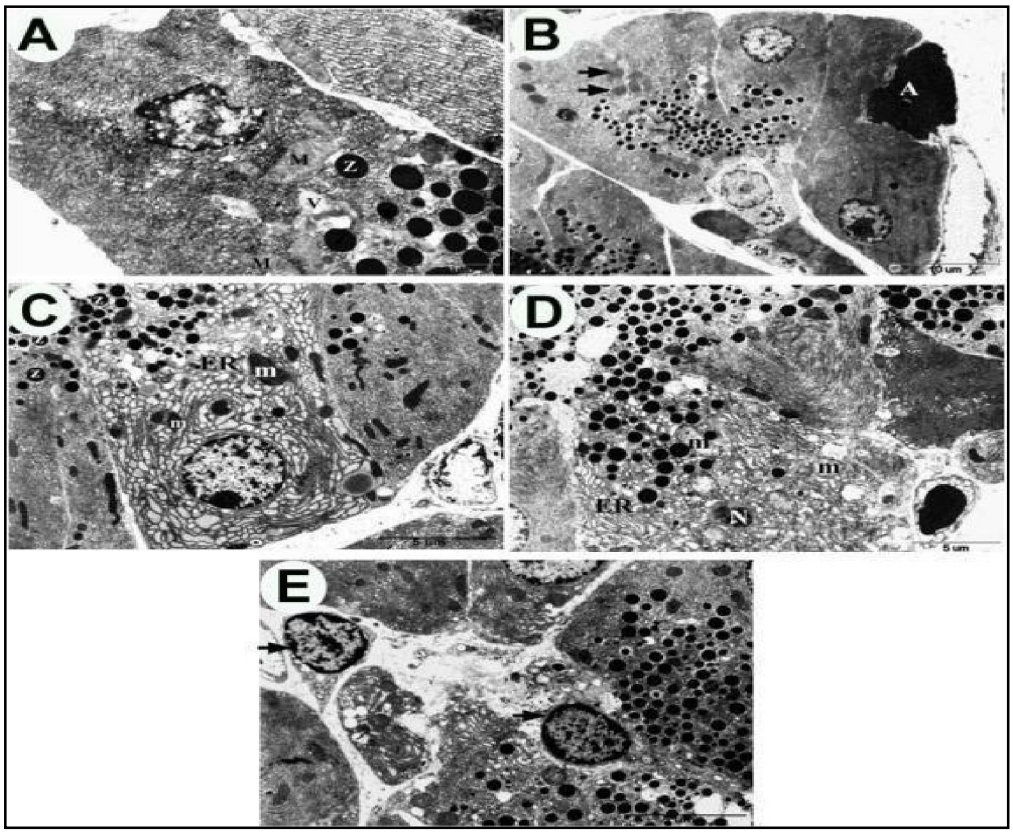

Fig. 13: TEM of ultrathin sections in the pancreas of a rat from group VI showing **(A)** acinar cells with swollen mitochondria (m) with ill-defined cristae which sometimes fuse with zymogen granules (Z)ending in formation of irregular cavities. **(B)** Partial degranulation in individual acinar cells, a necrotic acinar cell (A) is about to be engulfed by its neighboring cell. The mitochondria (arrows) show more electron-opaque irregular profiles**. (C)** Marked dilatation of rER cisternae **(D)** condensation and shrinkage of nuclear chromatin (pyknosis) (N). **(E)** infiltrating elements (arrows) within or around the affected acinar cells. [A bar=2µm; B bar =10µm; C-D-E bar=5µm]

Group VII (ASNase 1000 IU+ Octeotride): Electron microscopic examination of the ultrathin sections of the pancreas in this group showed that the majority of acini showed no morphological changes. However, mild focal ultrastructural changes manifested by dilated rER cisternae and few damaged mitochondria were observed in some acinarcells **(Fig.14).**

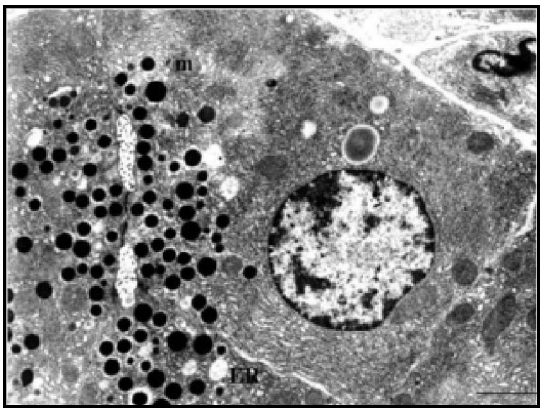

Fig. 14:TEM of an ultrathin section in the pancreas of a rat from group VII showing almost normal acinar cells. Mild focal slight dilated rER cisternae and few damaged mitochondria in few acinar cells. [bar=5µm]

4.Discussion

The enzyme ASNase has been used to treat ALL since the 1960s (Hill et al 1967**).** In the 1970s, use of ASNase significantly improved the rate of remission when employed withvincristine and prednisone for induction therapy in childhood (Ortega et al,1977).

Several recent reports have shown favorable outcomes for children with ALL, with long-term event-free survival rates of approximately 80% for an unselected patient population (Pui, et al 2004; Kearney et al 2009). Recently, some oncology groups have reported favorable outcomes in adults by adopting pediatric protocols (Storring et al, 2006)

However, one of the most serious adverse events of L-asparaginase is acute pancreatitis and it is the most common reason for stopping treatment with L-asparaginase (Kearney et al, 2009; Treepongkaruna et al, 2009).

Few reports showing that pancreatitis occurs after administration of ASNase by itself, because ASNase is usually used in combination with other chemotherapeutic agents and steroids, since the role ofdrug combinationwill increase the risk of pancreatitis as mentioned by (Altman et al 1982).

In the present study, we use a rat model to investigate the effects of ASNase on pancreatic acinar cells by examining histopathological changes in the pancreas and measuring the levels of serum pancreatic enzymes, this animal model was selected for the study as previously done by (Suzuki et al, 2008).There are a number of experimental models of (non-alcoholic) acute pancreatitis that reproduce the responses of human disease—animal models, such as pancreatitis induced in rats or mice by administration of caerulein (CCK-8 analog), bile acid (e.g.taurocholate) or L-arginine, or by feeding mice a choline-deficient, ethionine supplemented (CDE) diet; and the *ex vivo* model of isolated pancreatic acinar cells hyperstimulated with supramaximal CCK-8 (CCK) or cerulein (CR). **(**Lerch and Adler, 1994; Pandol et al 2007).

The severity of AAP in our experimental study is dose dependent, minimal damage occurs with 200IU, and the severity was detected with 500IUand 1000IU.

Hashimoto et al 2008 in their study of caerulein- induced experimental pancreatitis in mice that acute pancreatitis increased with the number of cerulein injections. With six and nine cerulein injections, mild edema and acinar cell degeneration were observed,with 12 caerulein injections, the pancreas showed severe acinar cell degeneration with significant edema and inflammatory cell infiltration in the interstitium. In accordance with histological changes, they observed a significant increase in serum amylase activity. The observed increase was proportional to the cerulein dose and the severity of acute pancreatitis**.**

The pathophysiology behind AAP is unknown (Vrooman et al, 2010) but is regarded to reflect systemic depletion of asparagines with a subsequent reduction of protein synthesis especially in organs with high protein turnover such as liver and pancreas.

Information from cellular and in vivo studies as well as genetic studies in humans suggests that pathologic events that begin in the pancreatic acinar cell often initiate this disease. This cell is designed to synthesize, store and secrete the enzymes required for nutrient digestion.

Under physiological conditions, most of these enzymes particulary proteases become active only when they reach the small intestine. Drugs as L-asparaginase that cause pancreatitis result in distinct changes in acinar cell signaling. These changes initiate a spectrum of pathologic changes withinacinar cells that include the activation of digestive enzymes, generation and release of inflammatory and vascular mediators, changes in paracellular permeability and stimulation of cell death pathways (Gorelick and Thrower, 2009).

Various *in vivo* and *in vitro* studies of experimental pancreatitis using cholecystokinin analogues (CCK analogues) such as caerulein or CCK-JMV-180 have established that the premature intraacinar activation of zymogens and increase secretion is a key event in the pathogenesis of pancreatitis (Mithofer *et al.,* 1998**)** followed by inhibition of secretion and retension of activated zymogens (Olegar et al, 2001).

This is in accordance with our study using asparaginase at a dose of 200IU, 500IU and 1000IU during the first days of experiments we observed by E/M as well as biochemically by estimation of serum amylase that the number of zymogen granules and the level of amylase increase significantly, than with further damage of pancreas with dose 1000IU and at the end of the duration of experiment (5 days), the number of zymogen granules as well as the level of serum amylase decrease significantly.

Also (Mithhofer et al, 1998) observed activation of trypsinogen and other zymogens in the pancreatic homogenate as early as 10 minutes after supramaximal stimulation by caerulein in rats and increase over time, in addition to other markers of pancreatitis e.g. hyperamylasemia, pancreatic edema and acinar cell vacuolization can be detected at 30 minutes after supramaximal stimulation.

Previous study of (Grady et al, 1996) strongly supports the paradigm that zymogen activation and increase exocrine pancreatic activity is the cause of pancreatitis and the autodigestion process.

Jugermam et al, 1995 postulated the factors involved in inhibition of secretion and retension of activated enzymes is the loss of terminal web and its associate intermediated filaments.

Another factor may also be due to disorders of exocytotic process related to SNARE proteins and small GTP binding proteins as proposed by (Gaisano et al., 2001) who explained that specific SNARE proteins (soluble-ethylmaleimide- sensitive fusion protein attachment protein receptor) located on the plasma membrane and zymogen granules membranes regulate exocytosis through their interactions and a high dose CCK-8 causes displacement of one of SNARE proteins

from the basal surface of acinar cell with a concomitant redirection of apical exocytosis to the basal surface.

Small GTP binding proteins of Rab family such as Rab27b have roles in exocytosis process these proteins are involved in vesicular traffic and membrane fusion in eukaryotic cells and are present in zymogen granule membranes of exocrine pancreas (Chen et al 2004).

Our data revealed accumulation of large vacuoles with variable content in acinar cells and characterized ultrastructurallyby containing partially degraded material, so these vacuoles have the characteristic of autophagic vacuoles which is a long noted feature of both experimental and human pancreatitis as mentioned previously by (Niederau and Grendell 1988; Hirota et al, 2006).The nature of these vacuoles and their mechanism of formation and their relation to other pathological responses of pancreatitis have been a matter of debate.

The mechanism was explained by (Saluja et al 2007) due to missorting of CatB which catalyze the conversion of trypsinoge to trypsin and become colocalized with trypsinogen in unindentified compartment (s).

In a recent study of (Hashimoto et al, 2008) they found that cytoplasmic vacuoles induced in pancreatic acinar cells by experimental pancreatitis were autophagic in origin, as demonstrated by immunohistochemical studies (microtubule-associated protein 1 light chain3 expression) and electron microscopy experiments.

In their experiments they used mice deficient in Arg5, a key autophagic protein in acinar cells. Acute pancreatitis was not observed except for very mild edema in a restricted area, in conditional knockout mice, unexpectedly, trypsinogen activation was greatly reduced in the absence of autophagy.

They suggested that autophagy exerts devasting effects in pancreatic acinar cells by activation of trypsinogen to trpsin in the early stage of acute pancreatitis through delivering trypsinogen to the lysosome.

The previous authors proposed that excessive autophagy is the cause of intra-acinartrypsinogen activation.

In the study of (Mareninova et al, 2009) they provide evidence by using in vivo and in vitro experimental models and both electron microscopic and immunofluorescent techniques that autophagy is activated by both pancreatitis and fasting, but unlike fasting acute pancreatitis cause inhibition of lysosomal degradation and retardation of autophagic flux and they further found that pancreatitis impairs processing/maturation and activities of CatL and CatB, which may underlie the inefficient lysosomal degradation of both trypsinogen and trypsin and their results indicate that this dysfunction, rather than missorting of CatB [75] or excessive autophagy [36] mediates the intra-acinar accumulation of active trypsin in autophagic vacuoles in acute pancreatitis.

In agreement with (Mareninova et al, 2009) and documented it, is the study of (Gukovsky and Gukovskaya, 2010); Gukovsky et al 2012. proved by several approaches using rodents as well as cell (*in vitro*) models of pancreatitis, in particular those induced by supraphysiological doses of cholecystokinin, or its analogue caerulin, that autophagy, the main cellular degradative, lysosome-driven process is activated but also impaired, this impairment mediates vacuolations in acinar cellsin acute pancreatitis and the abnormal maturation and activation of cathepsins(CatL and CatB) major lysosomal hydrolases leads to increase in intra-acinar trypsin, the hallmark of pancreatitis while lysosomal membrane associated proteins (LAMP-1 and LAMP-2) deficiency causes inflammation and acinar cell necrosis rather than blockage of autophagosome fusion with lysosomes.

Thus, the autophagic and lysosomal dysfunctions mediate key pathologic responses of pancreatitis.

Data from the present work demonstrated that the main acinar cell organellar damage observed in our present study of experimental asparaginase pancreatitis was the affection of mitochondria and rER.

The mitochondria show signs of degeneration in the form of swelling, loss of mitochondrial cristae, abnormal shapes, some of them contained amorphous and electron- dense material and myelin figures.

The rER showed dilatation of their channels, and even vesiculisationthese changes were similar to those observed ultrastructurally in the study of (Andrzejweska et al 1996**,** 1998), on acute pancreatitis of taurocholate treated rats group suggested that these

changes may reflect sublethal changes due to ATP deficiency during acute pancreatitis. The lack of sufficient energy supply may induce the morphological alterations such as dilatation of rER cisternae an Golgi apparatus.

In addition to mitochondrial morphological alterations which underlie cell organellar damage, mitochondria play a central role in regulating cell death since mitochondrial membrane permeabilization (MMP) is a universal trigger of both apoptosis and necrosis and is often considered as the point of no return in the chain of events leading to cell death (Duchen, 2004; Gukovsky et al 2012).

The molecular mediating mitochondrial permeabilization are not fully understood (Kroemer et al 2007; Richelli et al 2011)

explained that key manifestations of mitochondrial permeabilization triggering apoptotic and necrotic pathways are, respectively, the release of the mitochondria resident protein cytochrome c as well as other apoptosis-inducing factors into the cytosol and mitochondrial depolarization. Once in the cytosol, cytochrome c stimulates activation of specific cysteine proteases, the caspases, which mediate the downstream apoptotic events. On the other hand, loss of the mitochondrial membrane potential, ultimately leads to ATP depletion and necrosis.

Thus, mitochondrial permeabilization is a central event in both apoptotic and necrotic cell death.

The authors concluded that pancreatitis causes acinar cell mitochondria depolarization, mediated by the permeability transition pore (PTP) and that mitochondrial damage, increases the demand for efficient lysosomal degradation and therefore aggravates the pathologic consequences of lysosomal dysfunction.

Also mitochondria has an important role in maintaining Ca^{2+}homeostasis (Dolman et al 2005).Exocytosis requires large Ca^{2+}signals as well as energy in the form of ATP. Another role of mitochondria was to integrate local cytosolic Ca^{2+} that activates Kreb's cycle enzymes and drive ATP production and maintaining a mitochondrial membrane potential of 150-180mV across the inner mitochondrial membrane. Elevation of Ca^{2+}have been shown to be essential to the induction of apoptosis in isolated pancreatic acinar cells by ROS-producing oxidant menadione (Gerasimenko et al 2012) also toxic hypercalcaemia cause disruption of acinar cell ultrastructure and premature activation of serine proteases which is generally believedto be the crucial step in the escalation of acinar cell injury to acute pancreatitis (Pandol et al, 2007).

An interesting result morphologically of our study was the presence of many acinar cells showed ultrastructuralearly changes of apoptosis (pyknotic nuclei, cell shrinkage in the group of rats receiving 500IU of L-asparaginase as well as 1000IU.

Studies of (Kaiser et al,1996) have found that induction of apoptosis in pancreatic acinar cells attenuates the severity of experimental acute pancreatitis. Furthermore, they demonstrated that the inhibition of apoptosis by the administration of cyclohexamide was noted to worsen the severity of acute pancreatitis.

Biochemical and morphological examination of experimental models of AP has shown that severe AP is associated primarily with necrosis and little apoptosis, (e.g. that induced by pancreatic duct ligation in the opossum, by choline deficient andethionine supplemented diet in the mouse and by caerulin- hyperstimulation in the mouse) (Gukovskaya and Pandol, 2004). Apoptotic cell may play a significant role in affecting mortality and morbidity in severe AP. Control of apoptosis could be a potent strategy for improvement of the clinical outcome in severe AP (Takeyama, 2005).

The extent of pancreatic acinar cell apoptosis has been shown to be inversely related to the severity of the disease, so severe pancreatitis was noted to involve extensive acinar cell necrosis, but very little acinar cell apoptosis suggesting that apoptosis is a beneficial form of cell death in AP. (Kaiser et al 1995, Bhatia 2004)

Conceivably, medications or other interventions that favor the development of apoptosis may minimize the severity of pancreatitis, and they could, therefore be of substantial clinical value.

Our data showed no changes in body weight of different groups but the ratio of pancreatic weight relative to body weight (PW: BW) was significantly increased in groups IV and VI (ASNase 500 and 1000IU) then significantly reduced in the groups receiving octreotide groups (Vand VII).

The most probably explanation was due to increase interstitial pancreatic edema observed both lightly and ultrastructurally

Lynn et al, 1999 in their experimental studies of rats given subcutaneously injections of caerulein 24ug /kg every 8h for 2 days and the mean pancreatic weight and the pancreatic weight index were assessed by days 2,4,7 after the first injection found at day 2 no change compared to the saline controls group, by day 4 it had decreased by 42% and by 7days by 36% relative to saline controls.

They interpretated their results by explaining that the reduction in pancreaticweight index was due to acinar cell loss at 2 days and was compensated by stromal edema and increased connectivetissue deposition; reduction at 4 days mainly was due toreduced edema and stromal condensationseen morphologically as closer approximation of

theepithelial tissue elements. Even though the pancreas had a relatively normal histological appearance at 7 days, its weight remained markedly reduced compared with saline controls, indicating regeneration was incomplete.

In our study we observed hyperamylasemia (increase in serum amylase) with L-asparaginase 500 and 1000IU.

Destruction of the cell membrane, with release of pancreatic enzymes into the interstitial space, is a typical finding in human acute pancreatitis(Kloppel et al 1986).

Muller et al, 2007 using immunohitochemical tracers methods hypothesize that very early in the onset of the disease, amylase and other pancreatic enzymes leak out of the acinar cells through basolateral and basal cell membrane disruptions that allow albumin and IgG to penetrate into acinar cells as well as Ca2+ ions and exit of molecules such as enzymes.

Previous reports have shown that hyperstimulation by infusion of caerulein leads to hyperamylasemia within 30 minutes (Watanabe et al 1984)..

The role of the microvasculature, and especially alterations in the microvasculature that lead to the development of acute pancreatitis, has been emphasized by different authors. There is evidence from experimental models that pancreatitis is associated with vascular disorders such asreduced pancreatic flow, increased capillary permeability, and vascular leakage (Klar et al 1990**).** Mononuclear cell infiltration was also detected in our study and the release of cytokines and chemokines by these cells trigger the inflammatory response observed in the form of focal necrotic areas.

Multiple therapeutic modalities have been suggested for AP, but none have been unambiguously proven to be effective yet, and to date the treatment is essentially supportive.. However, the complex pathophysiology of the disease, which is still ill-defined, and the reality that numerous etiologic factors can initiate the disease through diverse mechanisms hinders the development of efficacious specific treatment **(**Greenberg et al, 2000).

The development of the long acting somato statin analogue, octreotide, introduced in the early 1980s has led to its use in experimental pancreatitis as well as in clinical patients, its longer half life allows subcutaneous administration avoiding the need for continuous intravenous infusion (Pless et al, 1986).

In our study after using octreotide with different doses of L-asparaginase we observe improvement in pancreatic histology with dose 500IU manifested by decrease zymogen granules in pancreatic acinar cells, decrease congestion and edema, mitochondria restore its normal shape.

Augelli et al, 1989 were able to show a reduction in histological severity inanimals given octreotide, 5ug/h, before the induction of pancreatitis although no effect was seen if treatment was delayed until pancreatitiswas established despite continuing treatment for 24h.

Murayama et al, 1990 giving octreotide immediately showed beneficial effects on both hitopathological and biochemical parameters in experimental pancreatitis. Less pancreatic edema, necrosis and inflammatory cell infiltration, reduction in serum amylase were also observed.

Kaplan et al, 1996 reported in their study of acute experimental pancreatitis that octreotide ameliorated pancreatic edema and histopathological injury score at different times, no deadline to begin octreotide treatment was indicated.

Results from clinical studies show evidence of a trend towards a reduction in death and complications rate with octreotide.

Paran et al, (1995) reported positive results for octreotide treatment in a small patient population with severe acute pancreatitis. The authors found significantly decreases in septic complications (74vs 26%, p=0.004) and in the development of adult respiratory distress syndrome (ARDS) (63%vs 18%) resulting in a significantly shorter hospital stay and the mortality rate was lower in the group treated with octreotide.

Octreotide significantly reduced the frequency of ARDS (40vs18%), circulatory shock and mortality.

Octreotide have been evaluated in the prophylaxis against acute pancreatitis after ERCP (Binmoeller et al 1992).

The proposed mechanisms of action of octreotide in acute pancreatitis in addition to inhibition of pancreatic secretion is the stimulation of the phagocytic cells of the reticuloendothelial system.In experimental acute pancreatitis, survival can be improved by stimulation of the reticuloendothelial system with either zymosan or glucan **(**Browder et al 1987). Conversely, depression of the reticuloendothelial system results in worsened survival. There is also some evidence of reticuloendothelial system depression in patients with acute pancreatitis (Banks et al 1991).

In our study we found decrease in inflammatory cells and mononuclear cells infiltration, since octreotide has been known to have an anti-inflammatory activity by reducing the inflammatory reactions and the release of inflammatory mediators such as TNF-α, IL-1β and IL-6, oxygen free radicals, thus prevent the progression of acute pancreatitis from mild to severe form and block the progression of

systemic inflammatory response syndrome and avoid multiple organ dysfunction.

Severe acute pancreatitis (SAP) is a common acute abdomen in clinical practice, and has become a hot spot of research in recent years because of its acute onset, rapid progression and high mortality.

Octreotide is one of the common SAP medications at present [15-78]**.**As a common used drug to treat SAP, octreotide provide good protection of multiple organs in SAP rats.

The underlying mechanisms include decreasing the release of inflammatory mediators, inhibiting inflammatory reaction, stabilizing cellular structure, reducing cell necrosis, improving microcirculation, and intensifying immunity It also alleviated injury to the intestinal mucosa and improved the survival of SAP rats.

In conclusion

The administration of ASNase by itselftends to directly aggravate histological damage in the pancreasand induce pre-pancreatitis. In addition, prior treatmentwith octreotide prevents the development of ASNaseinducedpancreatic injury. However, octreotide may providesignificant benefits to patients recovering from ASNaseinducedpancreatitis and improve treatment outcome in ALL.

Further research should focus on the mechanism of ASNase-associated toxicity and the unidentified risk factorsthat predispose some patients to pancreatitis.

References

1. Andrzejweska A, Dlugoszj W, Jurkowska G. The effect of antecedent acute ethanol ingestion on the pancreas ultrastructure in taurocholate pancreatitis in rats. 1998 Exp. MolPathol. 65, 64–77.
2. Altman A, Dindorff P and Quin J J Acute pancretitis in association with cytosine arabinosidetherapy.Cancer. **1982**;49: 1384-1386.
3. Alvarez OA and Zimmerman GPegasparaginase-induced pancreatitis. Medical and Pediatric oncology **2002;**34: 200-205.
4. Avramis VI, Sencer S and Periclou APA randomized comparison of native Esherichia Coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukaemia: a Children's Cancer Group study. Blood **2002**; 99:1986-1994.
5. Bai HX, Lowe ME and Hussain SZ What have we learned about acute pancreatitis in children.Journal of pediatric Gastroenterology and nutrition. 2011; 52: 262-270.
6. Banks RE, Evans SW, Alexander D *et al.,* Alpha 2 macroglobulin state in Acute pancreatitis. Raised values of alpha 2 macroglobulinproteasecomplexes in severe and mild attacks. Gut 1991; 32:
7. Bhatia M. Apoptosis versus necrosis in acute pancreatitis.Am J PhysiolGastrointest Liver Physio l286: G189–G196, 2004.
8. Bhatia M, Wong FL, Cao Y *et al.,* Pathophysiology of acutepancreatitis. (2005) Pancreatology 5(2–3):132–144.
9. Binmoeller KF, Harris AG, Dumas R et al**.** Does the somatostatin analogue octreotide protect against ERCP induced pancreatitis? Gut 1992; 33:1129-33
10. Bonatti H, Tabarelli W, Berger N *et al.,* Successful management of a proximal pancreatic duct fistula following pancreatic transplantation.Dig Dis Sci. 2006;51:2026–2030.
11. Browder WI, Sherwood E, Williams D, Jones E *et al.,* Protective effectofglucan enhanced macrophage function in experimental pancreatitis. AmJSurg 1987; 153: 25-33.
12. Canellos GP, Haskell C M, Arseneau J *et al.,* Hypoalbuminemic and hypocholesterolemic effect of L-asparaginase (NSC-109,229) treatment in man. A preliminary report. CancerChemother. Rep. 1969; 53: 67-69.
13. Chen X, Li C, Izumi T, Ernst SA, *et al.,* Rab27b localizes to zymogen granules and regulates pancreatic acinar exocytosis. BiochemBiophys Res Commun 2004;323:1157–1162.
14. Chnavov M, Petresen OH and Tepikin **A** Free radicals and the pancreatic acinar cells: role in physiology and pathology. Phil trans RSoc B 2005; 360:2273-2284
15. Czako L, Hegyi P, Takacs T, *et al.,* Effects of octreotideonacute necrotizing pancreatitis in rabbits. World J Gastroenterol2004; 10: 2082-6.
16. Duchen M R. Mitochondria in health and disease: perspectives on a new mitochondrial biology 2004 Mol.Aspect med.vol 25 pp. 2082-6
17. Duval M, Suciu S, Fester A *et al.,* Comparaison of Esherechia Coli-asparaginase with Erwinia-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European organization for research and treatment of Cancer-Children's Leukemia Group phase 3 trial. Blood. 2002;99:2734-273.
18. Dolman NJ, Gerasimenko JV, GerasimenkoOV *et al.,* Stable Golgmitochondria complexes and formation of Golgi Ca^{2+}gradients in pancreatic acinar cells. 2005; J Biol chem. 280: 15794-15799.
19. Falletta JM, Steuber CP, Hayes JW, *et al.,* Nonketotichyperglycemia due to prednisone (NS 10023) following ketotic hyperglycemia due toL-asparaginase (NSC 109299) plus prednisone. Cancer Chemother Rep.1972;56(1):781–2.
20. Flores-Calderon J, Exiga-Gonzalez Eand Moran-Villota S *et al.,* Acute pancreatitis in children with acute lymphoblastic leukemia treated with L-asparaginase. Journal of Pediatric Hematology/Oncology. 2009; 31:790-793.
21. Frick WTThe role of calcium in acute pancreatitis. Surgery2012;152(3S): S157-S163.
22. Gaisano HY, Lutz MP, Leser J, *et al.,* Supramaximal cholecystokinindisplaces Munc18c from the pancreatic acinar basal surface, redirectiapical exocytosis to the basal membrane. J Clin Invest 2001;108:1597–1611.
23. Gardner TB, Vege SS and Chari ST *et al.,*.Faster rate of initial fluid resuscitation in severe acute pancreatitis

diminishes in-hospitmortality.Pancreatology. 2009; 9: 770-776.
24. Garrington T, Bansard D, Ingram D, *et al.,* Successful managementwith octreotide of a child withL-asparaginase induced hemorrhagepancreatitis**.**1998; Med PediatrOncol, 30: 106-9
25. Gerasimenko OV and Gerasimenko JVMitochondrial function and malfunction inpathophysiology of pancreatitis. Eur J Physiol 2012; 464:89-99
26. Gorelick FS, Thrower E**.** The acinar cell and early pancreatitisresponses.ClinicalGastrenterologyand hepatology. 2009;7: S10-S14.
27. Gokbuget N, Hoelzer D**.** Recent approaches in acute lymphoblasticleukemia in adults. Rev ClinExpHematol 2002;6(2):114–41.
28. Grady T**,** Saluja A, Kaiser A, Steer M. Edema and intrapancreatictrypsinogen activation precede glutathione depletion during caeruleinpancreatitis. Am J Physiol 1996;271:G20–G26.
29. Greenberg R, Haddad R, Kashtan H, *et al.,* The effects ofsomatostatin and octreotideon experimental and human acutepancreatitis.J Lab Clin Med. 2000;135:112–121
30. Gugliota L, Mazzucconi MG, Leone G, *et al.,* Incidence of thrombotic complications in adult patients with acute lymphoblastic leukemia receiving l-asparaginase during induction therapy: a retrospective study. Eur J Hematol 1992;49:63–6.
31. Gukovskaya AS, Pandol SJ Cell death pathways in pancreatitis andpancreatic cancer. Pancreatology 2004; 4(6):567–586
32. Gukovsky I, Gukovskaya AS, Blinman TA *et al.,* Early NF--kβactivation is associated with hormone-induced pancreatitis. 1998 Am JPhysiol;275:G1402–G1414.
33. Gukovsky I and Gukovskaya AS**.** Impaired autophagy underlies key pathological responses of acute pancreatitis. Autophagy 2010 6: 428–429.
34. Gukovsky I, Pandol SJ and Mareninova AO *et al.,* impairedautophagy and organellar dysfunction in pancreatitis. Journal ofgastroenterology and hepatology 2012; 27(2): 27-32.
35. Hann I, Vora A, Richards S, *et al.,* Benefit of intensified treatment forall children with acute lymphoblastic leukemia: results from MRC UKALL XI and MRC ALL97 randomized trials. Leukemia 2000;14:356–63.
36. Hashimoto D, Ohmuraya M, Hirota M *et al.,*.Involvement of autophagy in trysinogen activation within the pancreatic acinar cells. The journal of cell biology 2008; 181(7):1065-1072
37. Hill JM, Roberts J and Loeb E. L-asparaginase therapy for leukemia and other malignant neoplasms. Remission in human leukemia Jama.1967;202: 882-888.
38. Hirota M, Ohmuraya S and Baba H genetic background of pancreatitis. Post graduate Med. J. vol. 82, pp.775-778.
39. Hjalgrim LL, Rostgaard K and Hjalgrim H *et al.,* Birth weight and risk for childhood leukemia in Denmark, Sweden, Norway, and Iceland. Journal of the National Cancer Institute. 2004; 96: 1549–1556.
40. Hoogerbrugge N, Jansen H, Hoogerbrugge PM. Transient hyperlipidemia during treatment of ALL with L-asparaginase is related to decreased lipoprotein lipase activity. Leukemia 1997;11(8):1377–9.
41. Jungermann J, Lerch MM, Weidenbach H**,** *et al.,* Disassembly of rat pancreatic acinar cell cytoskeleton during supramaximal secretagogue stimulation. Am J Physiol 1995;268:G328–G338.
42. Katz MD, Erstad BL. Octreotide, a new somatostatin analogue. ClinPharm. 1989; 8:255–273.
43. Kaiser AM, Saluja AK, Lu L, *et al.,* Effects of cycloheximide on pancreatic endonuclease activity,apoptosis, and severity of acute pancreatitis. Am. J. Physiol. 1996; 271: C982–93.
44. Kaplan O, Kaplan D, Casif E *et al.,* Effects of delayed administration of octreotide in acute experimental pancreatitis. J Surg Res 1996;62:109-17.
45. [45] Kearney SL, Dahlberg SE and Levy DE *et al.,*.Clinical course and outcome in children with acute lymphoblastic leukaemia and asparaginase-associated pancreatitis. Pediatric Blood and Cancer 2009; 53:162-167.
46. Klar E, Messmer K, Warshaw AL, *et al.,* Pancreatic ischaemia in experimental acute pancreatitis: mechanism, significance and therapyBr J Surg. 1990;77(11):1205Y1210.
47. Kloppel G, Dreyer T, Willemer S, *et al.,* Human acute pancreatitis: itspathogenesis in the light of immunocytochemical and ultrastructuralfindings in acinar cells. Virchows Arch A PatholAnatHistol. 1986;409(6):791Y803
48. Kroemer G, Galluzzi L, and Brenner C. Mitochondrial membranepermeabilization in cell death. Physiol Rev 2007 87: 99–163.
49. Leonard JV, Kay JDS. Acute encephalopathy and hyperammonaemia complicating treatment of acute lymphoblastic leukaemia with asparaginase. Lancet 1986;i:162–3.
50. Lerch MM, Adler G. Experimental animal models of acute pancreatitis. Int. J. Pancreatol. 1994; 15: 159–70.
51. Li ZS, Pan X, Zhang WJ, *et al.,* Effect of octreotide administration in the prophylaxis of post-ERCPpancreatitis and hyperamylasemia: Amulticenter, placebo-controlled, randomized clinicaltrial. Am J Gastroenterol. 2007;102:46–51.
52. Lorentz,K. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes.Part 9.IFCC method for alpha-amylase (1,4-alpha-D-glucan 4-glucanohydrolase, EC 3.2.1.2) International Federation of clinical chemistry and Laboratory medicine(IFCC). Committee on enzymes. Clin. Chem. Lab. Med 1998;36: 185-203.
53. Lynn RE and Walker IN acinar cell apoptosis and the origin of cellular complexes in caerulin induced pancreatitis Int J Exp Biology 1999 vol 20 pp.205-215.
54. Malmstrom ML, Hansen MB, Andersen AM, *et al.,* Cytokines and organ failure in acute pancreatitis: acute inflammatory response in acute pancreatitis. Pancreas 2012; 41:271-277.
55. Mareninova OA, Hermann K, French SW, *et al.,* Impaired autophagicflux mediates acinar cell vacuole

formation and trypsinogen activation in rodent models of acute pancreatitis. J Clin Invest 2009;119: 3340–3355.
56. Mithofer K, Fernandez-Del Castillo C, Rattner D, *et al.,* Subcellular kinetics of early trypsinogen activation in acute rodent pancreatitis. Am J Physiol 1998;274:G71–G79.
57. Muller WM, Paul L, Peter B *et al.,* Acinar Cell Membrane Disruption Is an Early Event inExperimental Acute Pancreatitis in Rats. Pancreas 2007; 35(4): e30-e40
58. Murayama KM, Drew JB, Joehl RJ. Does somatostatin analogueprevent experimental acute pancreatitis? Arch Surg 1990;125:1570-2.
59. Niederau C and Grendell JH. Intracellular vacuoles in experimental acute pancreatitis in rats and mice are an acidifiedcompartment. J Clin Invest1988; 81: 229–236.
60. Offman MN, Krol M, Patel N *et al.,* Rational engeneering of L-asparaginase reveals importance of dual activity for cancer cell toxicity. Blood 2011;117:1614 -2739.
61. T, Matej R, Zadinova M, *et al.,* Expression of proteinase- activated receptor 2 during taurocholate-induced acute pancreatic lesiondevelopment in Wistar rats. Int J Gastro intest Cancer 2001;30:113–121.
62. Ortega JA, Nesbit ME, Jr.Donaldson MH *et al.,* L-asparaginase, vincritine, and prednisone for induction of first remission in acute lymphocytic leukemia. Cancer research 1977; 37:535-540.
63. Pandol SJ Acute pancreatitis.Current opinion in Gastroenterology 2006; 22: 481-486.
64. Pandol SJ, Saluja AK, ImrieCW *et al.,* Acute pancreatitibench to the bedside. Gastroenterology 2007; 132: 1127–51.
65. Paran H, Klausner J, Siegal A *et al.,* Effect of the somatostatin analogue octreotide on experimental pancreatitis in rats. J Surg Res 1996;62:201-6.
66. Pastor CM, Matthay M A and Frossard JL Pancreatitis-associated acute lung injury: new insights.Chest 2003; 124:2341-2351.
67. Pless J, Bauer W, Briner U *et al.,* Chemistry and pharmacology ofSMS 201-995, a long-acting octapeptide analogue of somatostatin.Scand J Gastroenterol Suppl. 1986;119:54–64.
68. Pratt CB, Choi SI, Holton CP. Low-dosage asparaginase treatment of children acute lymphocytic leukemia. Am J Dis Child 1971;121:406–9.
69. Pui CH, Sandlund JT, Pei D *et al.,*. Improved outcome for children with acute lymphoblastic leukemia: results of Total Therapy Study XIIB at St. Jude Children's Research Hospital. Blood 2004; 104: 2690-2696.
70. Putter J Pharmacokinetic behavior of L-asparaginase in men and in animals. Recent results cancer Res. 1970 vol.33, pp. 64-74.
71. Raja AR, Schmiegelow K and Fandsen LT Asparaginase –associated pancreatitis in children. British journal of Haematology 2012;159: 18-27.
72. Raraty M, Ward J, Erdemli G, *et al.,* Calcium-dependent enzyme activation and vacuole formation in the apical granular region ofpancreatic acinar cells. Proc. Natl Acad Sci USA 2000;97:13126-31.
73. Rau B, Bertram P, Frank G *et al.,* Pathophysiologic role of oxygen free radicals in acute pancreatitis. Initiating Event or Mediator of Tissue Damage ? Annals of surgery 2000; 231:352-360.
74. Richelli F and Seleikete J. Shedding light on the mitochondrial permeability transition. Biochim. Biophys. Acta 2011 vol 1807, pp.482-90
75. Saluja AK, Lerch MM, Phillips PA, *et al.,* Why does pancreatic overstimulation cause pancreatitis? Annual Rev.Physiol 2007; 69:249– 269
76. Silverman LB, Gelber RD, Dalton VK *et al.,* Improved outcome for children with acute lymphoblastic leukemia: results of Dana-FarberConsortium Protocol 91-01. Blood 2001; 97: 1211–1218.
77. Storring JM, Brandwein J, Gupta V, *et al.,* Treatment of adult acute lymphoblastic leukemia (ALL) with a modified DFCI pediatric regimendThe Princess Margaret experience [abstract]. Blood. 2006;108(ASH Annual Meeting Abstracts): Abstract 1875.
78. Suzuki M, Shimizu T, Kudo T *et al.,* Octreotide preventsasparaginase-induced pancreatic injury in rats. Experimental Hematology2008; 36: 172–180.
79. Takeyama Y. Significance of apoptotic cell death in systemicomplications with severe acute pancreatitis. astroenterol2005; 40:1–10
80. Treepongkaruna S, Thongpak N, Pakakasama S *et al.,* Acutepancreatitis in children with acute lymphoblastic leukemia after chemotherapy. Journal of Pediatric Hematology/Oncology 2009; 31:812–815.
81. Van den Berg H. Asparaginase revisited. Leukemia and Lymphoma 2011; 52,: 168–178.
82. Vrooman LM, Supko JG, Neuberg DS *et al.,* Erwiniaasparaginaseafter allergy to E. coli asparaginase in children with acute lymphoblasticleukemia. Pediatric Blood and Cancer,54,199–205.
83. Watanabe O, Baccino FM, Steer ML, *et al.,* Supramaximalcaerulein stimulation and ultrastructure of rat pancreatic acinar cell: early morphological changes during development of experimental pancreatitis. Am J Physiol. 1984;246(4 Pt 1):G457YG467
84. Whitcomb DC and Cohn JA pancreatic exocrine dysfunction principles of molecular medicine, second edition 2006; 573-581.
85. Zaheer A, Singh VK, Qureshi RO *et al.,* The revised Atlanta classification for acute pancreatitis: updates in imaging terminology and guidelines. 2012 Abdominal Imaging, Epub ahead of print. doi:10.1007/s00261-012-9908-0.
86. Zavyalov T, KhoytsynaY, Tenner S.The role of antibiotics in the management of patients withacute necrotizing pancreatitis. Current infectious disease Reports 2010;12:13-18.