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A Protective Potential of Aprepitant on L-Arginine Induced Pancreatitis in Albino Wistar Rats

Sabarinath Chandrasekar[1*], Sukirtha Ramakrishnan[1], Sneha Sekar[1], Sudhakar Pachiappan[1], Gokulakannan Shanmugam[1]

[1]Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode, Tamilnadu – 637205, India

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***Corresponding author:** Sabarinath Chandrasekar
Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode, Tamilnadu – 637205, India

Abstract

The aim of the study is to evaluate the effective protective potential of aprepitant on L-arginine induced pancreatitis in albino wistar rats. Thirty albino wistar rats weighing 150-200 g were randomly divided into five groups. Each group contains six animals. Pancreatitis was induced by 250 mg L- Arginine in the normal saline and administer in the time interval of 4th, 7th, 10th, 13th, 16th and 19th days. Treatment with aprepitant 14 mg/kg and 8 mg/kg was evaluated by using body weight and serum parameters like amylase, lipase, creatinine, BUN, ALT, AST, inflammatory markers and histological study of pancreatitis induced rat's pancreas. The present study demonstrates that treatment with aprepitant with 14 mg/kg and 8 mg/kg had potential therapeutic effects on the treatment of pancreatitis. It was found that parental administration of the aprepitant shows the equal effectiveness in treating pancreatitis when compared with pancreatic rats treated with standard drug methylprednisolone. Histopathological studies of the pancreas sample also confirmed the damage in the pancreas reduced due to the aprepitant. This shows good anti- inflammatory activity against diseased group. It is concluded that the aprepitant showed siginificant anti- inflammatory activity in albino rats. Among that aprepitant 14 mg/kg showed distinguished effect than aprepitant 8 mg/kg. Therefore, the study results show that the aprepitant produces siginificant suppression of inflammation, cell damage in pancreas. Thus, it has the potential to be developed for clinical applications in the future.

Keywords: Pancreatitis, L-arginine, Aprepitant, neurokinin -1 receptor, Pancreas.

INTRODUCTION

Pancreatitis is a disease defined as acute or chronic inflammatory process of the pancreas characterized by premature activation of digestive enzymes within the pancreatic acinar cells and causing pancreatic auto digestion [1]. In pancreatitis, a local inflammatory process initiated by release of pro- and anti-inflammatory cytokines and chemokines recruit's granulocytes, monocytes and lymphocytes [2]. They are two types of pancreatitis Acute Pancreatitis: 1. acute pancreatitis 2. chronic pancreatitis

Acute pancreatitis: Acute pancreatitis (AP) is one of the most common gastrointestinal diseases requiring hospitalization worldwide, with a rising incidence ranging from 13 to 45 per 100,000 persons/ year [3]. Acute pancreatitis (AP) is an inflammation of the glandular parenchyma of the retroperitoneal organ that leads to injury with or without subsequent destruction of the pancreatic acini [4]. Although overall mortality of acute pancreatitis ranges from 2% to 10%,27 in 80% of patients the disorder is mild and self-limiting, with minimum mortality [5]. Acute pancreatitis is also associated with multiple rare causes such as drugs, infections, hyperparathyroidism, hyperlipidaemia, shock, trauma, and pregnancy [6].

Chronic pancreatitis (CP) is a progressive inflammatory disease of the pancreas characterized by destruction of parenchyma, fibrosis, and eventual exocrine and endocrine insufficiency. Common causes include alcohol abuse, smoking, and, rarely, genetic predisposition. It presents clinically as recurrent acute pancreatitis in early stages and later as persistent pain, calcification, diabetes, and steatorrhea due to impaired

digestion. Key pathological features include ischemia, fibrosis, reduced pancreatic enzyme secretion (<10% of normal), and decreased bicarbonate secretion, leading to maldigestion. CP also increases the risk of pancreatic ductal adenocarcinoma. Incidence in industrialized countries is 5–12/100,000 annually, with a prevalence of 50/100, 000 [8-10]. Herbs have been used as a source of drugs ever since man started treatment for various ailments, affecting human beings. Every herb has a range of secondary metabolites, which are provided as a result of metabolic activities in the plants. These compounds either alone or in combination with others are reported to be mainly responsible for the specific physiological changes or the therapeutic action in the human body when administrated as a medicament [11].

Autoimmune pancreatitis is a type of pancreatitis characterized by an autoimmune inflammatory process in which prominent lymphocyte infiltration with associated fibrosis of the pancreas causes organ dysfunction. Recently, the term "autoimmune pancreatitis" has become widely accepted, although it is apparent that autoimmune pancreatitis is a heterogeneous disease. prevalence of autoimmune pancreatitis as between 5 and 6% of all patients with chronic pancreatitis. Clinical or biochemical autoimmune stigmata are present in 40% of patients with idiopathic pancreatitis. Autoimmune pancreatitis occurs in both sexes, but it is at least twice as common in men as in women. Patients vary widely in age; most are older than 50 years. Immunologic abnormalities including hypergammaglobulinemia, elevated serum IgG4 levels, and the presence of autoantibodies against carbonic anhydrase and lactoferrin are important markers of the diseases [12].

Aprepitant is indicated for use with other antiemetic agents for the prevention of acute or delayed nausea and vomiting associated with initial or repeat courses of highly emetogenic cancer chemotherapy. Aprepitant (MK-869) is a centrally acting antiemetic. It works as a competitive antagonist of the substance P/neurokinin1 (NK1) receptor. Aprepitant is available as an oral agent. A water-soluble phosphoramidate prodrug of aprepitant is under development for IV administration. The time to peak concentration of aprepitant is 4 hours following oral administration. Aprepitant is extensively metabolized, primarily by CYP3A4 and to a lesser extent by CYP1A2 and CYP2C1. The terminal half-life is 9 to 13 hours.1 Aprepitant has a long duration of action due to its half-life and slow off-rate from the NK1 receptor [13].

Pathiophysiology of Acute Pancreatitis

Acute pancreatitis involves a complex cascade of events, which start in the pancreatic acinar cells. The exact mechanisms of the development of acute pancreatitis are still a subject of debate. The most common and widely accepted theory is that pancreatitis develops because of an injury or disruption of the pancreatic acini, which permit the leakage of pancreatic enzymes (trypsin, chymotrypsin and elastase) into pancreatic tissue. The leaked enzymes become activated in the tissue, initiating auto digestion and acute pancreatitis. The activated proteases (trypsin and elastase) and lipase break down tissue and cell membranes, causing edema, vascular damage, hemorrhage and necrosis [14].

Mechanism of Action

The early activaction of zymogens, and possible activation by cathepsin B, can cause injury to the pancreas through the the activation of proteases by trypsin, leading to autodigestion of the pancreas and subsequent injury and necrosis of acinar cells. Alcohol worsens this injury by generating the active toxic metabolites and activating more zymogens, in 36ddition to triggering a greater inflammatory response with the release of monocyte/macrophage pathways.

Phases of Acute Pancreatitis (AP):

1. **First Phase:** Enzymatic activation and calcium-mediated cellular injury cause abdominal pain and early symptoms.
2. **Second Phase:** Systemic inflammatory response syndrome (SIRS) arises from pro-inflammatory cytokines (IL-1, IL-6, TNF-α, etc.) and nitric oxide. Pancreatic necrosis correlates with organ failure and superinfection.
3. **Third Phase:** Complications develop, including bacterial translocation, secondary infections, and walled-off necrotic collections [15-17].

MATERIALS AND METHODS

Drugs and Chemicals:

- All the chemicals and drugs in the study were of analytical grade.
- Aprepitant was purchased from Sun Pharmaceutical Industries Ltd.
- L-Arginine were purchased from Loba Chemicals.

Selection of Animals

The study was conducted using healthy adult Wistar rats (150-200 g, either sex). The animals were placed in polypropylene cages under standard laboratory conditions (25±2°C, 50±15% humidity, 12 h of a light and dark cycle) with unrestricted access to standard pellet diet and water. The study was performed in the Central Animal House, Swamy Vivekanandha College of Pharmacy, Namakkal, in accordance with an Institution Ethical Committee (IAEC) approval. All of the procedures were in compliance with the CCSEA and the Indian National Science Academy guidelines for the care and use of experimental animals. [18] IAEC REFERENCE NO: SVCP/IAEC/PG/5/08/2024

Animal Grouping

Thirty albino Wistar rats weighing 150–200 g were randomly divided into five groups, each consisting

of six animals, as follows:Group I: Normal control (saline 0.5 ml, p.o.);Group II:L-arginine (250 mg, i.p.);Group III: L-arginine + methylprednisolone (30 mg/kg, p.o.);Group IV: L-Arginine + Aprepitant (8mg/kg,p.o) Group V: L-Arginine + Aprepitant (14 mg/kg,p.o)

Induction of Pancreatitis

Pancreatitis was induced by the Intraperitoneal administration of L-Arginine at the dose of 250mg/kg. L-Arginine was given in IP route in normal saline on 1, 4, 7, 10, 13, 16 and 19 days. Physical evaluation includes monitoring body weight, blood glucose levels, and enzymatic parameters, Determination of serum biochemical parameters.

Body Weight

Body Weight of each rat in all groups were measured daily till continuation of the treatment using a weighing balance and the changes were recorded. [19]

Blood glucose level

Blood samples were withdrawn from the tail vein of each animal aseptically, and blood glucose level (BGL) was measured by using Accu-chek blood glucometer. In the all causes, blood glucose level measurement was done on the 4th, 9th, 14th, 21th and the average value was taken.

Enzymatic parameters

The amylase, lipases were analyzed by Enzyme Linked Immunosorbent Assay method by using ELISA kit.

Determination of Serum Biochemical Parameters

Estimation of creatinine:

Principle:

Creatinine reacts with picric acid to produce coloured compound, creatinine alkaline picrate. The change in absorbance is proportional to the creatinine concentration.

Creatinine + alkaline picrate $\longrightarrow$ Orange coloured compound

Procedure

Pipette into tubes marked	Standard	Test
Working reagent	1000µl	1000µl
Standard	100µl	
Sample		100µl
Mix and read the optical density (T_1) 60 seconds after the sample or standard addition exactly 60 seconds after the first reading take second reading (T_2)		

Calculations:

$$\text{Creatinine concentration (mg/dl)} = \frac{\text{(T2-T1) sample}}{\text{(T2- T1) of std}} \times 2$$

The levels are expressed as mg/dl serum.

Estimation of liver function test:

Blood samples from the above animals were collected by retro orbital plexus from diethyl ether anaesthetized rat into clean non-heparinized bottles and allowed to clot. The serum was separated from the clot and centrifuged according to groups into clean bottles for biochemical analysis.

Estimation of serum glutamate oxaloacetate transaminase (SGOT)

Serum oxaloacetate transaminase, SGOT also called as Alanine transaminase AST was determined by using Reitman and Franker method.

SGOT (AST) Catalyses the following reaction:

α-ketoglutarate + L-Aspartate ↔ L-Glutamate + Oxaloacetate

Oxaloacetate so formed is coupled with 2,4-Dinitro phenyl hydrazine (2,4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium and this can be measured calorimetrically.

Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Standard: Standard calibration curve was given for finding the concentration of the test samples.

Summary of the Assay details

Pipetted in to test tube with test and add 0.25 ml of Buffered Aspartate a - KG substrate pH: 7.4 and Incubated at 37°C for 30minutes. Add 0.25 ml of DNPH Colour reagent mixed well and allowed to stand at room temperature (15-30°) for 20 minutes. Add 2.5 ml of 4 N NaOH diluted to 10 ml with distilled water and allowed to stand at room temperature for 10 min and O.D was read at 505 nm**.**

Calculation: Concentration of the test samples were found out from the given standard calibration curve.

Estimation of Serum Glutamate Pyruvate Transaminase (SGPT)

Serum Glutamate Pyruvate Transaminase, SGPT also called as Alanine transaminase ALT was determined by using Reitman and Franker method (Reitman S and Franker S, 1957)

Principle:

SGPT (ALT) catalyses the following reaction:

α-ketoglutarate + L-Alanine ↔ Glutamate + Pyruvate

Pyruvate so formed is coupled with 2,4-Dinitrophenyl hydrazine (2,4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium and this can be measured calorimetrically.

Procedure: Fresh clear and unhaemolysed serum was used for estimation.

Standard: Standard calibration curve was given for finding the concentration of the test samples.

Summary of Assay Details:

Pipette in to test tube with test sample and add 0.25ml of Buffered alanine a – KG substrate p:7.4 and incubated at 37°C for 5 minutes. Then add 0.05 ml of serum and incubated at 37° C for 30 minutes. Add 0.25 ml of DNPH colour reagent mixed well and allowed to stand at room temperature (15-30°C) for 20 minutes. Add 2.5ml of 4N NaOH diluted to 10ml with distilled water and allowed to stand at room temperature for 10 mins and O.D was read at 505 nm.

Calculation: Concentration of the test samples were found out from the given standard calibration curve. [20]

Determination of inflammatory biomarkers

Estimation of Pro-inflammatory cytokines like TNF-α, IL -6

The animals would be sacrificed using euthanasia method after the experimental period. ELISA (enzyme –linked immunosorbent assay) kits would be used to measure the levels of pro-inflammatory cytokines such TNF-α.IL-6 serum, according to the instructions provided by the manufacturer. [21]

Histopathology

For histopathological study, on the 21st day at the end if the experiment, all the animals were anesthetized under light ether anesthesia and sacrificed by cervical decapitation. Then, the pancreas removed from the rat, washed with normal saline and stored in 10% formalin. The fixed tissues were then stained with hematoxylin and eosin and viewed under 4* (×10) magnifications (scanner view) at doctors Diagnostic centre vivekanandha medical care hospital Namakkal. [22]

Statistical analysis

The data represents as Mean ± SEM of six replicated determinations. Results were analysed statistically by one-way ANOVA followed by Tukey's multiple comparison. The difference was considered significant when $p<0.05$. All statistical tests were carried out using Prism 9.0 (Graph Pad, San Diego CA, USA) statistical software.

RESULTS

Effect of Aprepitant on Body Weight Changes against Pancreatitis Induced Rats

S.NO	TREATMENT	DOSE	INITIAL BODY WEIGHT (g)	FINAL BODY WEIGHT (g)	CHANGE IN BODY WEIGHT (g)
1	Normal control	Normal saline	177±6.09	189±6.94	11.5±5.93
2	Disease control L-arginine	250 mg *(i.p)*	180±5.94	154±5.68^{a***}	-26.67±4.52 ^{a***}
3	Methylprednisolone	30 mg/kg(*p.o)*	170±5.97	181±6.40^{b**}	10.66±6.73 ^{b**}
4	Aprepitant	8 mg/kg(*p.o)*	174±5.84	181±7.10^{b**}	6.5±5.52 ^{b**}
5	Aprepitant	14 mg/kg(*p.o)*	180±6.78	185±7.62^{b**}	5.5±4.98 ^{b**}

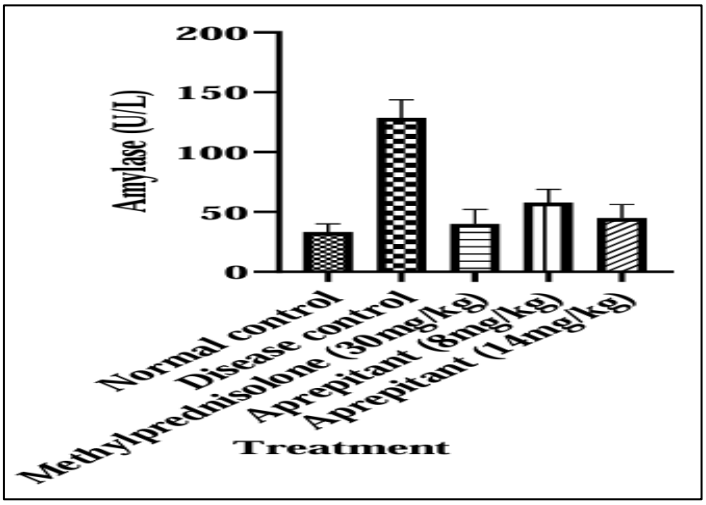
Effect of Aprepitant on Blood Glucose Level Changes against Pancreatitis Induced Rats

S.NO	TREATMENT	DOSE	BLOOD GLUCOSE (mg/dl)
1	Normal control	Normal saline	89.97±2.138
2	Disease control L-arginine	250 mg *(i.p)*	176.4±3.729^{a***}
3	Methylprednisolone	30 mg/kg(*p.o)*	102.3±3.428^{b***}
4	Aprepitant	8 mg/kg(*p.o)*	128.6±4.160^{b*}
5	Aprepitant	14 mg/kg(*p.o)*	111.2±3.189^{b**}

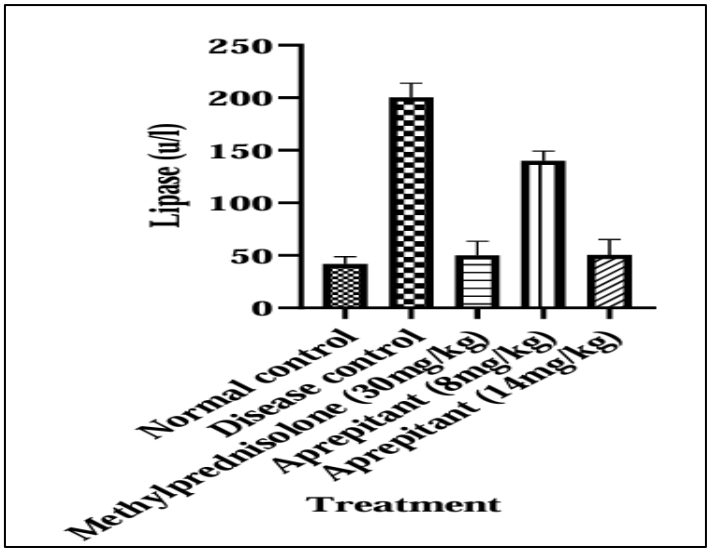
n =6; values expressed as Mean ±SEM; Comparison made as follows. a – Group I vs Group II, III, IV and V; b- Group II vs Group III, IV, and V; symbols represent statistical significance: ***P<0.05

Effect of Aprepitant on Amylase and Lipase Level Changes against Pancreatitis Induced Rats:

1. Pancreatic Amylase:



2. Pancreatic Lipase:



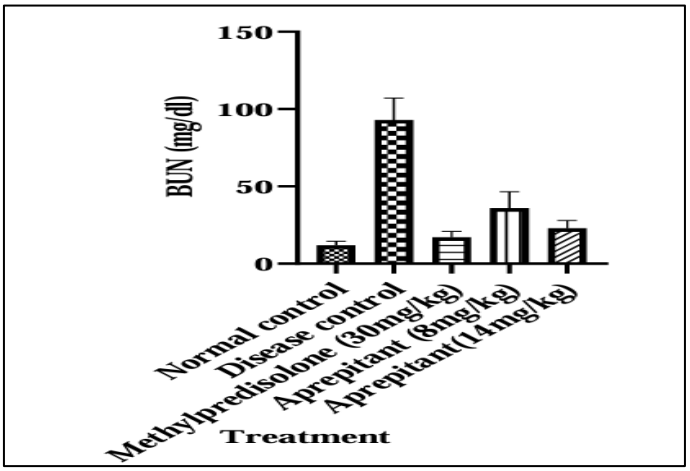
Effect of Aprepitant on Creatinine Level Changes against Pancreatitis Induced Rats:

S.NO	TREATMENT	DOSE	CREATININE (mg/dl)
1	Normal control	Normal saline	0.5117 ± 0.02386
2	Disease control L-arginine	250 mg *(i.p)*	1.422 ± 0.006009^{a***}
3	Methylprednisolone	30 mg/kg(*p.o)*	0.678 ± 0.01815 ^{b***}
4	Aprepitant	8 mg/kg(*p.o)*	0.8133 ± 0.02679 ^{b*}
5	Aprepitant	14 mg/kg(*p.o)*	0.7583 ± 0.02056 ^{b**}

n =6; values expressed as Mean ±SEM; Comparison made as follows.

a – Group I vs Group II, III, IV and V; b- Group II vs Group III, IV, and V; symbols represent statistical significance: ***P<0.001, **P<0.01, *P<0.05

Effect of Aprepitant on Bun Level Changes against Pancreatitis Induced Rats:



Graphical Representation of Effect of Aprepitant on BUN Level Changes against Pancreatitis Induced Rats

Effect of Aprepitant on Liver Function (Alanine Transaminase and Aspartate Aminotransferase) Level Changes against Pancreatitis Induced Rats:

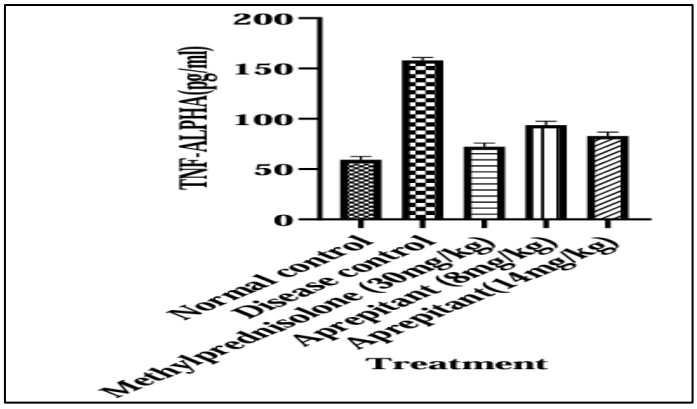
S.NO	TREATMENT	DOSE	ALT (U/L)	AST(U/L)
1	Normal control	Normal saline	60.90±3.120	26.22±2.411
2	Disease control L-arginine	250mg *(i.p)*	133.1±2.412^{a***}	64.52±3.265^{a***}
3	Methylprednisolone	30 mg/kg(*p.o)*	10.69±10.69^{b***}	40.05±3.550^{b***}
4	Aprepitant	8 mg/kg(*p.o)*	98.33±3.065^{b**}	58.95±3.224^{b**}
5	Aprepitant	14 mg/kg(*p.o)*	89.52±1.813^{b***}	46.47±3.338^{b**}

n =6; values expressed as Mean ±SEM; Comparison made as follows.

a – Group I vs Group II, III, IV and V; b- Group II vs Group III, IV, and V; symbols represent statistical significance: ***P<0.001, **P<0.01, *P<0.05

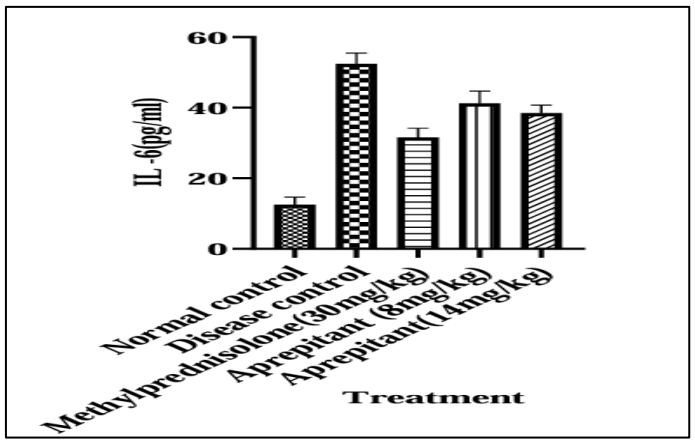
Effect of Aprepitant on Inflammatory Makers (Tumor Necrosis Factor-Alpha (TNF-α And Interleukin-6) Level Changes against Pancreatitis Induced Rats:

Tumor Necrosis Factor-α



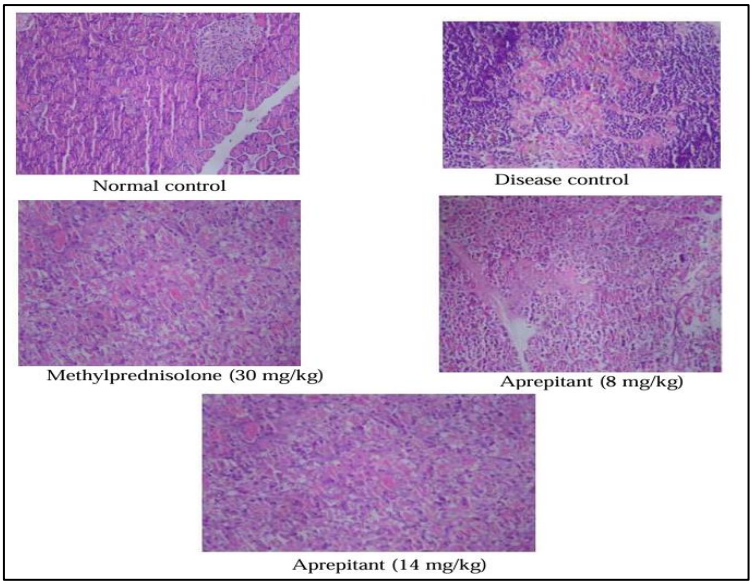
Graphical Representation of Effect of Aprepitant on TNF-α Changes against Pancreatitis Induced Rats

Interleukin-6



Graphical Representation of Effect of Aprepitant on IL-6 Changes against Pancreatitis Induced Rats

Histopathological Studies of Pancreas Activity of Aprepitant against L- Arginine Induced Pancreatits



Histopathological Studies of Pancreas Activity of Aprepitant against L- Arginine Induced Pancreatitis

DISCUSSION

Pancreatitis is caused by inflammation of the pancreas from the early activation of digestive enzymes, which leads to autodigestion, damage to acinar cells, and inflammation associated with neutrophils and macrophages. Severe inflammation can lead to tissue death/necrosis and/or organ failure. Pancreatitis can be classified as either acute or chronic; treatment includes pain management and fluid replacement. Aprepitant is a drug that prevents vomiting and nausea associated with chemotherapy, and it also impacts substance P that relates to inflammation.

In this study, we induced a model of pancreatitis in rats with a high dose of l-arginine, precipitating oxidative stress and inflammation. Disease control rats lost significant weight due to reduced total intake from pain and inflammation. Treatment with aprepitant (8 mg/kg and 14 mg/kg) improved body weight. Hyperglycemia was observed in disease control rats, likely due to impaired insulin release because of inflammation of the pancreas. Aprepitant treatment lowered blood glucose compared to the disease control group.

SUMMARY AND CONCLUSION

Rats were induced with L-arginine to produce pancreatitis, which occurs due to premature activation of pancreatic enzymes leading to inflammation. Aprepitant, an antiemetic with anti-inflammatory properties, reduced pro-inflammatory cytokines through antagonizing NK1 receptors. Treatment with 14 mg/kg of aprepitant exhibited a superior outcome than treatment with 8 mg/kg, and led to improved pancreatic function as evidenced by reduced levels of biochemical markers (amylase, lipase, TNF-α, IL-6, etc.) all while reducing inflammation, and cell injury seen in histological studies. Aprepitant is novel in the management of pancreatitis, and studies are still needed to establish further long-term safety and efficacy.

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