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RESEARCH ARTICLE

Serum Ghrelin and Plasma Insulin Levels were altered during Disease Course of L-Arginine induced Acute Pancreatitis

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Abstract

Objectives: To evaluate ghrelin serum level changes in animal model of L-arginine induced acute pancreatitis (AP) and the therapeutic effect of concomitant ghrelin administration.

Materials & Methods: 30 rats were divided into 3 equal groups: Group A; received intra-peritoneal injection (ipi) of sterile water and Groups B and C received ipi of L-arginine solution (500 mg/100 g) and group C received 2-days subcutaneous injections of ghrelin (10 nmol/kg). Fasting blood samples were obtained, prior to AP induction, 12, 24 and 48-hr after AP induction for estimation of fasting blood glucose (FBG), plasma insulin (FPI) and serum lipase, amylase, C.reactive protein(CRP) and ghrelin. At 48-hr, all animals were sacrificed and pancreas was excised for histopathological examination and scoring.

Results: Compared to control and pre-induction levels, serum amylase and lipase levels were significantly high at 12 and 24-hr and declined at 48-hr. FPI levels were significantly lower at 12 and 24-hr, but non-significantly elevated at 48-hr. FBG levels were significantly higher at 12 and peaked at 24-hr. Serum CRP levels at 12, 24 and 48-hr were significant high, while serum ghrelin levels were significantly lower at 12 and 24-hr, but started to increase at 48-hr. Regression analysis defined serum amylase as significant positive and FPI level as significant negative early predictor for histopathological injury. Serum amylase, lipase and CRP levels were significantly lower at 12, 24 and 48-hr and mean FPI levels were significantly higher with significantly lower FBG levels at 24 and 48-hr in group C compared to group B.

Conclusion: FPI and serum ghrelin levels were correlated negatively with pathological score. Serum ghrelin levels could be used for follow-up of cases with AP as a prognostic marker. Ghrelin administration ameliorated the altered pancreatic functions and aids subsidence of inflammation.

Introduction

Ghrelin, an acylated 28-amino acid polypeptide, is highly conserved among species and is classified into octanoylated (C8:0), decanoylated (C10:0), decenoylated (C10:1) and nonacylated ghrelin (des-acylated ghrelin, des-AG, dAG). Acylated ghrelin (AG) is the major active form of human ghrelin. Although the exact ratio of circulating AG to dAG varies depending on metabolic status, the majority of ghrelin circulates in the dAG form **(Khatib et al., 2014)**.

The primary production site of ghrelin is the stomach, and it interacts with stomach ghrelin as well as hypothalamic growth hormone (GH) releasing hormone and somatostatin in the regulation of pituitary GH secretion. Ghrelin is an endogenous ligand for the GH secretagogue receptors (GHSR) which are predominantly expressed in the pituitary and hypothalamus where it mediates AG-induced feeding and adiposity. Ghrelin stimulate GH release through the GHS receptor to increase intracellular Ca^{2+} levels via inositol triphosphate signal transduction pathway. Ghrelin provides a definitive proof of the occurrence of a GHS-GHS receptor signaling system in the regulation of GH secretion. Acting on GHS-R, ghrelin strongly and dose dependently stimulates release of GH from the anterior pituitary. The presence of an acyl side chain (mainly *n*-octanoic acid) attached to the ghrelin peptide is required for full agonism of GHSR **(Heppner et al., 2011; Yin et al., 2014)**.

Some in vitro evidence suggests that dAG interacts with GHSR, although this occurs at significantly lower levels compared with AG. Des-acylated ghrelin was initially considered as an inactive by-product of ghrelin secretion and degradation. However, multiple reports have suggested that dAG acts in peripheral tissues and in the brain to regulate biological actions, including the control of feeding, body temperature, muscle atrophy and lipid metabolism. All of these actions are attributed to GHSR-independent mechanisms **(Toshinai et al., 2006; Inoue et al., 2013; Porporato et al., 2013)**.

Ghrelin has been shown to suppress glucose-induced insulin release. This insulinostatic action is mediated by Gαi2 subtype of GTP-binding proteins and delayed outward K^+ channels. Interestingly, ghrelin is produced in pancreatic islets. The ghrelin originating from islets restricts insulin release and thereby upwardly regulates the systemic glucose level. Furthermore, blockade or elimination of ghrelin enhances insulin release, which can ameliorate glucose intolerance in high-fat diet fed mice and ob/ob mice **(Yada et al., 2014)**.

Acute pancreatitis is a disease which develops with perivascular infiltration and inflammation characterized by lipid necrosis, polymorphonuclear leukocyte infiltration, hemorrhage, acinar cell necrosis and tissue edema in the pancreas. The two most common etiological factors of acute pancreatitis are gallstones (including small gallstones or microlithiasis) and alcohol abuse. Acute pancreatitis is still a serious disease as its diagnosis and treatment is difficult. It is usually accompanied by mild and restricted clinical conditions; however, necrotizing acute pancreatitis may sometimes result in a serious outcome. Despite current intensive care techniques, the development of nutritional support applications, fluid-electrolyte replacement and mechanic ventilation facilities, the mortality rate may reach 10-20% **(Rebours, 2014; Rohan Jeyarajah et al., 2014)**.

The current prospective study aimed to evaluate serum ghrelin level changes in animal model of L-arginine induced acute pancreatitis (AP) compared to control animals and to evaluate the therapeutic effect of concomitant administration of ghrelin on serum diagnostic parameters of AP.

Materials & Methods

The current study was conducted at Department of Physiology, Faculty of Medicine, Benha University since Jan till April 2014. The study protocol was approved by the Local Ethical Committee, Benha Faculty of Medicine.

Animals

The present study included 30 male albino rats with weight range of 250-300 grams obtained from Faculty of Agriculture, Moshtohor. Rats were grouped and kept in separate animal cages, under the prevailing atmospheric conditions and maintained on a balanced diet (bread, barely, carrots, lettuce, and milk) and fresh-water supply with a 12:12-h light/dark cycle. At the end of the experiment and after collection of the samples we get rid of the animals in the incinerator of Benha university hospital.

Grouping

The animals were randomly divided into 3 equal groups (n=10):

- **Group A (Control group)** included rats received intra-peritoneal injection of sterile water in a volume of 2.5 ml/100 g body weight to exclude any effect of distilled water which was used as vehicle for L-arginine.
- **Group B** included rats received intra-peritoneal injection of L-arginine solution for induction of AP.
- **Group C** included rats received intra-peritoneal injection of L-arginine solution for induction of AP and concomitantly received subcutaneous injection of ghrelin in a dose of 10 nmol/kg for 2 days.

Rats of the three groups were kept on pre-test conditions without change and were scarified 48 hours after induction of AP. If an animal died spontaneously due to AP complications, it was replaced by another so as to keep the number of study animals sacrificed at 48-hr after AP induction equal to the control group.

Induction of pancreatitis

L-argininge (Sigma; Aldrich) was dissolved in distilled water immediately before use to give a final concentration of 200 mg/ml. Each of study animal received intra-peritoneal injection of the prepared solution of L-arginine in a dose of 500 mg (2.5 ml)/100 g body weight as previously described by **Tani et al., (1990)**.

Laboratory investigations

- Fasting venous blood samples, withdrawn from the tail vein, were obtained, prior to AP induction, 12, 24 and 48-hr after AP induction. Blood samples were divided into 2 parts:
  - A) The first was put in a tube containing sodium fluoride (2 mg sodium fluoride/ ml blood) to prevent glycolysis. Plasma was separated by centrifugation and used for estimation of glucose by enzymatic colorimetric assay, Infinity™ Glucose Oxidase Reagent (sensitivity 3.3 mmol/L; ThermoFisher, Loughborough, UK), **(Tinder, 1969)**.
  - B) The second part was divided into two parts the first was collected in EDTA containing tube for RIA estimation of fasting plasma insulin (FPI) **(Gordon et al., 1985)**. The second part was allowed to clot then serum was separated by centrifugation at 3000 rpm for 10 min. Serum was removed and used for ELISA estimation of: serum lipase **(Pezzilli et al., 1999)** and amylase **(Treacy et al., 2001)** using the commercially available kit (Randox Laboratories Limited, United Kingdom) and serum ghrelin using the commercially available kit (Linco Research Inc. St. Charles, 63304 MO, USA) **(Peterli et al., 2009)**.

Histopathological examination

At 48-hr after AP induction, animals of groups A and B were sacrificed and pancreas was excised and preserved in formalin solution. Paraffin sections were cut into 5 µm-thick slices and stained with hematoxylin and eosin. Sections were evaluated according to **Niederau et al., (1986)** using light microscope at magnification of 20 for histopathological changes. Assessment of pancreatic damage was scored by grading acinar cell degeneration, interstitial inflammation, edema and hemorrhage as described by Schmidt's standards **(Schmidt et al., 1992)** with modification as shown in table 1.

Statistical analysis

Obtained data were presented as mean±SD and ranges. Results were analyzed using Wilcoxon; ranked test for unrelated data (Z-test). Statistical analysis was conducted using the SPSS (Version 15, 2006) for Windows statistical package. P value <0.05 was considered statistically significant.

Results

Baseline levels of estimated parameters showed non-significant ($p>0.05$) difference between animals of the three groups. The injected dose of L-arginine was sufficient for AP induction as manifested by significantly ($p<0.001$) higher serum amylase and lipase levels estimated at 12-hr after injection compared both to control and pre-induction levels in both group B and C. Serum levels of both amylase and lipase reached peak level at 24-hr after AP induction and declined at 48-hr after AP induction in both groups. However, serum levels of both enzymes estimated at 24-hr and 48-hrs were significantly higher compared to control levels and to its respective levels estimated at pre-induction and 12-hr after induction, but levels estimated at 24-hr were significantly higher levels compared to at 48-hr levels. Serum levels of amylase and lipase were significantly lower at 12, 24 and 48-hr after AP induction in group C compared to group B, (Table 2, Fig. 1 & 2).

Induction of AP affected not only the exocrine function of pancreas but also affected its endocrine function. In group B, such effect was manifested by significantly lower FPI levels estimated at 12-hr and 24-hr after AP induction compared to control and pre-induction levels. On contrary, FPI levels estimated at 24-hr were non-significantly lower compared to levels estimated at 12-hr levels. FPI levels estimated at 48-hr after AP induction started to increase and were non-significant lower compared to control and pre-induction levels, but were non-significantly higher compared to levels estimated at 12 and 24-hr after AP induction. In group C, the ameliorative effect of therapeutic ghrelin was evident and manifested as non-significant decrease of FPI levels at 12, 24 and 48-hr after AP induction compared to control and pre-induction levels and at 24-hr compared to at 12-hr with non-significantly higher levels at 48-hr compared to at 12, 24 and 48-hr after induction of AP. Moreover, mean FPI levels estimated in group C were non-significantly higher at 12-hr, but were significantly higher at 24 and 48-hr compared to corresponding levels of group B, (Table 3, Fig. 3).

On opposite direction to FPI, at 12-hr FBG levels were significantly higher compared to control and pre-induction levels and peaked at 24-hr after AP induction. Despite the increased FPI at 48-hr after AP induction, FBG levels at 48-hr were still significantly higher compared to at 12 and 24-hr in both group B and C. However, mean

FBG levels estimated in group C were non-significantly lower at 12-hr, but were significantly lower at 24 and 48-hr compared to corresponding levels of group B, (Table 3, Fig. 4).

Induction of AP strenuously induced inflammatory response as manifested by significantly higher serum CRP levels estimated at 12, 24 and 48-hr after AP induction compared to control and pre-induction levels. Estimated serum CRP levels at 12 and 24-hr were significantly higher compared to levels estimated at 48-hr with significantly higher levels estimated at 24-hr compared to at 12-hr levels in both group B and C. However, mean serum CRP levels estimated in group C were significantly lower at 12, 24 and 48-hr after induction of AP compared to corresponding levels of group B, (Table 4, Fig. 5).

Estimated serum ghrelin levels in group B showed progressive decrease peaked at 24-hr after AP induction with significantly lower levels estimated at 24-hr compared control and pre-induction levels, but were non-significantly lower compared to levels estimated at 12-hr after induction. At 48-hr after AP induction, serum ghrelin levels started to increase and were non-significantly lower compared to control and pre-induction levels and non-significantly higher compared to levels estimated at 12 and 24-hr levels, (Table 5, Fig. 6).

Histopathological examination of control pancreatic tissue showed normal structure of pancreatic lobes and acini. Pancreatic tissue was formed of closely packed acini separated from each other by very little connective tissue septae. The acinar cells are pyramidal in shape with basophilic basal nucleus and acidophilic cytoplasm (Fig. 7). Histopathological examination of specimens of pancreatic tissue obtained from animals of group B showed marked changes in the form of massive inflammatory infiltrate, massive interlobular edema and dilated congested blood vessels with inflammatory cellular infiltrate around them (Fig. 8-10). All control pancreatic specimens were scored 0, while mean pathological score of pancreatic specimens of animals of group B was 6.2±1.2; range: 5-8.

Histopathological score determined at time of animal scarification (at 48-hr after AP induction) in group B showed positive significant correlation with serum levels of amylase and CRP, but showed negative significant correlation with serum ghrelin estimated at 12-hr after AP induction. Histopathological score showed positive significant correlation with serum amylase and lipase levels and negative significant correlation with serum ghrelin and FPI levels estimated at 24-hr after AP induction. At 48-hr after AP induction, histopathological scores showed negative significant correlation with serum ghrelin level (Table 6).

Regression analysis to verify blood levels of estimated parameters at 12-hr after AP induction as early predictor for histopathological injury as judged by histopathological scoring showed that serum level of amylase was significant positive predictor in one model, while FPI level was significant negative predictor in two models, while other estimated parameters were non-significant predictors (Table 7).

Discussion

The current animal model of acute pancreatitis (AP) showed some interesting data; firstly, AP affects not only exocrine pancreatic function manifested by significantly higher serum amylase and lipase levels, but also affects pancreatic endocrine function as manifested by decreased fasting plasma insulin (FPI) levels in association with hyperglycemia and all these changes were manifested early at 12-hr after AP induction.

In support of these findings, **Zechner et al., (2012)** using AP animal model induced in streptozotocin (STZ)-treated diabetic mice found diabetes aggravated AP, inhibited regeneration of the exocrine tissue and led to strong atrophy of the pancreas and during the regenerative phase, diabetes augmented inflammation, increased cell death, reduced acinar cell expansion, but administration of insulin reversed these changes in diabetic mice. Thereafter, **Zechner et al., (2014)** using animal model of chronic pancreatitis induced in STZ-treated diabetic mice, found diabetes has a detrimental influence on the progression of chronic pancreatitis by aggravating fibrosis, inflammation and pancreatic atrophy.

These experimental findings are supported clinically wherein **Solanki et al., (2012)** documented that complex pathogenetic connections exist between AP and factors involved in the development and therapy of diabetes mellitus and given the high morbidity associated with an attack of AP in a diabetic patient, therapy for hyperglycemia may help to reduce the risk of development of AP and may also help to reduce the severity of an established AP attack in a diabetic patient. **Li et al., (2012)** found intensive insulin therapy (IIT) in patients with severe AP could relieve patient's condition earlier and shorten the length of hospitalization without serious adverse effect. **Aryal et al., (2013)** presented a case of AP in a young woman due to hypertriglyceridemia who was successfully treated with insulin and heparin. **Du et al., (2014)** found IIT combined with low-molecular weight heparin for treatment of severe AP noticeably increased the white blood cell count, serum albumin level and the arterial partial oxygen pressure with markedly shortened intestinal recovery time and length of hospital stay and reduced multiple organ failure, surgery and fatality rates.

In trial to explore the pathogenesis of islet cell dysfunction concomitant with AP, **Deng et al., (2011)** using acute necrotizing pancreatitis (ANP) animal model reported that microscopic examinations indicated pancreatic β

cell dysfunction and death with concomitant elevation of serum glucose and using western blot and immuno-histochemisty assay found the expression of ORP150 (150kD oxygen-regulated protein taking part in the process of endoplasmic reticulum stress) mainly appeared on pancreatic β cells and decreased gradually during pathogenesis of AP and concluded that there is probable role of ORP150 in changes in appearance and function of pancreatic β cells following AP through endoplasmic reticulum stress pathway.

Secondly, in group B, serum ghrelin was found to be significantly decreased at 12-hr and 24-hr after AP induction, but started to increase at 48-hr after AP induction. Thus, estimated serum ghrelin could be used to follow-up patients with AP and could be considered as an early marker for recovery.

These findings are supported clinically by **Ma et al., (2013)** who documented that serum level of ghrelin during early-stage AP showed significant differences between patients had mild and severe AP and it may become an early predictor of pancreatic necrosis and a degree marker of clinical severity. Also, **Panek et al., (2014)** found serum ghrelin concentrations on admission of patients with acute biliary pancreatitis (ABP) were significantly lower than in controls, but showed steadily increasing levels in patients with mild and severe AP during the course of disease and concluded that rising serum ghrelin levels during the course of ABP may be a marker of recovery and an indicator of the healing process.

Continuous consumption of normally secreted ghrelin may underlie its lower levels early in disease course and starting levels to increase indicted lessened consumption secondary to starting recovery; such explanation may indicate an anti-inflammatory or inflammation controlling role of ghrelin. In support of this attribution, serum ghrelin levels estimated at 12, 24 and 48 hours showed negative significant correlation with histopathological score indicating more consumption in animals with severe tissue destruction.

Depending on what previously documented by **Lin et al., (2002)**, that the coincidence of acute gastritis with AP is a frequent event and more than half of patients with AP may complicate with upper gastrointestinal ulcers and the occurrence of ulcer was positively correlated with the severity of pancreatitis, mucosal inflammation may alter mucosal secretion of ghrelin and this could be another explanation for altered ghrelin levels in association with AP and starting increase of serum ghrelin levels may indicate starting mucosal regeneration with concomitant secretion of ghrelin. In support of this assumption, **Liang et al., (2014)** found that in L-arginine induced ANP melatonin levels in serum or gastric tissue peaked at 6 h and returned to normal levels at 12 h after melatonin was administered, while ghrelin remained at low levels during the first 12 h, but it recovered at 24 h and continued increasing, while the levels of oxidative stress damage markers and activity of inflammatory factors were decreased and concluded that the protective effects of melatonin on acute gastric injury during the early stages of ANP may be mediated through anti-oxidative and anti-inflammatory activities, while at advanced stages of ANP, it may be mediated through the recovered endogenous ghrelin.

In group C, ghrelin administration concomitant with induction of AP resulted in amelioration of markers' alterations, wherein serum amylase, lipase and CRP levels were significantly lower in group C compared to group B at 12, 24 and 48-hr after AP induction, and mean FPI levels were significantly higher with significantly lower FBG levels at 24 and 48-hr in group C compared to group B. These findings spotlight on the ameliorative effect of ghrelin administration on severity of AP and support the data reported in group B concerning the relationship between starting increased levels of ghrelin and improvement of disease markers.

In support of these data, **Warzecha et al., (2010)** found ghrelin treatment after development of caerulein-induced AP in animal model reduced morphological signs of pancreatic damage, led to earlier pancreatic regeneration and significant reduction of biochemical indexes of disease severity and serum level of pro-inflammatory interleukin-1b (IL-1b). **Turk et al., (2012)** reported significant difference in blood glucose levels in newborn-STZ-diabetic rats compared to ghrelin treated diabetic rats at weeks 1, 2 and 4 with decreases in pancreatic non-enzymatic glycosylation and lipid peroxidation levels, while glutathione levels and enzymatic activities were increased and insulin peptide and mRNA (+) signals in islets of Langerhans showed an increase and concluded that administration of ghrelin to newborn rats may prevent effects of diabetes. Also, **Pantic et al., (2013)** found ghrelin administration increases exocrine pancreas fractal dimension and textural entropy, and decreases lacunarity.

In trial to explore the underlying mechanism for the therapeutic effect of ghrelin for AP; **Ceranowicz et al., (2010)** found ghrelin treatment of cerulein-induced AP in rats with intact pituitary reduced biochemical indexes of AP severity and morphological signs of pancreatic damage, leading to faster pancreatic regeneration, reduction in serum IL-1β levels and decrease in serum activity of amylase and lipase with improvement of pancreatic blood flow and an increase in pancreatic DNA synthesis, while hypophysectomy of rats with cerulrin-induced AP, delayed the pancreatic healing and abolished the therapeutic effect of ghrelin, but treatment with insulin-like growth factor-1 (IGF-1) exhibits therapeutic effect similar to that observed in ghrelin-treated rats with intact pituitary and concluded that therapeutic effect of ghrelin in AP is indirect and depends on growth hormone and IGF-1 release.

It could be concluded that AP induced a diabetic-like state characterized by early decreased FPI. Serum ghrelin levels were significantly decreased at 12-hr and 24-hr after AP induction, but started to increase at 48-hr after AP induction. Both serum ghrelin and FPI levels correlated negatively with pathologically confirmed disease severity. Serum ghrelin levels could be used for follow-up of cases with AP as a prognostic marker. Concomitant administration of ghrelin with AP induction ameliorated the altered pancreatic functions and aids subsidence of pancreatic inflammation.

Table (1): Grading of histopathological changes secondary to AP (Schmidt et al., 1992)

Score	Edema	Inflammation	Acinar cell degeneration	Parenchymal hemorrhage
0	Absent	Absent	Absent	Absent
1	Edema in interlobular space	Mild	Focal (<5%)	Mild
2	Edema in intralobular space	Moderate	Sublobular degeneration (<20%)	Moderate
3	Isolated island-shape of pancreatic acinus	Severe	Lobular degeneration (>20%)	Severe

Table (2): Mean (±SD) levels of serum amylase and lipase estimated in groups B and C at 12, 24 and 48-hr after induction of AP compared to Group A and pre-induction levels

parameter	Groups			Group A	Time of sampling			
					Pre-	12-hr	24-hr	48-hr
Serum amylase (U/L)				448.1±19.6				
	Group B	Level			456.4±34.6	902.4±87.4	1245.5±64	768±56.7
		P value	P1		>0.05	<0.05	<0.05	<0.05
			P2			<0.05	<0.05	<0.05
			P3				<0.05	<0.05
			P4					<0.05
	Group C	Level			468.4±43.9	760±82.4	945.5±89.1	675±84
		P value	P1		>0.05	<0.05	<0.05	<0.05
			P2			<0.05	<0.05	<0.05
			P3				<0.05	<0.05
			P4					<0.05
			P5		>0.05	<0.05	<0.05	<0.05
Serum lipase (U/L)				116.2±9				
	Group B	Level			114.8±6.6	209.5±21.6	354.4±22.8	183.1±23.3
		P	P1		>0.05	<0.05	<0.05	<0.05

		value	P2			<0.05	<0.05	<0.05
			P3				<0.05	<0.05
			P4					<0.05
	Group C	Level			117.3±6.8	185.5±17.1	246.2±51.3	162±21
		P value	P1		>0.05	<0.05	<0.05	<0.05
			P2			<0.05	<0.05	<0.05
			P3				<0.05	<0.05
			P4					<0.05
			P5		>0.05	<0.05	<0.05	<0.05

P1: significance versus control levels; P2: significance versus pre- induction level in the study groups; P3: significance versus 12-hr post-induction level in the study groups; P4: significance versus 24-hr post-induction level in the study groups; P5: significance of difference between groups B and C.

Table (3): Mean (±SD) levels of FPI and FBG estimated in Groups B and C at 12, 24 and 48-hr after induction of AP compared to Group A and pre-induction levels

parameter	Groups			Group A	Time of sampling			
					Pre-	12-hr	24-hr	48-hr
FPI (µIU/ml)				0.94±0.17				
	Group B	Level			0.93±0.17	0.82±0.11	0.76±0.12	0.89±0.16
		P value	P1		>0.05	<0.05	<0.05	>0.05
			P2			<0.05	<0.05	>0.05
			P3				>0.05	>0.05
			P4					>0.05
	Group C	Level			0.96±0.18	0.915±0.17	0.89±0.17	0.933±0.1
		P value	P1		>0.05	>0.05	>0.05	>0.05
			P2			>0.05	>0.05	>0.05
			P3				>0.05	>0.05
			P4					>0.05
			P5		>0.05	>0.05	<0.05	<0.05
FBG (mg/dl)				77.5±9.2				
	Group	Level			77.3±8.7	114.5±13.2	159.9±23.5	144±28

	B	P value	P1		>0.05	<0.05	<0.05	<0.05
			P2			<0.05	<0.05	<0.05
			P3				<0.05	<0.05
			P4					<0.05
	Group C	Level			79.8±9.1	109.5±10.4	140.3±13.5	124.6±10
		P value	P1		>0.05	<0.05	<0.05	<0.05
			P2			<0.05	<0.05	<0.05
			P3				<0.05	<0.05
			P4					<0.05
			P5		>0.05	>0.05	<0.05	<0.05

FPI: Fasting plasma insulin; FBG: Fasting blood glucose; P1: significance versus control levels; P2: significance versus pre- induction level in the study groups; P3: significance versus 12-hr post-induction level in the study groups; P4: significance versus 24-hr post-induction level in the study groups; P5: significance of difference between groups B and C.

Table (4): Mean (±SD) levels of serum CRP estimated in Groups B and C at 12, 24 and 48-hr after induction of AP compared to Group A and pre-induction levels

				Time of sampling			
				Pre-	12-hr	24-hr	48-hr
Group A			1.12±0.54				
Group B	Level			1.1±0.42	19.2±2.34	24.6±2.5	17.6±2.63
	P value	P1		>0.05	<0.05	<0.05	<0.05
		P2			<0.05	<0.05	<0.05
		P3				<0.05	>0.05
		P4					<0.05
Group C	Level			1.13±0.34	16±2.87	20.8±3.59	15±1.71
	P value	P1		>0.05	<0.05	<0.05	<0.05
		P2			<0.05	<0.05	<0.05
		P3				<0.05	>0.05
		P4					<0.05
		P5		>0.05	<0.05	<0.05	<0.05

P1: significance versus control levels; P2: significance versus pre- induction level in the study groups; P3: significance versus 12-hr post-induction level in the study groups; P4: significance versus 24-hr post-induction level in the study groups; P5: significance of difference between groups B and C.

Table (5): Mean (±SD) levels of serum ghrelin estimated in Group B at 12, 24 and 48-hr after induction of AP compared to Group A and pre-induction levels

				Time of sampling			
				Pre-	12-hr	24-hr	48-hr
Group A			290.3±88.2				
Group B	Level			300.1±77.9	237.5±60.1	218.5±52.1	254±66.4
	P value	P1		>0.05	>0.05	<0.05	>0.05
		P2			>0.05	<0.05	>0.05
		P3				>0.05	>0.05
		P4					>0.05

P1: significance versus control levels; P2: significance versus pre-induction level in group B; P3: significance versus 12-hr post-induction level in group B ; P4: significance versus 24-hr post- induction level in group B

Table (6): Spearman correlation between histopathological score and laboratory parameters estimated at 12, 24 and 48 hours after AP induction in group B

	12-hr after AP induction		24-hr after AP induction		48-hr after AP induction	
	Rho	p	Rho	p	Rho	p
Serum amylase (U/L)	0.669	<0.05	0.669	<0.05	0.493	>0.05
Serum lipase (U/L)	0.556	>0.05	0.720	<0.05	0.215	>0.05
FPI (µIU/ml)	-0.608	>0.05	-0.788	<0.05	-0.469	>0.05
Serum CRP (g/L)	0.657	<0.05	0.379	>0.05	0.152	>0.05
Serum ghrelin (pg/ml)	-0.633	<0.05	-0.788	<0.05	-0.665	<0.05

FPI: Fasting plasma insulin; CRP: C-reactive protein

Table (7): Regression analysis of laboratory parameters estimated at 12-hrs after AP induction in Group B for prediction of disease severity as judged by histopathological score determined at 48-hrs after AP induction

	Parameter	Standardized coefficient	t	p
Model 1	Serum amylase	0.578	3.030	<0.05
	FPI	-0.617	3.325	<0.05
Model 2	FPI	-0.642	2.367	<0.05

FPI: Fasting plasma insulin

Figures

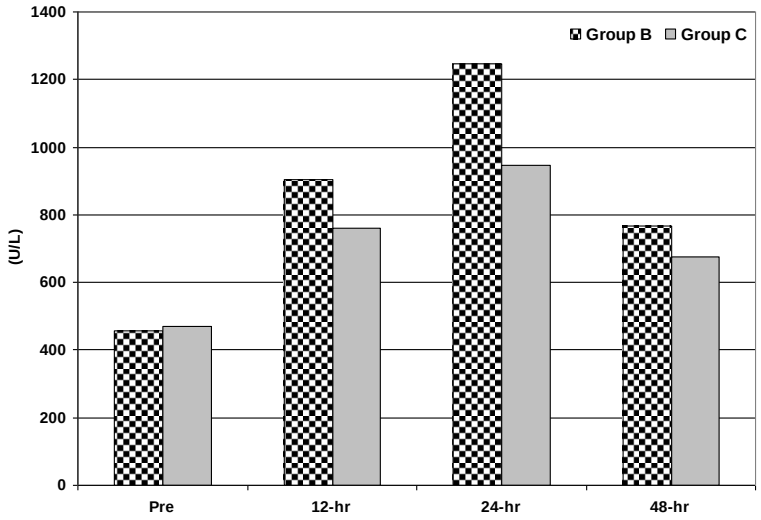

Fig. (1): Mean serum amylase levels estimated in AP groups throughout 48-hr after AP induction

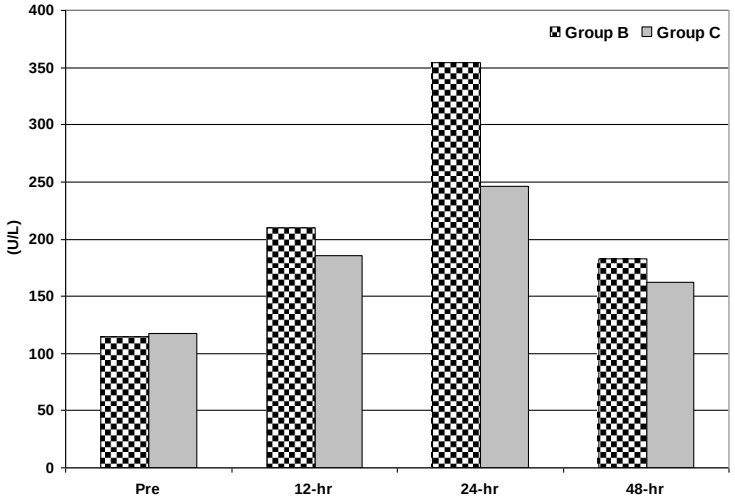

Fig. (2): Mean serum lipase levels estimated in AP groups throughout 48-hr after AP induction

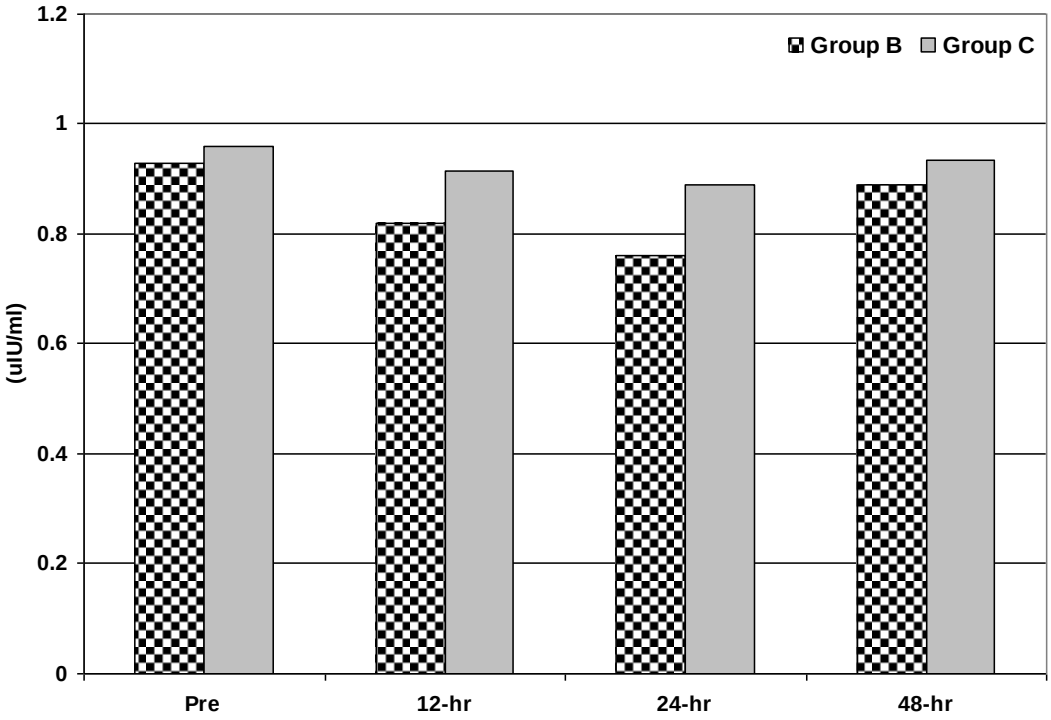

Fig. (3): Mean FPI levels estimated in AP groups throughout 48-hr after AP induction

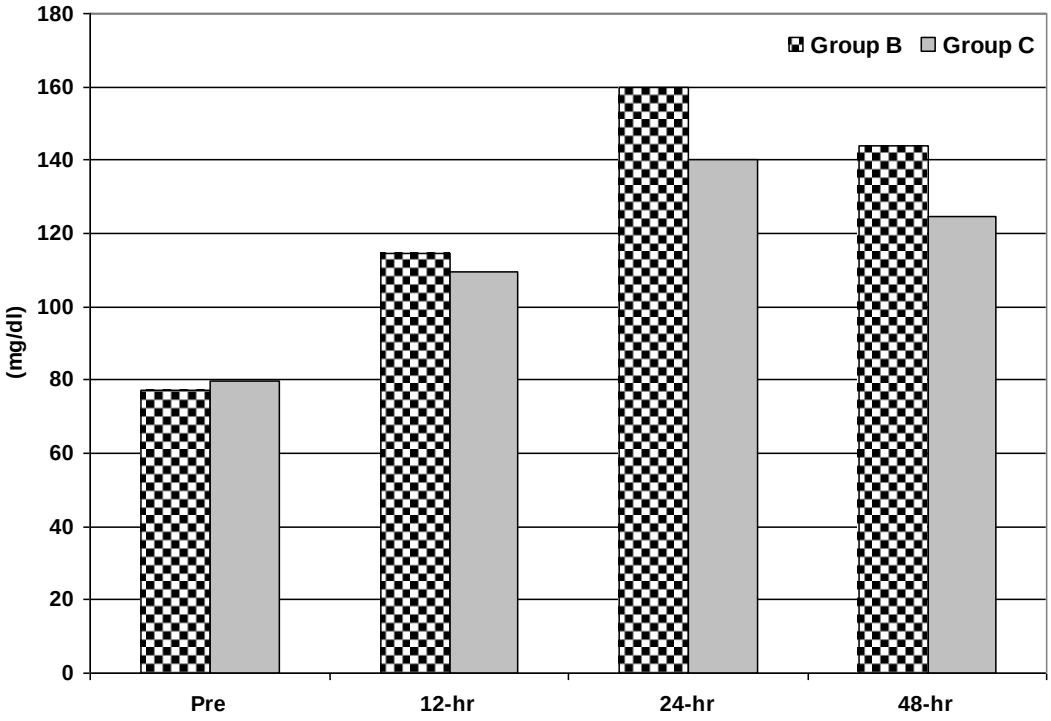

Fig. (4): Mean FBG levels estimated in AP groups throughout 48-hr after AP induction

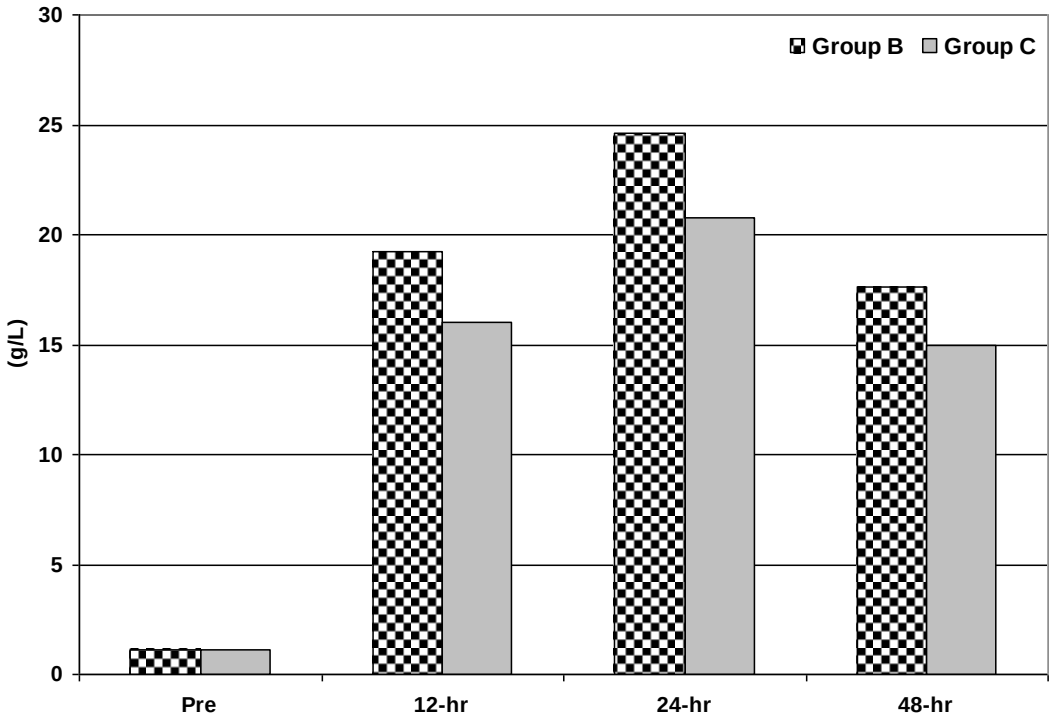

Fig. (5): Mean serum CRP levels estimated in AP groups throughout 48-hr after AP induction

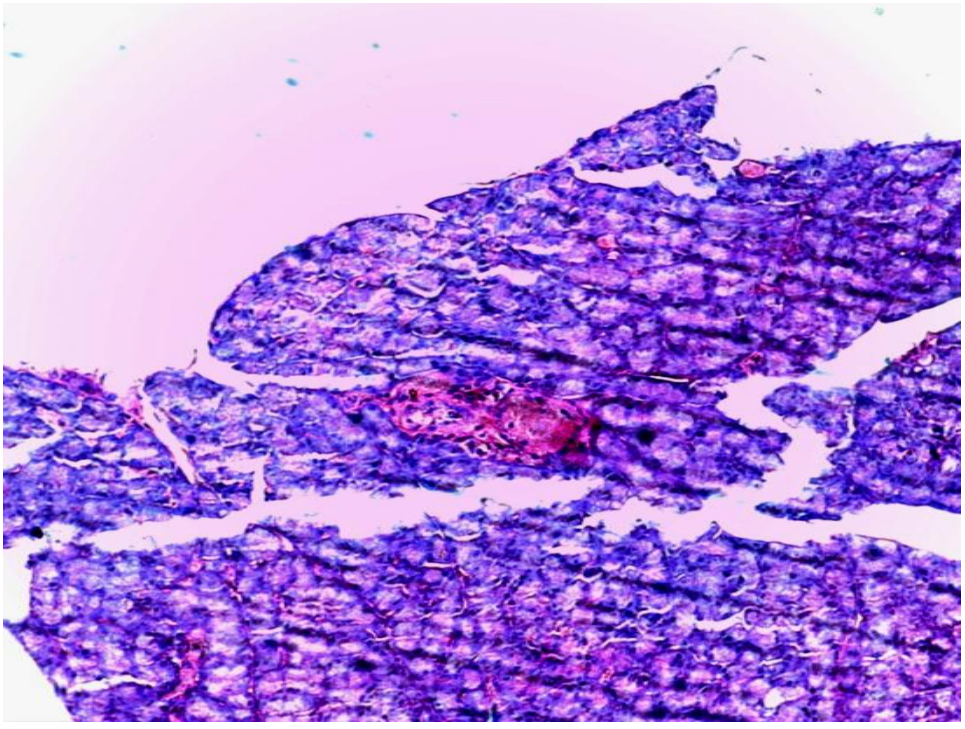

Fig. (6): Light microscopy of Group A animal revealed normal histological structure of the pancreas.(H&E x20)

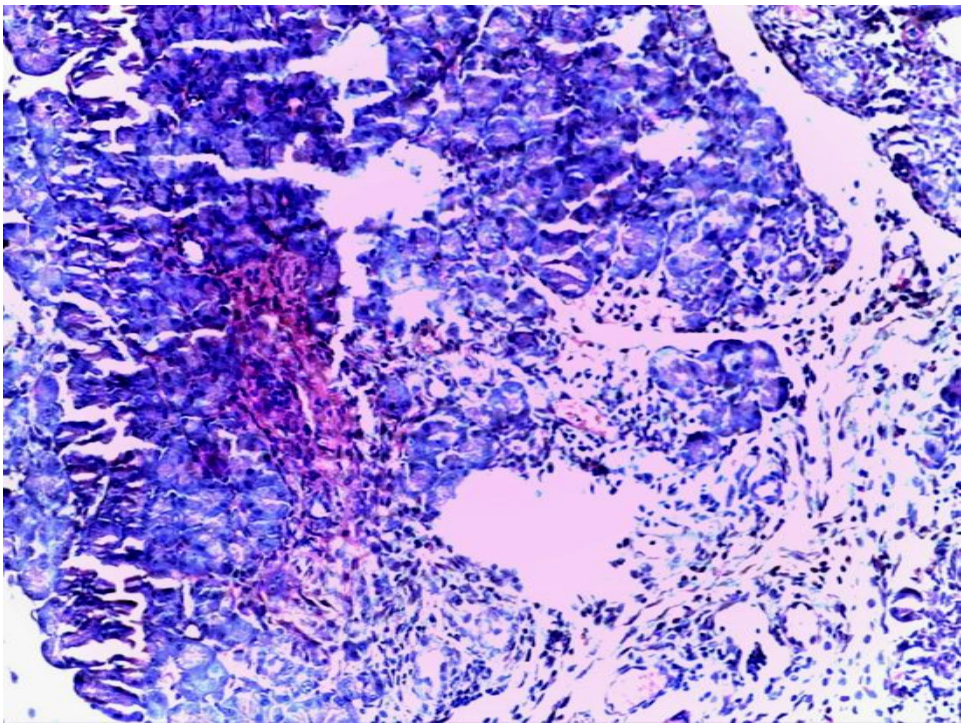

Fig. (7): Light microscopy of an animal had AP in Group B showed massive inflammatory infiltrate, massive interlobular edema and dilated congested blood vessels with inflammatory cellular infiltrate around blood vessels. (H & E x20)

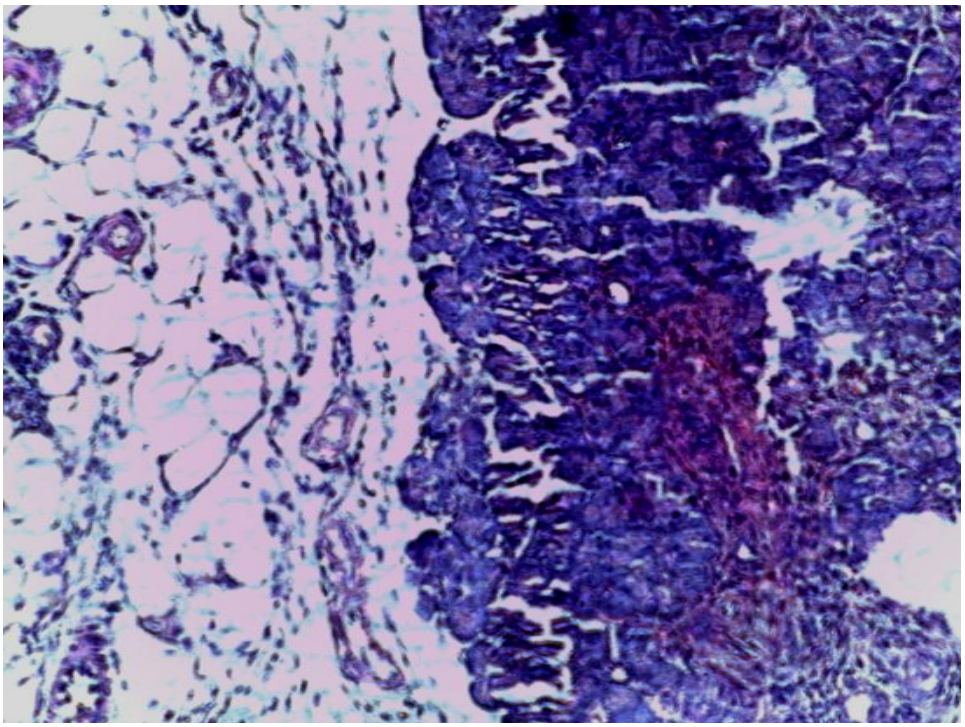

Fig. (8): Light microscopy of an animal had AP in Group B showed moderate inflammatory infiltrate, massive interlobular edema and dilated congested blood vessels. (H & E x20)

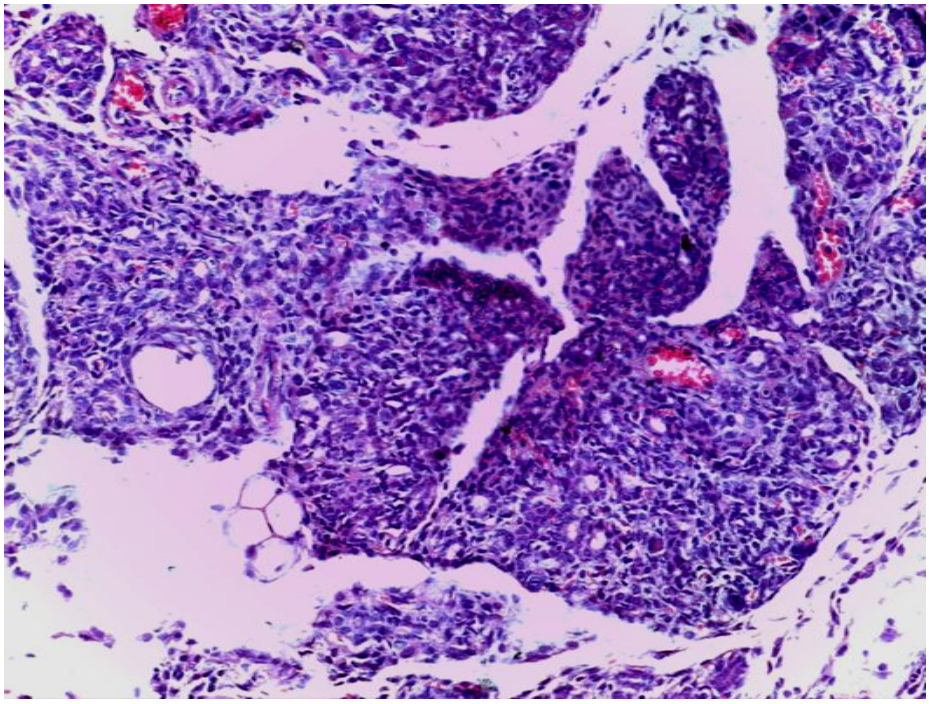

Fig (9): Light microscopy of an animal had AP in Group B showed moderate inflammatory infiltrate, massive interlobular edema and dilated congested blood vessels. (H & E x20)

References

1. Aryal MR, Mainali NR, Gupta S, Singla M: Acute pancreatitis owing to very high triglyceride levels treated with insulin and heparin infusion. BMJ Case Rep. 2013; 2013.
2. Ceranowicz D, Warzecha Z, Dembinski A, Ceranowicz P, Cieszkowski J, Kusnierz-Cabala B, Tomaszewska R, Kuwahara A, Kato I: Role of hormonal axis, growth hormone - IGF-1, in the therapeutic effect of ghrelin in the course of cerulein-induced acute pancreatitis. J Physiol Pharmacol. 2010;61(5):599-606.
3. Deng WH, Chen C, Wang WX, Yu J, Li JY, Liu L: Effects of ORP150 on appearance and function of pancreatic beta cells following acute necrotizing pancreatitis. Pathol Res Pract. 2011; 207(6):370-6.
4. DU JD, Zheng X, Huang ZQ, Cai SW, Tan JW, Li ZL, Yao YM, Jiao HB, Yin HN, Zhu ZM: Effects of intensive insulin therapy combined with low molecular weight heparin anticoagulant therapy on severe pancreatitis. Exp Ther Med. 2014; 8(1):141-146.
5. Gordon C, Yates AP, Davies D: Evidence for a direct action of exogenous insulin on the pancreatic islets of diabetic mice: islet response to insulin pre-incubation. Diabetologia 28, 291–4, 1985.
6. Heppner KM, Tong J, Kirchner H, Nass R, Tschöp MH: The ghrelin O-acyltransferase-ghrelin system: a novel regulator of glucose metabolism. Curr Opin Endocrinol Diabetes Obes 2011; 18:50–5.
7. Inoue Y, Nakahara K, Maruyama K, Suzuki Y, Hayashi Y, Kangawa K, Murakami N: Central and peripheral des-acyl ghrelin regulates body temperature in rats. Biochem Biophys Res Commun 2013;430;278–283.
8. Khatib N, Gaidhane S, Gaidhane AM, Khatib M, Simkhada P, Gode D, Zahiruddin QS: Ghrelin: ghrelin as a regulatory Peptide in growth hormone secretion. J Clin Diagn Res. 2014; 8(8):MC13-7.
9. Li J, Chen TR, Gong HL, Wan MH, Chen GY, Tang WF: Intensive insulin therapy in severe acute pancreatitis: a meta-analysis and systematic review. West Indian Med J. 2012; 61(6):574-9.
10. Liang ZH, Qin MB, Tang GD, Yang HY, Su J, Huang JA: Melatonin reduces inflammation and recovers endogenous ghrelin in acute necrotizing pancreatitis in rats. Mol Med Rep. 2014; 9(6):2599-605.
11. Lin CK, Wang ZS, Lai KH, Lo GH, Hsu PI: Gastrointestinal mucosal lesions in patients with acute pancreatitis. Zhonghua Yi Xue Za Zhi (Taipei). 2002; 65(6):275-8.
12. Ma J, Fan H, Kou JT, Li LX, He Q: Clinical significance for monitoring of serum ghrelin in acute pancreatitis severity assessment. Zhonghua Yi Xue Za Zhi. 2013; 93(48):3864-6.

13. Niederau C, Liddle RA, Ferrell LD, Grendell JH: Beneficial effects of cholecystokinin-receptor blockade and inhibition of proteolytic enzyme activity in experimental acute hemorrhagic pancreatitis in mice. Evidence for cholecystokinin as a major factor in the development of acute pancreatitis. J Clin Invest 1986, 78:1056–63.
14. xPanek J, Bonior J, Pieton J, Jaworek J: Serum leptin and ghrelin levels in patients in the early stages of acute biliary pancreatitis and different degrees of severity. Pol Przegl Chir. 2014; 86(5):211-7.
15. Pantic I, Nesic D, Stevanovic D, Starcevic V, Pantic S, Trajkovic V: Effects of ghrelin on the structural complexity of exocrine pancreas tissue architecture. Microsc Microanal. 2013; 19(3):553-8.
16. Peterli R, Wolnerhanssen B, Peters T, von Flüe M, Ackermann C: Improvement in glucose metabolism after bariatric surgery: comparison of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy: a prospective randomized trial. Ann Surg. 2009; 250:234–41.
17. Pezzilli R, Morselli-Labate AM, Miniero R, Barakat B, Fiocchi M, Cappelletti O: Simultaneous serum assays of lipase and interleukin-6 for early diagnosis and prognosis of acute pancreatitis. Clin Chem. 1999; 45(10):1762-7.
18. Porporato PE, Filigheddu N, Reano S, Ferrara M, Angelino E, Gnocchi VF, Prodam F, Ronchi G, Fagoonee S, Fornaro M, Chianale F, Baldanzi G, Surico N, Sinigaglia F, Perroteau I, Smith RG, Sun Y, Geuna S, Graziani A: Acylated and unacylated ghrelin impair skeletal muscle atrophy in mice. J Clin Invest 2013; 123:611–22.
19. Rebours V: Acute pancreatitis: An overview of the management. Rev Med Interne. 2014; 35(10):649-55.
20. Rohan Jeyarajah D, Osman HG, Patel S: Severe acute pancreatitis attacks are associated with significant morbidity and mortality. Curr Probl Surg. 2014; 51(9):370-2.
21. Schmidt J, Rattner DW, Lewandrowski K, Compton CC, Mandavilli U, Knoefel WT, Warshaw AL: A better model of acute pancreatitis for evaluating therapy. Ann. Surg., 1992; 215: 44–56.
22. Solanki NS, Barreto SG, Saccone GT: Acute pancreatitis due to diabetes: the role of hyperglycaemia and insulin resistance. Pancreatology. 2012; 12(3):234-9.
23. Tani S, Itoh H, Okabayashi Y, Nakamura T, Fujii M, Fujisawa T, Koide M, Otsuki M: New model of acute necrotizing pancreatitis induced by excessive doses of arginine in rats. Dig Dis Sci. 1990; 35(3):367-74.
24. Tinder P: Determination of blood glucose. Ann. Clin. Biochem.; 6:24, 1969.
25. Toshinai K, Yamaguchi H, Sun Y, Smith RG, Yamanaka A, Sakurai T, Date Y, Mondal MS, Shimbara T, Kawagoe T, Murakami N, Miyazato M, Kangawa K, Nakazato M: Des-acyl ghrelin induces food intake by a mechanism independent of the growth hormone secretagogue receptor. Endocrinology 2006;147: 2306–14.
26. Treacy J, Williams A, Bais R, Willson K, Worthley C, Reece J, Bessell J, Thomas D: Evaluation of amylase and lipase in the diagnosis of acute pancreatitis. ANZ J Surg. 2001; 71(10):577-82.
27. Turk N, Dağistanli FK, Sacan O, Yanardag R, Bolkent S: Obestatin and insulin in pancreas of newborn diabetic rats treated with exogenous ghrelin. Acta Histochem. 2012; 114(4):349-57.
28. Warzecha Z, Ceranowicz P, Dembinski A, Cieszkowski J, Kusnierz-Cabala B, Tomaszewska R, Kuwahara A, Kato I: Therapeutic effect of ghrelin in the course of cerulein-induced acute pancreatitis in rats. J Physiol Pharmacol. 2010; 61(4):419-27.
29. Yada T, Damdindorj B, Rita RS, Kurashina T, Ando A, Taguchi M, Koizumi M, Sone H, Nakata M, Kakei M, Dezaki K: Ghrelin signalling in β-cells regulates insulin secretion and blood glucose. Diabetes Obes Metab. 2014;16 Suppl 1:111-7.
30. Yin Y, Li Y, Zhang W: The growth hormone secretagogue receptor: its intracellular signaling and regulation. Int J Mol Sci. 2014; 15(3):4837-55.
31. Zechner D, Spitzner M, Bobrowski A, Knapp N, Kuhla A, Vollmar B: Diabetes aggravates acute pancreatitis and inhibits pancreas regeneration in mice. Diabetologia. 2012; 55(5):1526-34.
32. Zechner D, Knapp N, Bobrowski A, Radecke T, Genz B, Vollmar B: Diabetes increases pancreatic fibrosis during chronic inflammation. Exp Biol Med (Maywood). 2014; 239(6):670-676.