Original Article

The Possible Effects of Caffeine and Nicotine on the Pathogenesis of Experimentally Induced Alcoholic Pancreatitis: A Histological and Immunohistochemical Study

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ABSTRACT

Introduction: The incidence of pancreatitis has increased in recent decades and alcohol was recognized as one of the major causes of pancreatitis as well as gall stones. Nicotine is a major toxin in tobacco and cigarettes and may influence the development of pancreatitis. Caffeine is a natural stimulant widely consumed psychoactive drug.

Aim: This study was done to evaluate the influence of nicotine and caffeine on the pathogenesis of alcohol-induced pancreatitis.

Materials and Methods: Forty adult male Wister albino rats were divided into four equal groups; control group, alcoholic pancreatitis group, alcoholic pancreatitis with nicotine group and alcoholic pancreatitis with caffeine group. At the end of the experimental period, blood samples were collected for serological study (serum amylase, lipase and blood sugar) and pancreata were processed for light microscopic examination.

Results: Histological examination of the pancreas in alcoholic pancreatitis group showed marked pancreatic distortion with marked cytoplasmic vaculations and small deeply stained nuclei of acinar cells. In between the acini there were inflammatory cell infiltration and congested blood vessels. Immunohistochemically, NF-κB and Caspase-3 were increased, while insulin was decreased. These histological/immunohitochemical changes were increased in alcoholic pancreatitis with nicotine group and decreased in alcoholic pancreatitis with caffeine group. Blood glucose, serum amylase and lipase levels were also increased in alcohol pancreatitis group compared to controls. The increase in these enzymes was elevated by alcohol pancreatitis with nicotine group and decreased by alcohol pancreatitis with caffeine group.

Conclusion: Nicotine exaggerates the adverse effects of alcoholic pancreatitis while caffeine improves these changes.

Key Words: Alcohol, caffeine, nicotine, pancreas, pancreatitis.

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INTRODUCTION

Pancreatitis can be classified into acute and chronic pancreatitis; however, discrepancy exists on whether two distinct conditions really exist or whether acute pancreatitis (AP) leads to chronic pancreatitis (CP). The most common symptom of both is severe abdominal pain[1,2]. The mortality rate of acute pancreatitis is especially high and has not decreased since the 1970s, which is most likely because treatment has not improved and is mainly directed toward pain control. The majority of pancreatitis cases are associated with either cholelithiasis or heavy alcohol intake, relationships widely considered to be causal[3]. Additional specific factors for pancreatitis risk include other obstructive bile duct conditions, endoscopic retrograde cholangio-pancreatography (ERCP), hyperlipidemia (types I, IV, and V), certain viral infections including human immunodeficiency virus (HIV), pancreas divisum, ischemic damage, genetic predilection and abdominal trauma. In most reported series 10% to >30% of cases are considered idiopathic with medications playing an unclear role in some of these[4].

Among men in developed countries, alcohol-associated disease is generally the largest subset. Since only a small proportion of chronic heavy alcohol users develop pancreatitis. It seems likely that other traits are involved in pancreatic susceptibility to heavy drinking. Cofactors or promoters of alcohol-associated pancreatitis have been relatively little studied, especially in prospective analyses. Hypothetical possibilities include drinking pattern, beverage type, and genetic predilection, nutrients in the diet, drug use, adiposity and other habits[5].

Nicotine is a toxin occurs in tobacco and cigarettes and may affect the progress of pancreatitis and pancreatic cancer. It is rapidly absorbed in the lungs and is removed from the body within two to three hours[6]. Nicotine metabolism occurs mainly through the cytochrome P450 (CYP) 2A6 pathway as well as aldehyde oxidase 1, UDP-glucuronosyltranferases, flavin-containing monooxygenase 3 and other CYPs. Polymorphisms in CYP2A6 have been shown with racial and genetic differences in the metabolism of nicotine[7].

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Caffeine is a purine alkaloid, 1, 3, 7-trimethylxanthine, belongs to methylxanthine family. It is naturally present in the seeds, leaves and fruits of more than 63 plant species. It is mostly found in coffee and tea; it is also present in kola nut which is chewed in Africa, yerba mate (usually taken with hot water in South America) as well as guarana seeds which are primarily added to soft drinks in Brazil. At least one caffeinated product is daily consumed by nearly 80% of the world population[8].

Caffeine gets readily absorbed in the body, and it reaches the brain within 5 minute after its consumption and is also eliminated with an average half-life of five hours from the body. It performs various physiological functions involving its effect on the respiratory, cardiovascular, gastrointestinal and the central nervous system. Caffeine is generally consumed for its role to work as a mild nervous chemical stimulant towards drowsiness and fatigue[9].

Although alcohol abuse is the major cause of pancreatitis, the pathogenesis of alcoholic pancreatitis remains obscure. So, this work aimed to study histological changes resulting from the acute use of alcohol on pancreas of adult rat and the possible effects of nicotine and caffeine on modulating these changes.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were purchased from Sigma chemical company, Cairo, Egypt.

1) Ethanol and Palmitoleic acid (POA): Palmitoleic acid (254 mg, 1 mmol) was added oxalyl chloride (1.5 mL, excess). The mixture was stirred at room temperature for 2 h and excess oxylyl chloride was evaporated off. The crude product (palmitoleic acid chloride) was dissolved in ethyl acetate (5 mL) and fluorescein (400 mg, 1.20 mmol) was added. . To establish a novel alcoholic acute pancreatitis model induced by fatty acid ethyl ester (FAEE, FAEE-AP), the dose of ethanol was firstly optimised to 1.35 g/kg, which alone did not cause any obvious morphological changes in the pancreas. Palmitoleic acid ethyl ester (POAEE) was dissolved in pure ethanol to make stock solutions for injection. Mice received two intraperitoneal injection of POAEE (150 mg/kg) and ethanol (1.35 g/kg) at 1 hr interval[10].

2) Nicotine, hydrogen tartrate, freshly prepared nicotine in normal saline was injected subcutaneously (SC) as 10 mg/kg body weight 3 times / week for 4weeks starting an hour after the second POA/ethanol injection. The prepared solutions were stored in foil-wrapped glass bottle at 4ĉ for just about 4-5 days[11].

3) Caffeine, anhydrous extra pure (99%), Caffeine dissolved in saline. Animals were treated by IP injection of 100mg/kg body weight once daily for 4 weeks starting an hour after the second POA/ethanol injection. The dissolved caffeine was filtered through a disposable sterile filter membrane immediately before injection[12].

Animals

Forty healthy adult Wister male albino rats (4-6 months) weighing 180-200 g were used in this study. The animals were obtained from the Animal House, Faculty of Medicine, Zagazig University, Egypt. They were fed standard balanced diet and allowed water ad-libitum. They were housed in hygienic cages in 12 h light/12 h dark cycle at room temperature according to the guidelines for animal research issued by the National Institute of Health and approved by Animal Ethics Committee, Zagazig University, Egypt.

Experimental Design

Rats were equally divided into four groups; I, II, III and IV.

Group I (Control group): animals were injected with normal saline subcutaneously (SC) 3 times/week and intraperitoneal (IP) daily.

Group II (alcoholic pancreatitis group): animals were receiving ethanol IP as previously mentioned.

Group III (alcoholic pancreatitis with nicotine group): animals were given ethanol IP and nicotine (SC) as previously mentioned.

Group IV (alcoholic pancreatitis with caffeine group): animals were given ethanol IP and caffeine IP as previously mentioned.

During the experimental period (4 weeks), clinical signs and general appearance, which included the amount of food and water consumed, were checked daily. Mortalities of the rats were recorded as it occurred. At the end of the experiment, the rats were fasted overnight. They were sacrificed with IP injection of phentobarbitone sodium 60 mg/kg body weight. Plasma glucose, amylase, lipase were measured. Pancreas was dissected out, rinsed and prepared for light microscope examination.

Biochemical study

The collected blood was divided into two portions. One portion was used to estimate blood glucose level by the glucose oxidase method[13] using Accu-chek Active (Roch Diagnostics, Mannheim, Germany). The other portion was transferred to a clean non-EDTA-added tube, allowed to clot, and centrifuged for 10 minutes at 3000 rpm to obtain sera. The sera were stored at −20°C and were used to

determine lipase and amylase levels[14,15]. A fully automatic biochemical analyzer (Olympus AU5400; Olympus Corp, Tokyo, Japan) was used to determine the plasma amylase and lipase levels (IU/l).

Histological study

Specimens for light microscopic examination were fixed in 10% neutral formol saline, processed for paraffin block preparation, cut into 5 µm thick sections, and subjected to H&E & Mallory's trichrome staining[16].

Immunohistochemical study

This study was carried out applying the peroxidase labeled- streptavidin Biotin technique for detection of Caspase-3 as a marker for apoptotic cells, nuclear factor kappa B (NF-κB) as a marker for inflammatory cells and insulin protein as a marker for β cells. All markers appear as cytoplasmic brown coloration. Paraffin sections were deparaffinized, rehydrated and washed in phosphate buffered saline (PBS) for 3-5 minutes; peroxidase activity was quenched using 3% H_2O_2 for 5 minutes and the sections were then rinsed with PBS for 15 minutes. Sections were blocked with 1.5% normal goat serum in PBS and were then incubated for 45 minutes at room temperature with the primary antibody. Sections were subsequently incubated with a secondary biotinylated antibody (biotin- conjugated goat anti- rabbit Immunoglobulin G (IgG), 1:200, 1h, at room temperature). After rinsing in PBS, the reaction products were visualized by immersing the sections into the chromogen diaminobenzidine. Finally, the sections were counterstained with hematoxylin, dehydrated, and mounted. For the negative control, the same steps were followed, but the primary antibody was replaced with PBS. The primary antibodies used in the study were:

(1) Monoclonal mouse antibody of IgG type against Caspase-3 delivered from Sigma Laboratories Ltd., (Catalog number: 14751).

(2) Nuclear factor kappa B (NF-κB) rabbit polyclonal antibody of IgG type delivered from GeneTex, Inc. North America (Catalog number: GTX103261).

(3) Insulin protein rabbit monoclonal antibody delivered from Dako Laboratories (UK), (Catalog number: 275-R17).

Avidin biotin peroxidase system (Novacastra Labaratories Ltd.-UK) was used to detect the reaction[17].

Histo-morphometrical analysis

Morphometric analysis for Cellular infiltration, blood vessels congestion and pancreatic disorganization were done. In H&E stained pancreatic sections by a histologist who did not know the experimental design. The analysis was blindly done in all experimental groups. Pancreatic scoring system was performed in details according to[18].

The image analyzer computer system Leica Qwin 500 (Leica Ltd, Cambridge, UK) at the Image Analyzing Unit of the Pathology Department, Faculty of Dentistry, Cairo University, Egypt, was used to evaluate pancreatic scoring system, area percentage of collagen fibers. The expression of NF-κB, and Caspase-3 was measured by counting the number of positive cells. Insulin protein was measured as area. The measurements were done using the interactive measure menu. Measuring frame of a standard area equal to 118 476.6 mm² was chosen. Ten readings from five non-overlapping sections from each rat of all groups were examined.

2.7 Statistical analysis:

All data were expressed as mean ± SD. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software, version 13.00 (Chicago, Illinois, USA). Statistical significance was determined by one-way analysis of variance for differences between the means of different groups. Further analysis was carried out using the post-hoc test to compare the parameters between the different groups with each other. Probability of P less than 0.05 was considered statistically significant.

RESULTS

General observations

During the experimental period, the general appearance of rats in each group was checked. Alcoholic pancreatitis group showed lethargy, decreased locomotor activity, decreased food consumption. Alcoholic pancreatitis with nicotine group showed an increase in the incidence and severity of the previous signs. No such signs were observed in alcoholic pancreatitis with caffeine group. A mortality rate of about 10% was observed in alcoholic pancreatitis and alcoholic pancreatitis with nicotine-administered rats which were not compensated.

Biochemical assays

Serum amylase and lipase levels were significantly elevated by alcoholic pancreatitis compared to control rats. The increase in both enzymes was drastically elevated by nicotine and decreased dramatically by caffeine (Fig. 1). Blood sugar level was increased in alcohol pancreatitis rats compared to their control mates and markedly increased by nicotine while returned back near to normal by caffeine (Fig. 2).

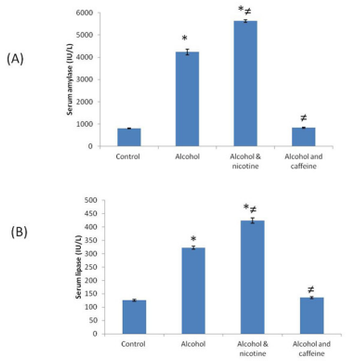

Fig. 1: Serum amylase (A) and lipase (B) in different experimental groups. Data were expressed as mean ± SD, *$p<0.001$ compared to control group, ≠$p< 0.001$ compared to alcohol-treated group.

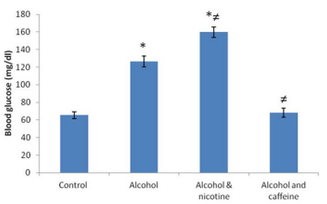

Fig. 2: Blood glucose level was measured in different experimental group. Data expressed as mean±SD. *$p<0.001$ compared to control group, ≠$p< 0.001$ compared to alcohol-treated group.

Histological results using H&E

The control group showed normal parenchymal pattern of the pancreas. The pancreas had plentiful islets of Langerhans surrounded by the pancreatic exocrine acini. The islets appeared rather lightly stained than the surrounding acinar cells. Ill-defined interlobular connective tissue is present between densely packed pancreatic lobules. Each islet consisted of lightly stained polygonal cells arranged in cords separated by a network of blood capillaries. The pancreatic acinar cells were characterized by apical acidophilic and basal basophilic cytoplasm (Fig. 3a).

Alcoholic pancreatitis group was associated with disorganized pancreatic architecture with obvious vacuoles among the acini. (Fig.3b-1) Congested blood vessels and cellular infiltration were also noticed (Fig.3b-2). Alcoholic pancreatitis with nicotine group showed obvious acinar vaculations and cellular infiltration (Fig. 3c). Interestingly, alcoholic pancreatitis with caffeine group showed nearly normal slightly shrunken pancreatic acini Islets of Langerhans with minimal connective tissue (CT) in-between (Fig. 3d).

Detailed histopathological examination of the pancreatic tissue in alcoholic pancreatitis with nicotine group is presented in (Fig. 4).

The total pancreatic injury score (in terms of cellular infiltration, blood vessels congestion and pancreatic disorganization) of the alcoholic pancreatitis and the alcoholic pancreatitis with nicotine groups were significantly higher com¬pared with that of the control group ($P<0.001$) On the other hand caffeine administration with alcohol prevent the deterioration of pancreatic injury score that was almost near to normal (Fig. 5).

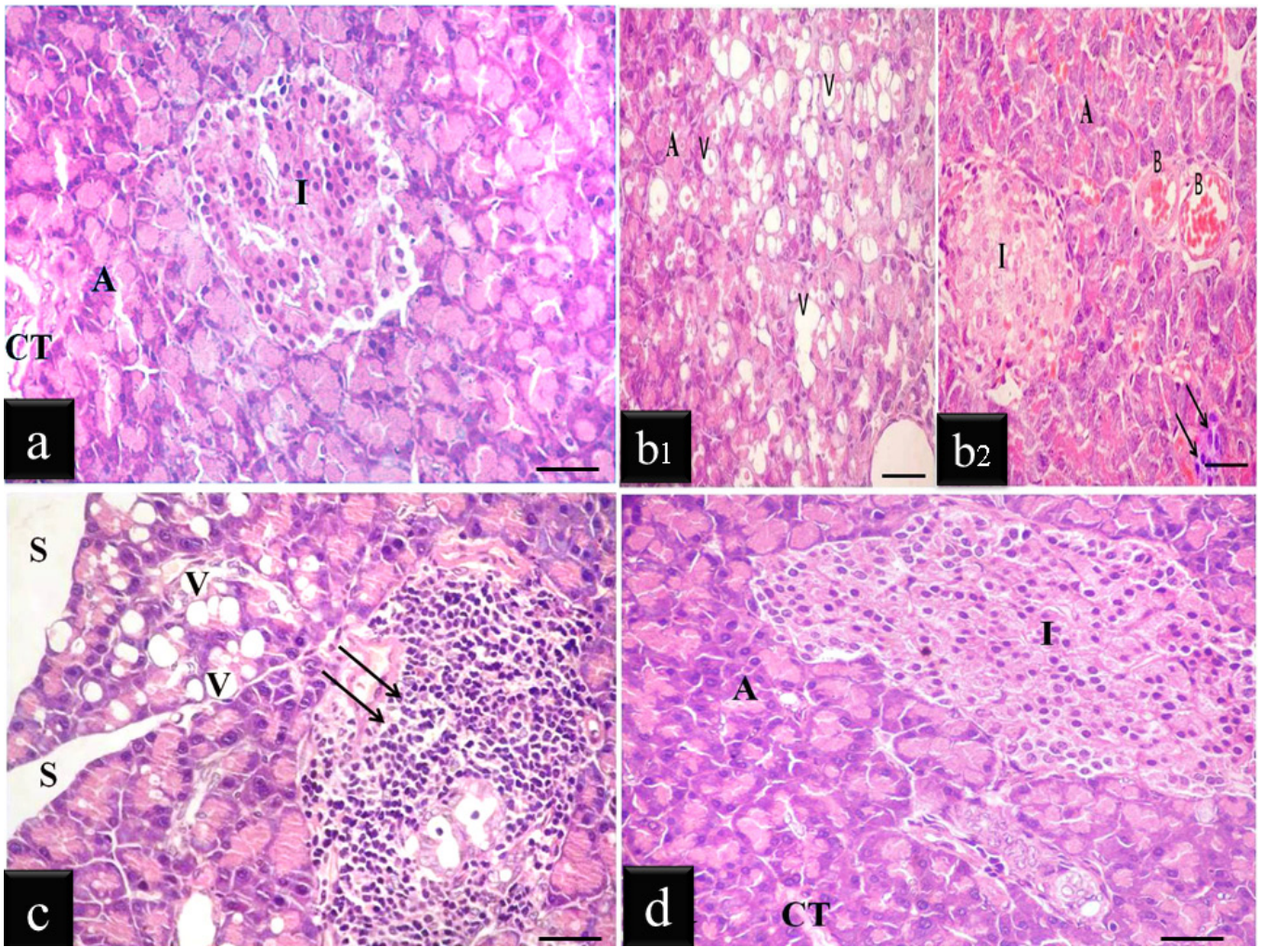

Fig. 3: Pancreatic sections stained with H and E from different experimental group. (a) Control groups showing normal pancreatic acini (A) and Islets of Langerhans (I) with minimal interlobular connective tissue (CT) in-between. (b) Alcoholic pancreatitis group exhibited disorganized pancreatic acini (A) with obvious vacuoles (V) among the acini (b1). Congested blood vessels (B) and cellular infiltration (arrow) are also seen (b2). (c) Alcoholic pancreatitis with nicotine-treated group presented obvious acinar vaculations (V), cellular infiltration (arrow). (d) Alcoholic pancreatitis with caffeine-treated group showed nearly normal slightly shrunken pancreatic acini (A), Islets of Langerhans (I) with minimal connective tissue (CT) in-between. Scale bar 20 µm, x400, H & E.

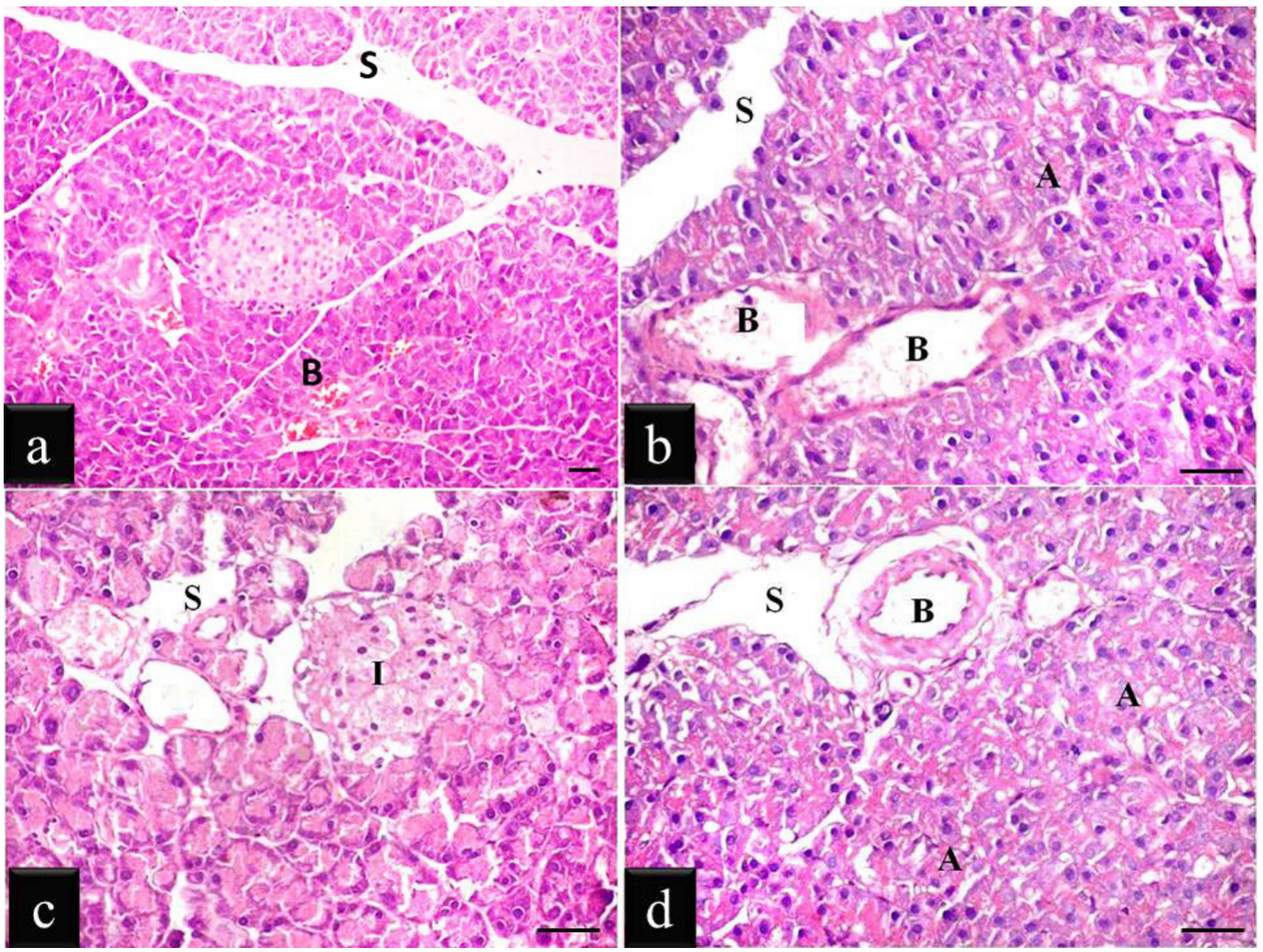

Fig. 4: Representative micrographs of alcoholic pancreatitis with nicotine-treated group (a-d). Widely spaced lobules and increased connective tissue septa (S) and congested blood vessels (B) (b, d) Marked acinar distortion (A), (c) shrunken pancreatic islet (I). Scale bar 20 µm (a: x200, b-d: x400, H&E).

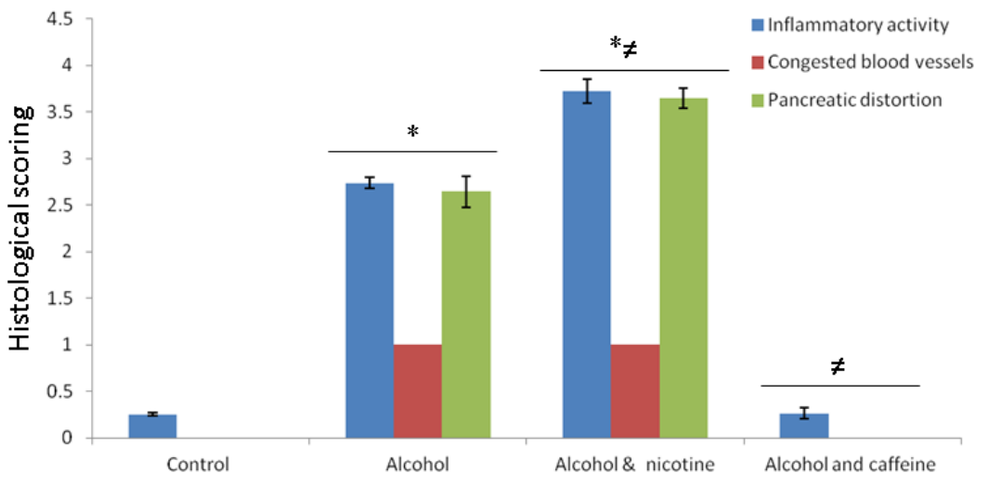

Fig. 5: Pancreatic scoring system. Pancreatic injury was associated with by cellular infiltrates, congested blood vessels and marked pancreatic disorganization. Histopathological scoring of the pancreatic tissue in H&E stained sections of all groups was done according to18. *p<0.001 compared to control group, ≠p< 0.001 compared to alcohol-treated group.

Histological results using Mallory's trichrome stain

Minimal collagen fibers were observed between pancreatic lobes and lobules in control group (6a). Increased collagen deposition in were demonstrated in alcoholic pancreatitis and alcoholic pancreatitis with nicotine group (6b, c). Less collagen fibers deposition were revealed in alcoholic pancreatitis with caffeine group (6d). The extent (area %) of fibrosis is represented in Fig. (7).

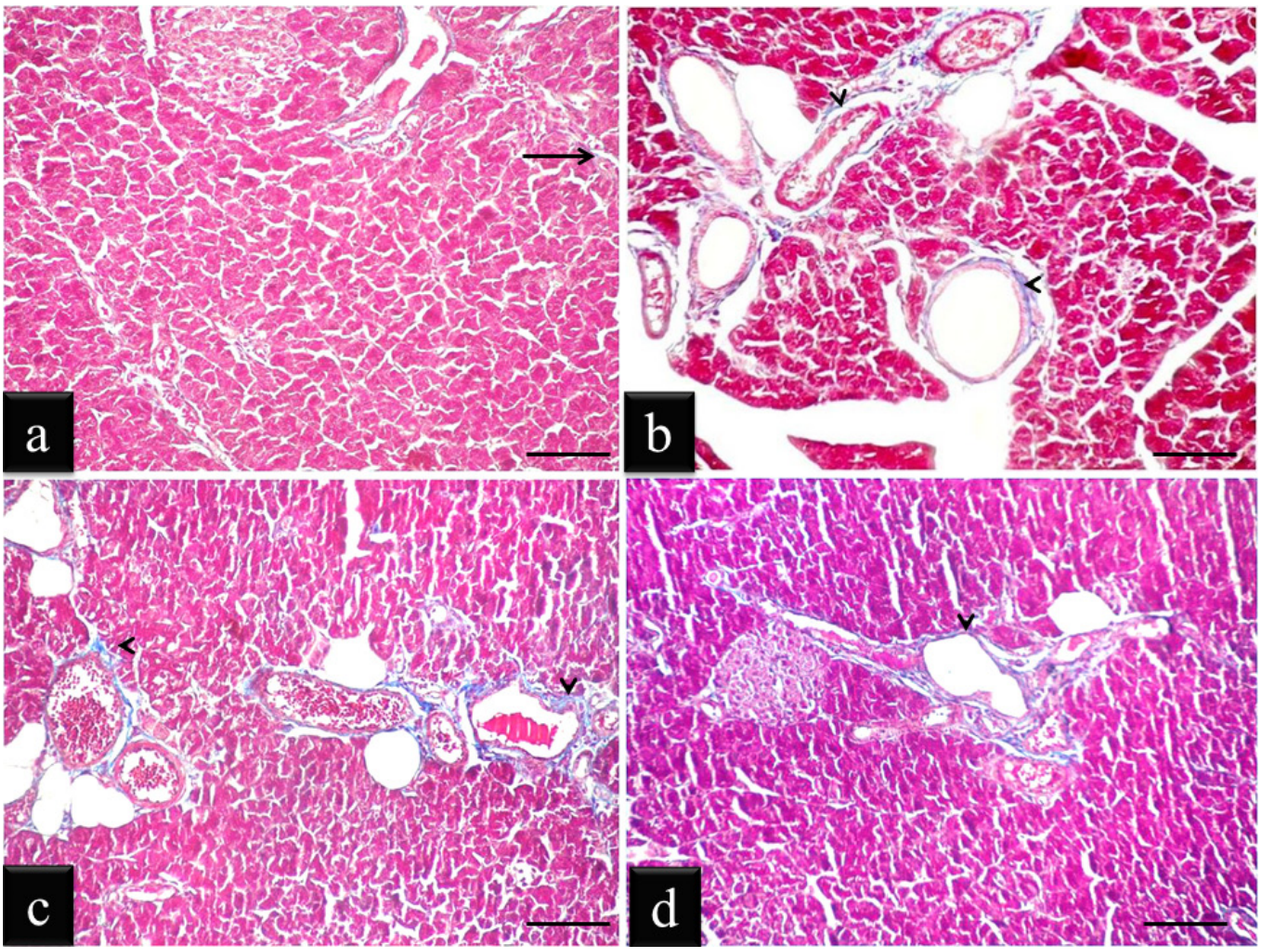

Fig. 6: Mallory's trichrome stained sections showing collagen deposition in the pancreatic tissue in different experimental groups. (a) Control group showing minimal collagen fibers (arrow) between pancreatic lobes and lobules. (b) Alcoholic pancreatitis group exhibited increased collagen deposition between pancreatic lobes and lobules (arrowheads) (C) Alcoholic pancreatitis with nicotine-treated group presented with increased collagen fibers deposition (arrowheads) in the pancreatic tissue. (d) Alcoholic pancreatitis with caffeine-treated group showed less collagen fibers deposition (arrow head). Scale bar 40 µm, x200, Mallory's trichrome.

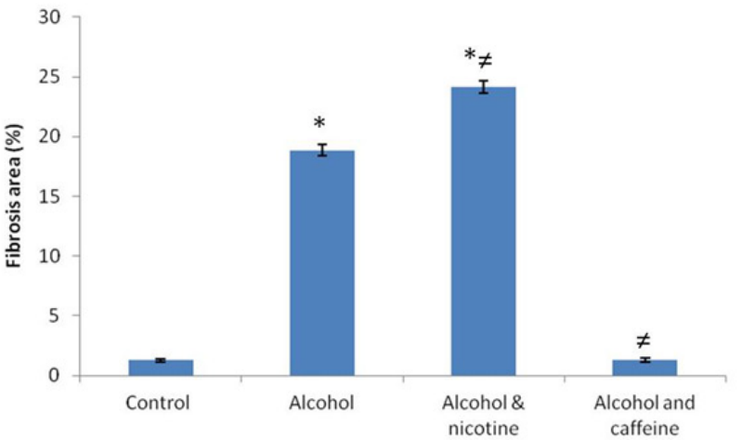

Fig. 7: The extent of fibrous tissue deposition following pancreatic injury was assessed in Mallory's trichrome stained sections. Quantitation of fibrosis area percent was done by Leica Qwin software. *$p<0.001$ compared to control group, ≠$p< 0.001$ compared to alcohol-treated group.

Immunohistological results using NF-κB, Caspase-3 and Insulin protein

Higher NF-κB expression was obvious in alcoholic pancreatitis and alcoholic pancreatitis with nicotine groups compared to control group. Less NF-κB positive cells were observed in alcoholic pancreatitis with caffeine. Numbers of NF-κB positive cells was noticeably decreased in alcoholic pancreatitis with caffeine (Figs. 8 and Fig. 9).

Caspase-3 expression in the islets of Langerhans and among pancreatic acini was increased in alcoholic pancreatitis and alcoholic pancreatitis with nicotine groups compared to control mates less Capsase-3 positive cells were observed in alcoholic pancreatitis with caffeine group. This increased expression (area %) of Caspase-3 positive cells was noticeably decreased in alcoholic pancreatitis with caffeine (Fig 10 and Fig. 11).

Lastly, the insulin expression was obvious in an islets of Langerhans in control group while less insulin expression was detected in alcoholic pancreatitis group but marked reduction in insulin expression in alcoholic pancreatitis with nicotine. The insulin expression level (area %) was markedly enhanced in alcoholic pancreatitis with caffeine (Fig. 12 and Fig. 13).

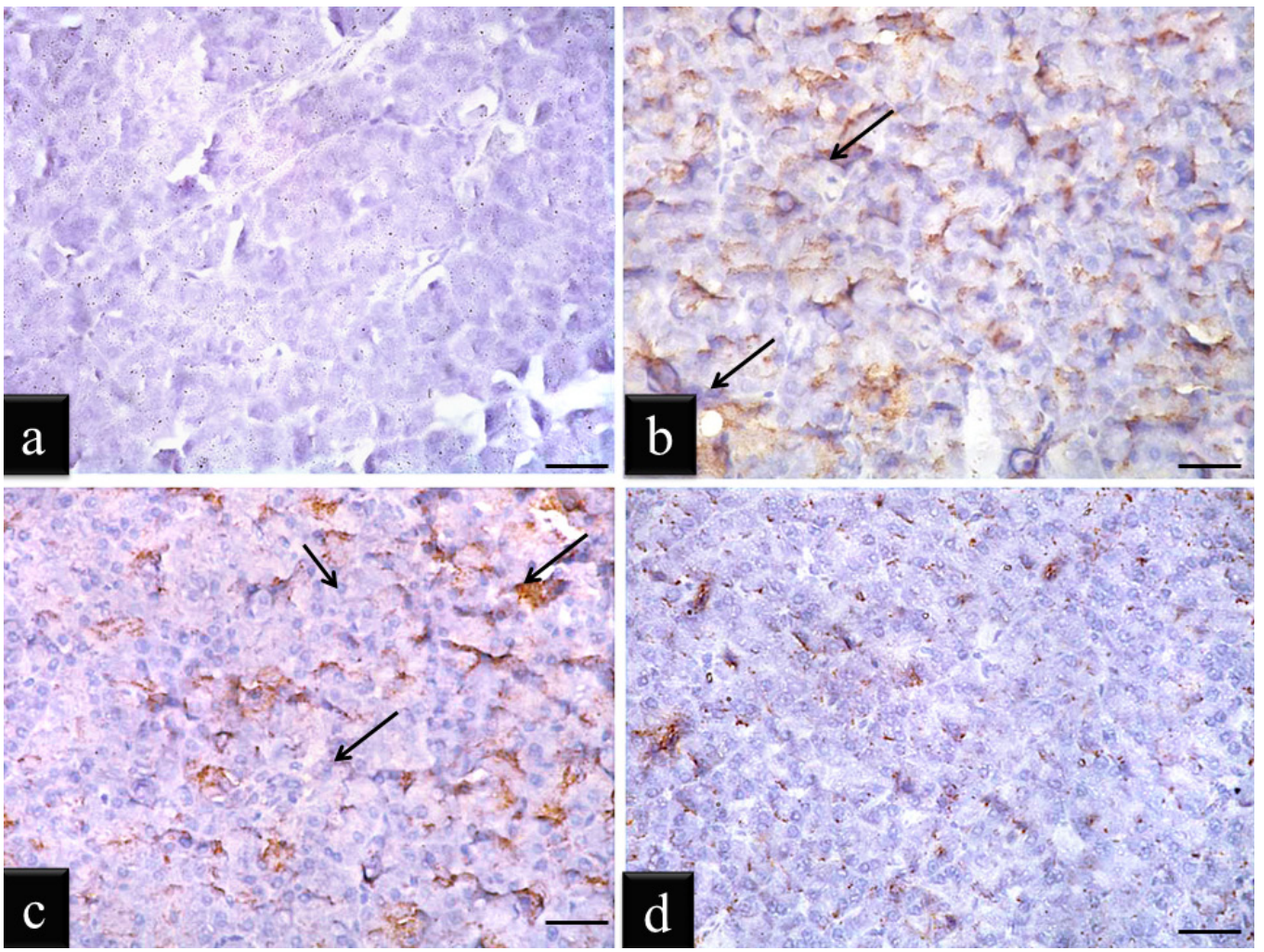

Fig. 8: NF-κB expression in different experimental groups. (a) Control group showed negative NF-κB expression (b) Alcoholic pancreatitis group exhibited obvious NF-κB expression (arrows). (C) Alcoholic pancreatitis with nicotine group presented with higher NF-κB expression (arrows). (d) Alcoholic pancreatitis with caffeine group presented with less NF-κB positive cells (arrow). Scale bar 40 µm, x200, NF-κB immunoreaction.

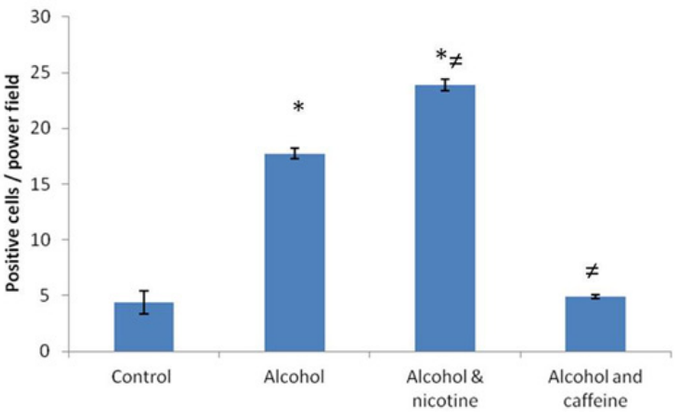

Fig. 9: The number of NF-κB positive cells was counted in different experimental rats using Leica Qwin software. *$p<0.001$ compared to control group, ≠$p< 0.001$ compared to alcohol-treated group.

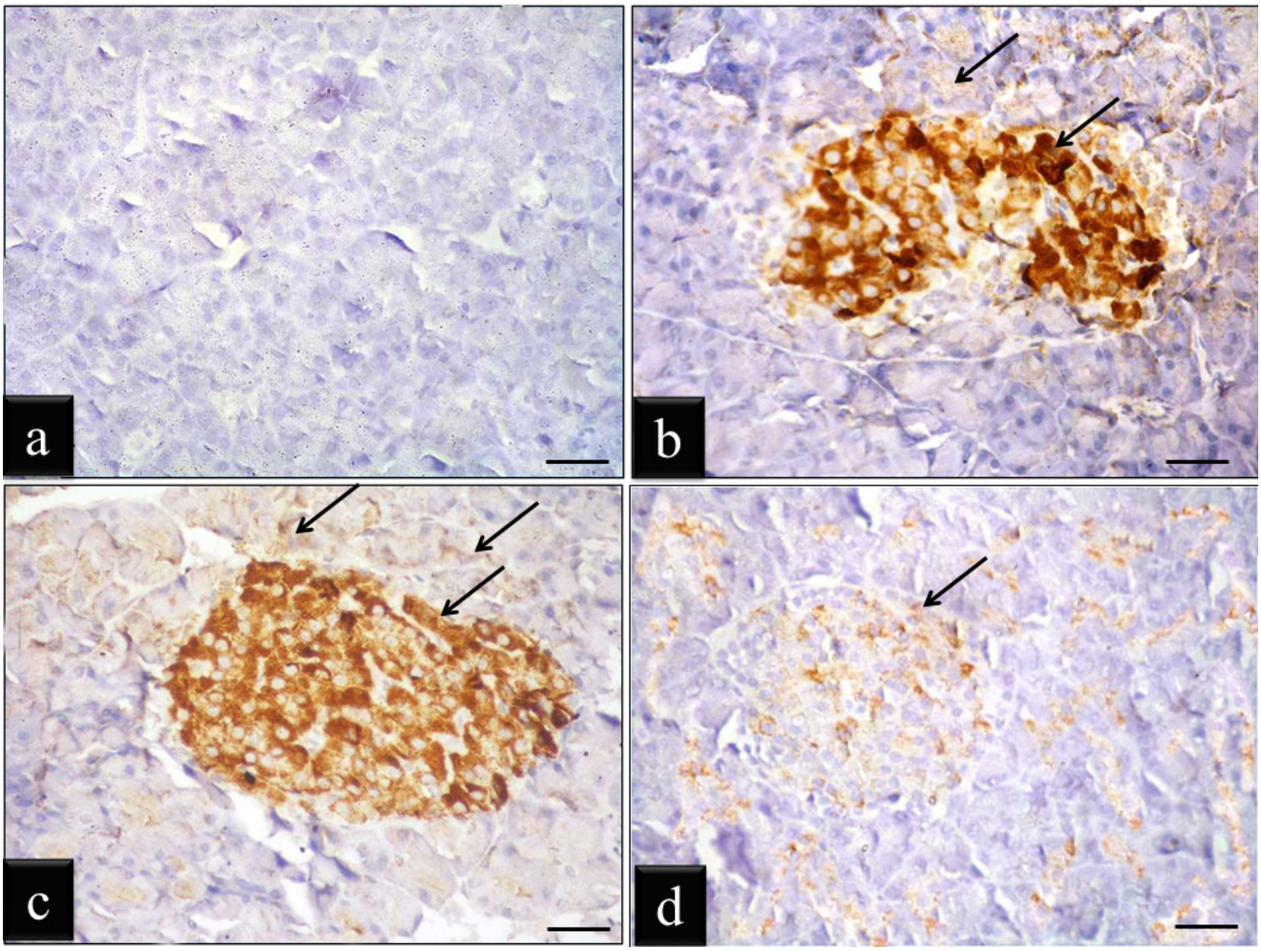

Fig. 10: Caspase-3 expression was studied in different experimental groups. (a) Control group exhibited negative expression for Caspase-3. (b) Alcoholic pancreatitis group presents with increased Caspase-3 expression among the pancreatic acini and an islet (arrows). (c) Alcoholic pancreatitis with nicotine-treated group showed marked increase in Caspase-3 expression (arrows). (d) Alcoholic pancreatitis with caffeine-treated group showed less Capsase-3 expression (arrow). Scale bar 40 µm, x200, Caspase-3 immunoreaction.

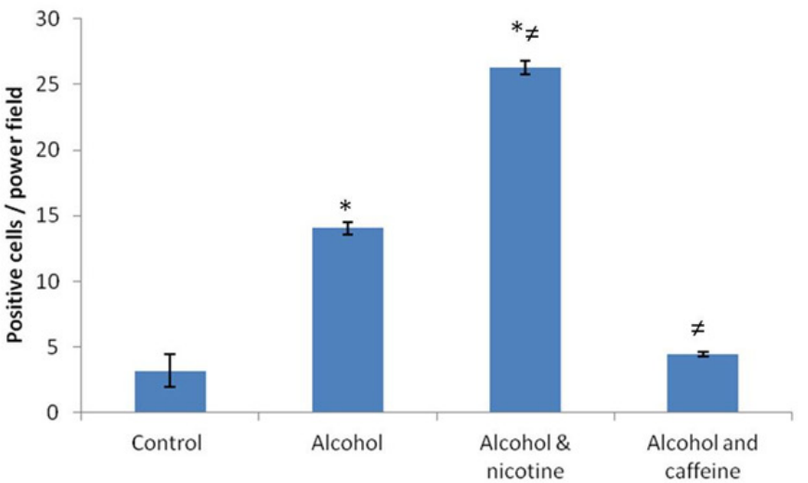

Fig. 11: The area % of Caspase-3 expression was assessed, in different experimental rats, *$p<0.001$* compared to control group, *≠$p< 0.001$* compared to alcohol-treated group.

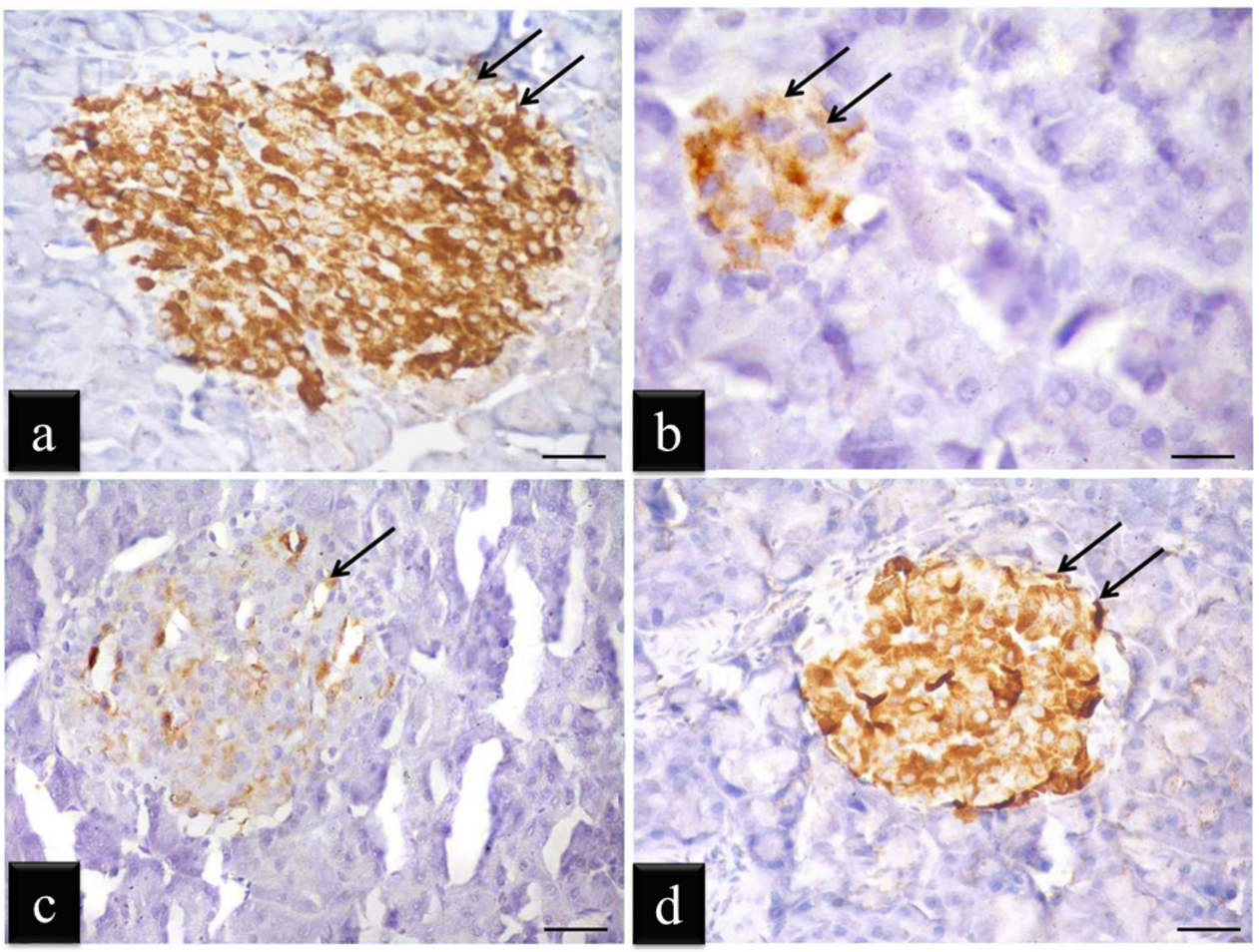

Fig. 12: Insulin expression in the pancreatic islets in different experimental groups. (a) Control group showed obvious insulin expression (arrows) in an islets of Langerhans. (b) Alcoholic pancreatitis group showed less insulin expression (arrows) (C) Alcoholic pancreatitis with nicotine-treated group presented with marked reduction in insulin expression (arrows). (d) Alcoholic pancreatitis with caffeine-treated group showed increased insulin immunoreaction (arrows). Scale bar 40 μm, x200, Insulin protein immunoreaction.

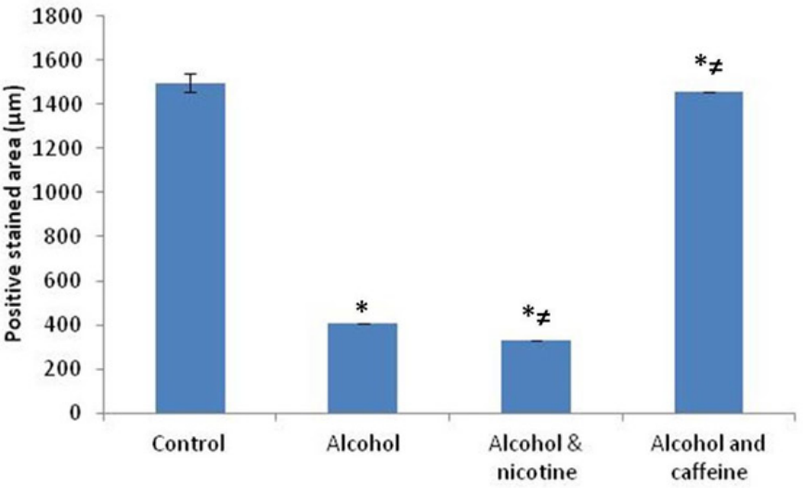

Fig. 13: The area of insulin expression was assessed, in different experimental rats, *$p<0.001$ compared to control group, ≠$p< 0.001$ compared to alcohol-treated group.

DISCUSSION

While alcohol is a well-known trigger of pancreatitis, smoking has been considered as a major risk factor. The mechanism by which smoking contributes to pancreatic injury or by which smoking accelerates the pancreatic inflammatory process is still unknown[19]. Caffeine is a frequently used psychotropic remedy to overcome the effects of tiredness and to increase physical activity[20].

The IP route of ethanol administration was chosen in this study to separate direct effects (e.g., hyperemia) that might result from its contact with the gastric mucosa or the small bowel. Interestingly, the most effective method at delivering caffeine was IP route[21].

Blood glucose, serum amylase and lipase levels, were elevated in alcoholic pancreatitis group this is in agreement with[22]. The latter values were highly elevated in alcoholic pancreatitis with nicotine group. However, these levels were improved in alcoholic pancreatitis with caffeine group.

Cigarette smoking has been shown a cause insulin resistance in peripheral tissues. Heavy smoking, which has been identified as a risk factor for non-insulin dependent diabetes mellitus in several studies, can be related to long-standing diabetes and developing cancer of pancreas[23]. Caffeine is a well-known agent to increase the rate of glucose transport in the absence of insulin in rodent skeletal muscle[24,25]. It also activates 5′-AMP-dependent protein kinase through an insulin-independent mechanism, which increases AMP kinase-1 to promote glucose transport. Moreover, caffeine antagonizes the A1 adenosine receptors in rat skeletal muscles, which improves glucose compliance[26].

The present histological finding in alcoholic pancreatitis group showed disorganized pancreatic architecture with obvious vacuoles among the acini. Also, in between the acini there were congested blood vessels and cellular infiltrations. The total injury score of alcoholic pancreatitis group was significantly higher compared with that of the control group ($P<0.003$) as morphometrically confirmed.[27] reported that, the manifest acute pancreatitis event theory suggests that the first insult occurs in acinar cells, stimulating release of inflammatory cells and secretion of cytokines. Inflammatory cells as well as cytokines, chemokines, and their receptors have different biological functions including provocative response, angiogenesis and metastasis.

The alcoholic pancreatitis with nicotine-treated group showed of obvious acinar vaculations, blood vessels congestion and cellular infiltration.[28,29] Pancreatic acinar damage; the triggers, thresholds, immunologic response, and cellular mechanisms of CP are unclear. Traditional theories to explain the pathogenesis of CP include: oxidative stress, toxic-metabolic factors, ductal obstruction, and necrosis-fibrosis. There is an evidence of a toxic, even though nonspecific, effect of tobacco smoking on the exocrine pancreas. Smoking inhibits pancreatic secretory activity in vivo and in vitro. There are elevated levels of pancreatic proteins in smokers, but not in nonsmokers after secretin injection.

It looks likely that increased duration and/or levels of tobacco intake can lead to recurrent attacks of AP which finally progress into CP. Moreover, the consequence of smoking on the course of autoimmune pancreatitis and after ERCP induced-pancreatitis has been considered. Importantly, nicotine can reduce contraction of the sphincter of Oddi and obstruction which may cause pancreatitis[30].

Interestingly, alcoholic pancreatitis with caffeine-treated rats showed nearly normal slightly shrunken pancreatic acini. Islets of Langerhans with minimal connective tissue in-between and significant improvement of the pancreatic scoring system. These results were in accordance with[31].

Collagen fibers were increased in alcoholic pancreatitis and alcoholic pancreatitis with nicotine-treated groups while decreased in alcoholic pancreatitis with caffeine-treated group. This is in line with what has been documented by[32]. They found that cytokine secretion; fibrogenic pancreatic stellate cells secrete collagen and deposit the stage for fibrosis with pancreatitis. Nicotine stimulates oxidative stress in rat pancreas and this was associated with inflammation and increased IL-6 secretion in the pancreas. Expression of inducible nitric oxide synthase (iNOS) is increased during the progression of pancreatic cancer as well as in inflamed tissues[33]. Caffeine may also act as an antioxidant. Therefore, it helps to prevent different diseases by protecting cells in the body against oxidative damage and raise the overall immunity of a person[34,35].

NFκB is a well-known transcription factor that controls pro-inflammatory gene expression. It may mediate pancreatitis responses through a direct interaction with α7 nAChR on the acinar cell surface, but it might also wield influence over inflammatory cells during pancreatitis. α7nAChRs are expressed on macrophages, and both NNK (4-[Methylnitrosamino]-1-[3-Pyridyl]-1-Butanone) and nicotine could potentially modulate immune responses. Nicotine delays generation of pro-inflammatory cytokines from macrophages by inhibiting the NFκB pathway, which mediates macrophage activation[36,37].

Chronic nicotine exposure stimulates activation of Erk and Akt kinases, both are key pro-cancer pathways. Nicotine treatment in the drinking water for four weeks considerably diminished the response of xenografts mouse model to gemcitabine. This was coupled with decreased gemcitabine-induced caspase-3 cleavage and inhibition of phosphorylated/activated forms of Erk, Akt and Src in xenograft tissues[38].

The present immunohistochemical findings, decreased number of insulin immunoreactive cells in the pancreatic islets of alcoholic pancreatitis-treated group and alcoholic pancreatitis with nicotine-treated group while increased numbers of insulin immunoreactive cells in alcoholic pancreatitis with caffeine-treated group. NFκB and Caspase-3 expression were increased in alcoholic pancreatitis group and marked increase in alcoholic pancreatitis with nicotine-treated group but these expression levels were decreased in alcoholic pancreatitis with caffeine-treated group, the morphometric measurements presented in our study confirmed the results.[39] found that a decreased number of insulin immunoreactive cells in the pancreatic islets of alcoholic pancreatitis group, along with a disrupted β-cells ultrastructure. Additionally,[40] showed that caffeine at a dose of 100 mg/kg/BW/day has a protective effect against pancreatic β -cells damage caused by oxidative stress, and prevent membrane damage.

CONCLUSION

At the moment there is no specific pharmacological treatment for pancreatitis. Caffeine ameliorates experimental acute pancreatitis so; it is a suitable starting point for the manufacture of new drug therapy. We are also hoping that our findings can be used to warn against the dangers of being drinking and smokers.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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