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APROTININ INHIBITED ACUTE PANCREATITIS INDUCED BY HYDROCHLOROTHIAZIDE AND FUROSEMIDE IN EXPERIMENTIAL ANIMALS

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ABSTRACT

The effect of the Kallikrein inhibitor, aprotinin, on hydrochlorothiazide and furosemide inducing pancreatitis has been studied in male rats. Treatment of animals with hydrochlorothiazide and furosemide for two weeks significantly increased serum lipase, amylase and glucose levels. The drugs therapy markedly reduced serum potassium level. Concurrent administration of aprotinin with hydrochlorothiazide and furosemide succeeded to ameliorate the elevated levels of serum lipase, amylase and glucose. Aprotinin failed to correct the hypokalemic effect of diuretics therapy. Histopathological studies revealed that aprotinin improved the picture of the pancreas and beta-cells which were partialy destroyed by hydrochlorothiazide and furosemide.

INTRODUCTION

Diuretics are indispensable group of therapeutic agents that are used to adjust the volume and/or composition of body fluids. Therefore, diuretics remain the cornerstone for the treatment of a variety of clinical situations including hypertension, acute and chronic heart failure, renal failure, nephrotic syndrome, and cirrhosis[1-3]. The thiazide type of diuretics is more effective as antihypertensive agents than loop diuretics such as furosemide especially in patients who have normal renal function[4]. On the other hand, furosemide has been found to be safer than thiazides in elder patients with congestive heart failure[5,6].

However, hypokalemia is the most serious adverse effect of thiazide and loop diuretics which are related to abnormalitis of fluid and electrolytes balance[7,8]. Acute pancreatitis with hyperamylasemia has been also described frequently in patients treated with thiazide and loop diuretics[9-11].

A variety of factors including acute pancreatitis has been found to activate series of proteolytic reactions that generate bradykinin and kallidin in tissues[12]. These peptides are autocoids that act locally to poduce pain, vasodilatation, increased vascular permeability, and prostaglandins synthesis. Thus, they comprise a subset of large number of mediators that contribute to the inflammatory response[13-15].

Kallikrein inhibitors such as aprotinin has been evaluated for the treatment of certain conditions including acute pancreatitis, sepsis, carcinoid syndrome, and brain edema[16,17]. Thus,the aim of the present work was directed to study the effects of hydrochlorothiazide and furosemide alone and in combination with aprotinin (trasylol) on some pancreatic functions; serum lipase (EC 3.1.1.3), amylase (EC 3.2.1.1), insulin and serum glucose, potassium as well as histopathological changes in pancreas.

MATERIALS AND METHODS

Animals:

Male albino rats of both sex, weighing 200 ± 20 g bred at The King Saud Research Center, Riyadh, Saudi Arabia were used. Food and water *ad Libitum* were allowed.

Work Design:

Rats were divided into five groups, 10 rats each and treated for two weeks as follows:

Group 1: was given saline orally and kept as control.

Group 2: was treated orally with hydrochlorothiazide (Esidrex, Ciba) in a dose of 4.5 mg/kg.

Group 3: was given furosemide (Lasix, Hoechst, Ger.).orally in a dose of 3.6 mg/kg

Group 4 and 5: were treated as mentined in groups 2 and 3, respectively in combination with aprotinin (Bayer) in a daily dose of 22,500 KIU/kg (Kallikrein Inactivatory Units) IM.

At the end of the experimental period blood samples were withdrawn from the tail vein and serum was separated. Animals were sacrificed by cervical dislocation and pancreas was removed immediately.

Histopathology:

Sections of pancreas were fixed in formole saline (10%), embedded in paraffin, cut at 3-5 μm and stained with modified aldehyde fuchsin.

Biochemical determinations:

Standard analytical procedures were used for determination of the different activities of serum lipase[18], amylase[19] as well as serum glucose by the oxidase method[20], insulin by enzyme immunoassay[21] and serum K^+ by atomic absorption flame emission spectrophotometer according to **Parker et al.** [22].

RESULTS

As shown in Table 1, administration of hydrochlorothiazide to rats for two successive weeks

significantly increased serum glucose, lipase and amylase levels by approx. 19.8%, 30% and 153% in comparison with control respectively. Hydrochlorothiazide, decreased ($P < 0.001$) serum K^+ level by 57.6% of control value, while serum insulin level was insignificantly reduced.

Treatment of rats with aprotinin in combination with hydrochlorothiazide (Table 1) significnatly increased serum glucose, lipase and amylase levels by 10.9%, 18.7% and 98.8% of control values, respectively. While serum K^+ was reduced by 45.8% of control value. In comparison with hydrochlorothiazide alone, combination of aprotinin with hydrochlorothiazide significantly reduced serum glucose, lipase and amylase by 8.04%, 8.82% and 21.67%, respectively and increased serum K^+ by 27.81% (Table 1).

Data in table 2 showed that, administration of furosemide to animals significantly increased serum glucose, lipase and amylase by approx. 17.26%, 31.39% and 167.25% of control values respectively. The drug significantly decreased serum K^+ by about 31.37% of control value. Non significant reduction in serum insulin was observed.

Treatment of rats with aprotinin in combination with furosemide induced a marked increase in serum glucose, lipase and amylase levels by 21%, 18.37%, 118% and 40.59%, and a decrease in K^+ level by 40.59% of control data, respectively. Combination of aprotinin with furosemide decreased serum levels of glucose, lipase, amylase and K^+ by 8%, 10%, 18.41% and 9.34% in comparison with furosemide alone, respectively (Table 2).

Histopathological studies revealed that, hydrochlorothizaide induced a mild destruction in the B-cell of pancreas with the development of perivascular mast cells (Fig. 1). Few mast cells were shown in pancreas of rats treated with furosemide (Fig. 2). Sections of pancreas of rats receiving aprotinin in combination with hydrochlorothiazide showed an improved picture of B-cell (Fig. 3) and with furosemide showed nearly normal cells (Fig. 4).

Table 1: Effect of administration of hydrochlorothiazide (4.5 mg/kg) alone and in combination with aprotinin (22, 500 KIU/kg) for two weeks on serum glucose, insulin, potassium, lipase and amylase levels.

Parameters	Treatment		
	Control ($\bar{X} \pm$ S.E.)	Hydrochlorothiazide ($\bar{X} \pm$ S.E.)	Hydrochlorothiazide + aprotinin ($\bar{X} \pm$ S.E.)
Glucose (mg/dL)	128.94 ± 1.69	154.47 ± 2.78 (a)	142.05 ± 1.71 (a)
Insulin (IU/L)	16.08 ± 0.66	13.37 ± 0.45 (a)	17.33 ± 0.60
Potassium (mEq/L)	4.41 ± 0.88	1.87 ± 0.09 (a)	2.39 ± 0.11 (a *)
Lipase (IU)	70.38 ± 2.19	91.63 ± 1.76 (a)	83.55 ± 1.59 (a)
Amylase (U/L)	59.75 ± 1.30	151.30 ± 3.09 (a)	118.49 ± 1.78 (a, b)

Mean values ± SEM; (n = 10)
(a) Significantly different from control at $P < 0.001$.
(b) Significantly different from hydrocholorothiazide at $P < 0.001$, $P < 0.05$ (*).

Table 2: Effect of administration of furosemide (3.6 mg / kg) alone and in combination with aprotinin (22, 500 KIU / kg) for two weeks on serum glucose, insulin, potassium, lipase and amylase levels.

Parameters	Treatment		
	Control ($\bar{X} \pm$ S.E.)	Furosemide ($\bar{X} \pm$ S.E.)	Furosemide + aprotinin ($\bar{X} \pm$ S.E.)
Clucose (mg/dL)	128.94 ± 1.69	156.20 ± 2.69(a)	144.23 ± 1.85(a)
Insulin (IU/L)	16.08 ± 0.66	13.00 ± 0.54	14.16 ± 0.51
Potassium (mEq/L)	4.41 ± 0.88	2.89 ± 0.10(a)	2.62 ± 0.05(a)
Lipase (IU)	70.38 ± 2.19	92.47 ± 1.66(a)	83.31 ± 1.81(a)
Amylase (U/L)	59.75 ± 1.30	159.68 ± 2.91(a)	130.29 ± 1.91(a,b)

Mean values ± SEM; (n = 10).
(a) Significnalty different from control at $P < 0.001$,
(b) Significantly different from furosemide at $P < 0.001$, $P < 0.05$(*).

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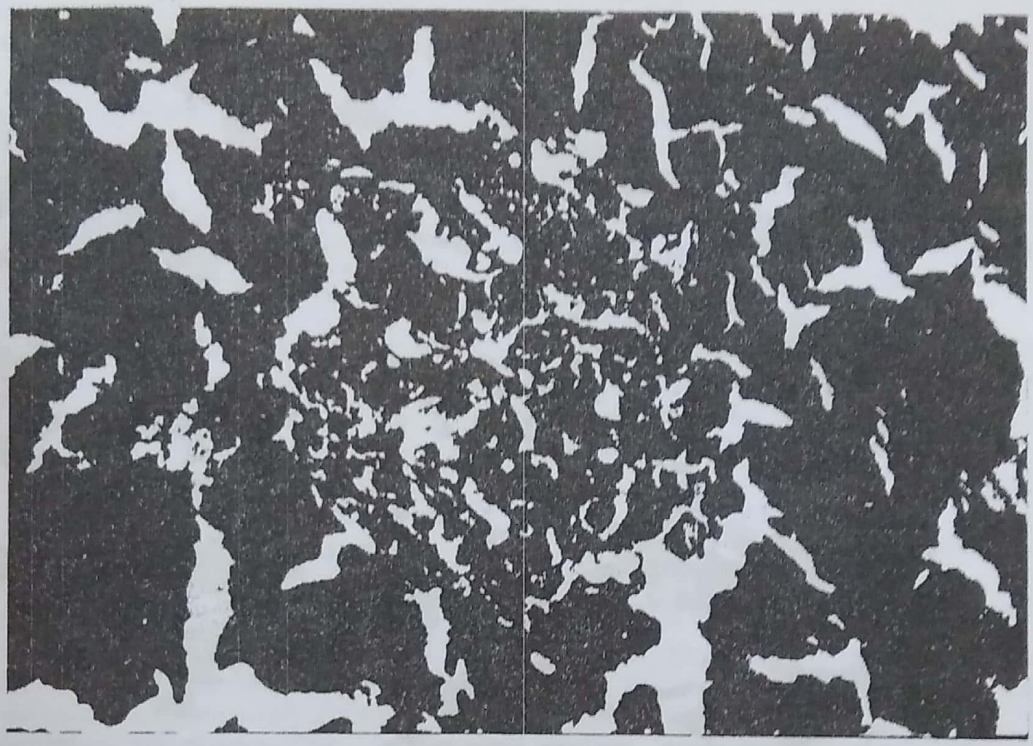

Fig. (1): Section of pancreas of rats treated with hydrochlorothiazide, showing mild destruction of B-cells and appearance of perivascular mast cells. (Aldehyde fuchsin, X 400).

Fig. (2): Section of pancreas of rats treated with furosemide,showing few mast cells due to slight destruction of B-cells (Aldehyde fuchsin, X 400).

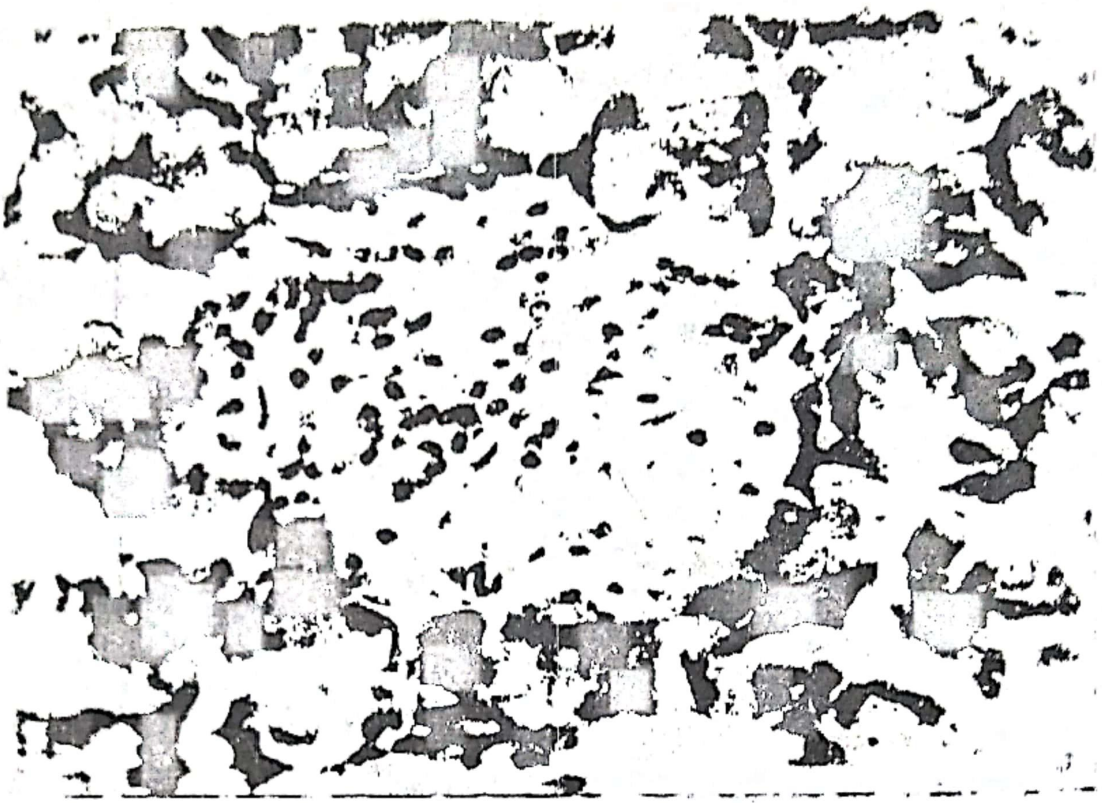

Fig. (3): Section of pancreas of rats treated with aprotinin in combination with hydrochlorothiazide, showing an improved tissue picture. (Aldehyde fuchsin, X 400).

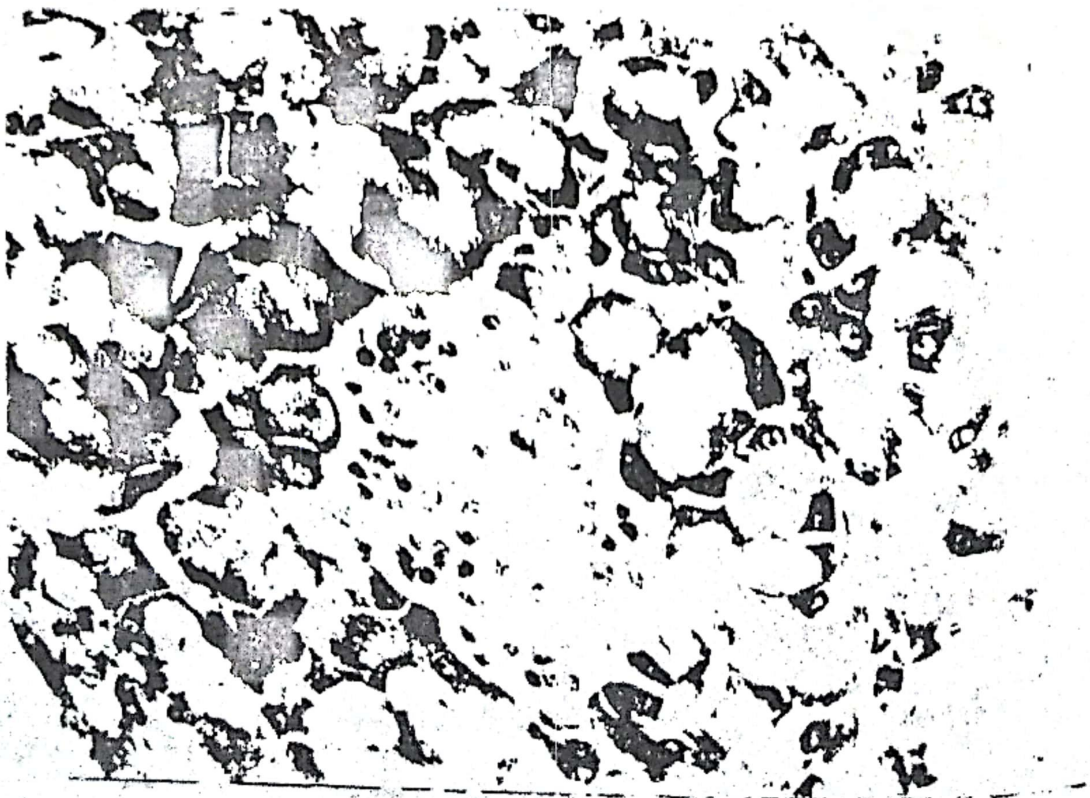

Fig. (4): Section of pencreas of rats treated with aprotinin in combination with furosemide, showed normal picture. (Aldehyde fuchsin X 100)

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DISCUSSION

Pancreatic dysfunctions following the widely used diuretics, hydrochlorothiazide and furosemide were submitted[23]. Evidence of pancreatitis in the present study was observed after two weeks on diuretics therapy used with a significant rise in serum amylase, lipase and glucose levels.

The possibility that thiazide diuretics can induce a diabetes like state was first observed by Wilkins[24] who reported that, hyperglycemia and glucosure a were developed in patients taking such drugs. Positive family history of diabetes is not necessary for post hydrochlorothiazide hyperglycemia[25].

The mechanism of the reduced glucose tolerance by thiazide diuretics is not completely understood but appears to involve, reduced insulin secretion, decreased tissue sensitivity to insulin, increased insulin output resulting in insulin depletion in the prediabetic state and alterations in glucose metabolism[26, 27]. Hyperglycemia may also be related in some way to K^+ depletion, in that hyperglycemia is reduced when K^+ is given along with diuretics[28].

Impairment of glucose tolerance has been observed also in patients receiving furosemide[29]. It has been suggested that glucose intolerance induced by furosemide may be in part due to its direct effect on pancreatic B-cell[11].

Our results demonstrated that, hydrochlorothiazide and furosemide insignificantly reduced serum insulin. These data strongly points to direct effect of diuretics upon the B-cell of pancreas.

The results of the present study also presented that, serum K^+ level was markedly decreased during administration of hydrochlorothiazide and furosemide. This finding was in accordance with previous reports[8, 30]. Thiazide and loop diuretics increase the delivery of sodium to the late distal tubule and collecting duct, a situation that is often associated with increased K^+ and hydrogen ion excretion[17].

Our data also showed that when kallikrein inhibitor, aprotinin concomitantly administered to the animals with hydrochlorothiazide and furosemide a significant protective effect was noticed and serum amylase, lipase and glucose were ameliorated. The insignificant reduction in serum insulin induced by diuretics therapy was normalized by aprotinin administration. These data support our finding that hydrochlorothiazide and furosemide induced pancreatitis which aprotinin inhibit it. Since it was reported that, aprotinin successfuly used in treatment of acute pancreatitis through intention of inhibiting proteolytic activity[16]. Combination of aprotinin with hydrochlorothiazide and furosemide did not protect the animals against the hypokalemic effect of diuretics as hypokalemia may be mainly due to renal dysfunction of diuretics.

The histopathological studies supported our finding in that, aprotinin inhibited the production of pancreatitis during diuretics therapy and produced normal picture of pancreas and B-cell. Also, aprotinin improved the partial destuction in B-cell induced by hydrochlorothiazide and furosemide.

In conclusion, results of the present study point out that diuretics such as hydrochlorothiazide and furosemide can induce acute pancreatitis which may be inhibited by kallikrein inhibitor, a protinin.

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أبروتينين نمط النهاب البنكرياس الحاد الحادث بالهيدروكلوروثيازيد الفيروسوسمد فى حيوانات التجارب

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فى هذا البحث تم دراسة تأثير كل من الهيدروكلوروثيازيد والفيروسيميد كلاً على حدا ومجتمعين مع أبروتينين فى فئران التجارب. أحدث العلاج بكل من الهيدروكلوروثيازيد والفيروسيميد ارتفاعاً ملحوظاً فى معدلات الزيمات الليبز والأميليز وكذلك السكر فى الدم. وقد أدى أيضاً العلاج بالعقارين إلى انخفاض ملحوظ فى معدل البوتاسيوم فى الدم. أما بالنسبة لهرمون الأنسولين فلم يكن التغير ملحوظاً به.

أحدث الحقن بالأبروتين مع كل من الهيدروكلوروثيازيد والفيروسميد إلى إرتفاع معدل كل من الليبز والأميليز فى الدم الحيوانات التجارب بالمقارنة بالمجموعات الضابطه. بينما انخفضت معدلاتهم بالمقارنة بتأثيرات الهيدروكلوروثيازيد والفيروسميد كلاً على حدا.

أثبتت نتائج فحص الأنسجة أن كلاً من الهيدروكلوروثيازيد والفيروسيميد أحدث تغيراً فى أنسجة البنكرياس وخاصة فى خلاياه بيتا التى تفرز الأنسولين وأن اعطاء الأبروتنين مع كل منهم أدى إلى اعادة صورة الخلايا إلى حالتها الطبيعية. والخلاصة أن كل من الهيدروكلوروثيازيد والفيروسيميد عنده القدرة على إحداث التهاب بالبنكرياس وارتفاع فى سكر الدم وأن تعاطى الأبروتنين يقلل من هذه الأعراض.