Protective Role of Diadzein in L-Arginine-Induced Acute pancreatitis in Rats

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ABSTRACT

L-arginine is a naturally occurring quasi amino acid. The treatment of high amounts of L-arginine was already demonstrated to cause acute pancreatitis. Daidzein is a naturally occurring isoflavone flavonoid discovered from Pueraria mirifica and belongs to the group of physiologically active secondary metabolites. Anti-tumor, anti-diabetic, anti-obesity, and cardiac actions are all established uses. The objective of this paper is to see if diadzein can prevent rats from developing acute pancreatitis caused by L-arginine. Five groups of Wistar rats were assigned randomly i.e., saline, L-arginine, methyl prednisolone, and diadzein with L-arginine. Rats were killed three days after receiving the final dosage of diadzein, and blood was taken for biochemical examination of amylase, lipase, C-reactive protein, TNF-α, IL-6, superoxide dismutase, lipid peroxidation, glutathione, and catalase. The isolated pancreatic tissue was subjected to histopathological examinations. When contrasted to the disease and normal control groups, diadzein therapy dramatically reduced amylase, lipase, C-reactive protein, TNF-α, IL-6, and lipid peroxidation while increasing superoxide dismutase and glutathione levels. Furthermore, histological examination revealed that diadzein administration reduced pancreatic acinar cell damage compared to rats given L-arginine alone, perhaps due to the reduction of oxidative stress. More research on the actual molecular process is required.

Keywords: *L-arginine, pancreatitis, diadzein, amylase, antioxidant enzymes*

INTRODUCTION

Acute pancreatitis (AP) is a trypsin-activated pancreatic inflammatory disease with varying degrees of activation of other local organs. With rising indications of maturity rates over the earlier decades, AP is recognized as one of the most common acute illnesses globally.[1]Despite the fact that it is self-limiting, upto to 20percent of individuals may develop moderate edematous to extreme necrotizing forms. It is quite prevalent in the Western world, with a yearly prevalence of 20 cases per 100,000 persons. Worldwide, 4.58 lakh reported cases of pancreatic cancer were recorded in 2018, with an anticipated 3.55 lakh new cases occurring through 2040.[2,3] Systemic inflammatory response syndrome or multiple organ failure syndromes can cause death as a result of AP. Acute abdominal discomfort and a rise in serum lipase and amylase concentrations are the most prevalent signs of AP. Around 80percent of total of cases are caused by biliary tract illness or alcoholism.[4]

Microvascular abnormalities that cause oedema, lipid necrosis, acute inflammatory response, loss of pancreatic parenchyma, and degradation of blood vessels that contribute to interstitial hemorrhage are all symptoms of AP changes. AP has been described as a multifactorial pathophysiological program that requires autodigestion of the pancreas by intrapancreatic trypsin, interrupted microcirculation, enhanced calcium, excessive inflammation, oxidative stress, necrosis, significant discharge of proinflammatory cytokines, and pancreatic cell apoptosis.[5,6]Increased reactive oxygen species (ROS) generation is associated with AP, which causes inflammation and pancreatitis via zymogen degranulation, granulocyte migration, tissue necrosis, and elevated amylase and lipase activity.[7]

Daidzein is a naturally occurring isoflavone flavonoid derived from Pueraria Mirifica. It belongs to a group of physiologically active bioactive compounds generated by soybeans and other legumes.[8] In traditional Chinese medicine, it is also the most active bioactive component. Daidzein has a chemical structure that is comparable to that of mammalian estrogens, and it has a dual-directional action that involves replacing/interfering with estrogen and the estrogen-receptor (ER) complex. Daidzein has a variety of pharmacological actions, including anti-tumor, anti-diabetic, anti-obesity, and cardiac actions.[9-11]Fat loss, bowel movement decrease, and inflammation linked with histopathological abnormalities are some of the additional medicinal applications of daidzein. These positive benefits are mostly related to immune response modulation, oxygen free radical scavenging, and proliferation inhibition, among other things.[12]

There is presently no definitive cure, and medication is primarily supportive, aimed at preventing local pancreatic damage and systemic inflammatory consequences. As a result, we postulated that diadzein might be useful in the treatment of AP due to its well-known antioxidant and antiapoptotic properties, as well as its safety and lack of harmful side effects.

MATERIAL AND METHODS

Chemicals

L-arginine was purchased from Sigma Aldrich Pvt. Ltd., Mumbai, India. Amylase, lipase, TNF-α, IL-6,and C-reactive protein estimation kits were purchased from Erba Diagnostics Inc., USA. All other chemicals used in the study are of analytical grade and were purchased from Sigma Aldrich Pvt. Ltd., Mumbai, India.

Animals

Albino male Wistar rats weighing 200-250 g were used in the experiment. At a temperature of 25±2°C, the animals were kept in polypropylene cages. They were fed a normal pellet diet with unlimited water. They were given a week to acclimate before the trial begun. With reference number GPRCP/IAEC/23/19/02/PCL/AE-13-Rats-M-42, the Institutional Ethics Committee, G. Pulla Reddy, College of Pharmacy, Hyderabad, India authorized all of the research.

Study Protocol

The animals were divided into 5 groups (n=6) each at random Group I(Normal Control) received normal saline, Group II(disease control) received two doses of L-Arginine (2.5 g/kg i.p)1 hour apart. Group III(standard control) received methyl prednisolone (30 mg/kg/day p.o). Treatment groups IV and V (Diadzein-I and Diadzein-II, respectively) received 10 and 20 mg/kg/day i.p. All the groups received respective treatment one days before and after administration of L-Arginine and 1hour after second dose of L-Arginine.[13]

24hours after 2nd dose of L-Arginine blood samples were collected under mild anaesthesia and estimated serum biochemical parameters. Finally animals were sacrificed and isolated Pancrease, Lungs, Liver, Heart and Kidney for biochemical estimation and histopathological examinations.

Estimation of Inflammatory Biomarkers

Two key indicators for pancreatitis are serum amylase and lipase levels.Serum amylase, lipase, TNF-α, IL-6 and C-reactive protein (CRP) levels were measured with the help of kit obtained from Sigma Aldrich Pvt. Ltd., Mumbai, India.

In a potassium phosphate buffer (10 mM pH), pancreatic tissue was homogenized (7.4). The weight of homogenized tissue to the buffer used was 1:5. The produced homogenates were then centrifuged at 4000 g for 10 minutes at 4 °C to extract the supernatant, which was used to measure pancreatic amylase, lipase, TNF-α, IL-6, and CRP.[14]

Estimation of Endogenous Antioxidants

In serum and pancreatic homogenate, endogenous antioxidant activity were calculated. Animal pancreas were extracted and homogenized for 15 minutes in ice-cold phosphate buffer (pH 7.4) using a remimotor at 15000 rpm. On the obtained homogenates, colorimetric assays of Superoxide Dismutase (SOD), Malondialdehyde (MDA), glutathione (GSH), and Catalase (CAT) were conducted. Marklund&Marklund established a technique for determining SOD activity.[15] and the concentration of lipid peroxides was determined by utilizing the Buege&Aust technique to estimate MDA.[16] The GSH level was evaluated using the Rahman et al., 2006 technique.[17]Hadwan and Abed's method was used to determine the catalase enzyme's activity.[18]

Histopathological Studies

The samples were fixed in paraffin wax and partitioned into five-millimeter complex shapes for light microscopy. Hematoxylin and Eosin were employed as histological stains in this

investigation (H&E). The stained slices were overlaid with cover slip and viewed under a CX31 microscope.[14]

Statistical Analysis

All of the data were reported in mean±standard error of the mean (SEM) and subjected to one-way analysis of variance followed by Tukey's Multiple Comparision Test with a significant threshold of $P< 0.05$. The Graph Pad Prism program (Version 5) was used to analyze the data (Graphpad Software Inc., San Diego, California, USA).

RESULTS

Effect of Diadzein on Pancreatic Edema

As shown in table 1, There was no animal mortality observed during the trial. There was a significant ($P<0.01$) rise in the weight of the pancreas, confirming pancreatic edema,

Table 1: Effect of Diadzein on Organ Weights

Treatment	Group I (NC)	Group II (DC)	Group III (DZ I)	Group IV (DZ II)	Group VII (PRED)
Body wt (g)	216.8±39.22	209.2±12.86	210.2±10.32	207.8±8.75	209.0±12.1
Pancrease wt(mg)	859.5±27.0	1130±53.2$^{\alpha}$	998.2±26.3$^{\alpha,a}$	892.8±38.5^{a}	917.5±62.1^{a}

Data presented as Mean ± SEM (n=6).$^{\beta}P<0.01$compared to the normal control (One-way ANOVA, Tukey's Multiple Comparison Test).

Effect of Diadzein on Inflammatory Biomarkers

According to Table 2, the disease control group of rats showed a significant ($P < 0.001$) increase in the levels of amylase, lipase, TNF-α, and CRP both in the serum and pancreatic tissue when compared to the normal control group, which indicates pancreatic inflammation. There was less significant ($P < 0.01$) difference found in the levels of IL-6 in disease control group when compared to normal control, decrease in the levels of amylase, lipase, TNF-α, and CRP was observed in animals treated with diadzein in both serum and pancreatic tissue,when compared to the disease control group($P < 0.001$). Diadzein therapy increased the preventive role on the pancreas, dose dependently against L-arginine by lowering these inflammatory markers substantially ($P<0.001$) when compared to normal control. Diadzein at a dosage of 20 mg/kg body weight produced better outcomes and is comparable to the standard control group.

Table 2: Effect of Diadzein on Inflammatory Biomarkers in Serum and Pancreatic Tissue

Treatment	Group I (NC)	Group II (DC)	Group III (DZ I)	Group IV (DZ II)	Group VII (PRED)
Serum Amylase(IU/L)	1281±55.53	4998±256.5 $^{\alpha}$	3310±412.5$^{\alpha,a}$	1757 ±169.60$^{\beta,a}$	1490±93.96 ^{a}
Serum Lipase (U/L)	68.87±2.78	1388±33.81 $^{\alpha}$	230.5±19.69$^{\alpha,a}$	154.7 ±26.31$^{\beta,a}$	116.8,±9.326 ^{a}

Pancreatic Edema(mg/g)	5.9±0.72	38.92±3.36$^{\alpha}$	13.62±1.35$^{\alpha,a}$	8.03±0.88^{a}	4.40±1.12^{a}
Serum TNF–α(ng/L)	18.94± 0.42	23.62± 0.73$^{\alpha}$	21.40 ±0.59$^{\alpha,a}$	20.20 ±0.56$^{\gamma,a}$	19.90 ±0.13$^{\gamma,a}$
Serum IL6 (Pg/ml)	102.8± 4.16	162.2 ± 4.70 $^{\alpha}$	132.0 ±7.82$^{\alpha,a}$	118.0 ± 9.31$^{\beta,a}$	112.2 ±4.95 ^{a}
Serum CRP (mg/L)	407.0± 54.13	16064± 18 $^{\alpha}$	4654±10 $^{\alpha,a}$	1577 ± 272.80 ^{a}	580.3 ±64.40 ^{a}

Data presented as Mean ± SEM (n=6).$^{\alpha}P$<0.001, $^{\beta}P$<0.01, $^{\gamma}P$<0.05 compared to the normal control; ^{a}P<0.001, ^{b}P<0.01, ^{c}P<0.05 compared to the disease control(One-way ANOVA, Tukey's Multiple Comparison Test).

Effect of Diadzein on Endogenous Antioxidants

According to Table 3, the disease control group of rats showed a significant (P < 0.001) decrease in the antioxidant status when compared to normal control group which was observed as reduced levels of SOD, GSH, CAT, and a rise in MDA levels in the pancreatic tissue, indicating that oxidative stress is involved in pancreatitis. However, when diadzein was administered to the animals, there was a significant (P < 0.001 &P < 0.01) increase in the levels of SOD, GSH, and reduction (P < 0.01) in the levels of MDA, CAT levels when compared to disease control group. By boosting these antioxidants, diadzein therapy significantly (P<0.01) enhanced the protective impact on the pancreas against L-arginine dose dependently. Diadzein, given at a dose of 10 mg/kg body weight, significantly increased antioxidant levels and may be considered comparable to the standard control group.

Table 3: Effect of Diadzein on Endogenous Antioxidants

Treatment	**Group I (NC)**	**Group II (DC)**	**Group III (DZ I)**	**Group IV (DZ II)**	**Group VII (PRED)**
MDA(nmol/mg protein)	7.983±0.5	96.00±5.9 $^{\alpha}$	37.05 ±6.9$^{\alpha,a}$	14.38±2.5 ^{a}	9.905±0.7 ^{a}
SOD (U/g tissue)	22.43±1.8	6.967±0.5 $^{\alpha}$	13.0±1.7 $^{\alpha,a}$	17.97±0.6 $^{\alpha,a}$	18.13±0.7 $^{\alpha,a}$
Catalase (U/mg protein)	0.045±0.02	1.70±0.15 $^{\alpha}$	0.27±0.16 $^{\alpha,a}$	0.11±0.03 ^{a}	0.079±0.01 ^{a}
GSH (µmol/L/g tissue)	827.3±44.01	350.8±158.2$^{\alpha}$	555.0±32.3$^{\alpha,b}$	710.7±59.8 ^{a}	746.8±68.4 ^{a}

Data presented as Mean ± SEM (n=6).$^{\alpha}P$<0.001, $^{\beta}P$<0.01, $^{\gamma}P$<0.05 compared to the normal control; ^{a}P<0.001, ^{b}P<0.01, ^{c}P<0.05 compared to the disease control (One-way ANOVA, Tukey's Multiple Comparison Test).

Histopathological Studies

There were no histological changes inside acinar cells in pancreatic tissues from treated groups with saline or methyl prednisolone, according to histological investigation. Results revealed a normal pancreatic morphology. L-arginine caused a substantial disturbance of normal architecture, including cell vacuolization, interstitial edema, fat necrosis in adipose

tissues, and widespread acinar cell destruction. Diadzein treatment protected the pancreas against L-arginine-induced histological damage, resulting in reduced interstitial edema, no vacuolar degeneration, and typical structural design.

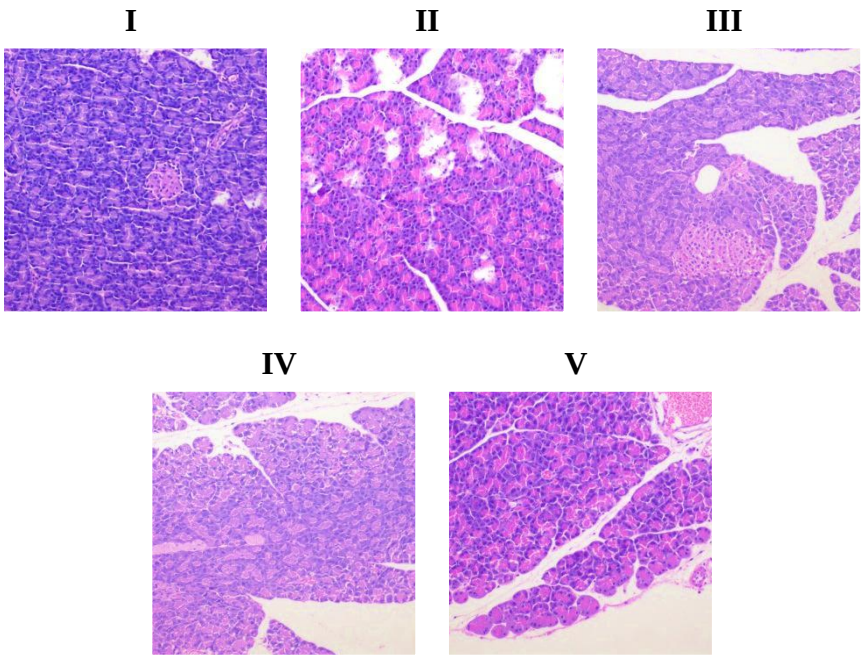

Figure 1: Histopathological findings of formalin-fixed paraffin embedded section of pancreas with hematoxylin and eosin. (I) Photomicrograph of the normal pancreas; (II) L-arginine induced pancreas showing atrophic islets, extensive acinar cell damage with interstitial edema &vacuolated acini; (III) Methyl prednisolone treated animal pancreas showing normal echotexture; (IV & V) Protective effect of Diadzein on pancreas showing normal acinar cells, islets of Langerhans with islet cells (H&E X 400; Inset X1000).

DISCUSSION

The objective of this paper was to see if a higher dosage of L-arginine (as a model) might cause acute pancreatitis (AP) in rats and if diadzein could prevent it. L-arginine produced acute pancreatitis, as indicated by a significant rise in plasma amylase and lipase concentrations in the L-arginine administered animal group, which was consistent with earlier studies.[19,20] Delaney and Weaver, demonstrated that large doses of L-arginine produce acute pancreatitis in a model, indicating that high doses of L-arginine were suitable for inducing acute pancreatitis.[21]L-arginine-induced AP was used in this investigation because it is a reliable, non-invasive paradigm that causes dose-dependent necrosis in pancreatic acinar cells with distinct alterations within 24 hours after delivery, although the exact process is unknown,findings suggests that oxygen free radicals, nitric oxide (NO), and inflammatory mediators all play a role in the disease progression.[22-26]

Following ingestion, daidzein and its glycoside derivative, daidzein, are converted to O-desmethylangolensin (O-DMA) and equol. When compared to daidzein, these metabolites have higher antioxidant capabilities and may thus be advantageous to human health.[27] Two key diagnostic indicators for interpreting the severity of AP are serum amylase and lipase.

Lipase has been proven to be a more important biological marker for acute pancreatitis identification than amylase.Pre-administration of diadzein prevented the rise in lipase levels, suggesting that diadzein may block a signaling pathway implicated in the development of AP.[28,29] L-arginine treatment increased the levels of amylase and lipase in the current research, indicating exocrine pancreatic injury, which is consistent with earlier studies.[13] In vitro, diadzein has been shown to block alpha-amylase and alpha-glucosidase glucose absorption.[30] In a dose-dependent manner, diadzein administration decreased the levels considerably by triggering their protective action through radical scavenging activity.

Because anti-oxidant enzyme production in pancreatic islet cells is exceedingly low, pancreatic tissue is more vulnerable to oxidative stress than other tissues. This triggers inflammatory reactions in the pancreas, which are defined as a continuum from acinar cell damage to local and systemic inflammation. Pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP) are generated first in the pancreas and then in distant organs in acute pancreatitis, resulting in a systemic inflammatory response syndrome (SIRS).[31]C-Reactive Protein (CRP) is a non-specific acute phase-reactive protein seen in the bloodstream following an inflammatory response. Treatment with diadzein resulted in a substantial drop in serum TNF-α, IL-6, and CRP concentrations, as well as a reduction in the severity of acute pancreatitis, according to the results of our present study. Furthermore, earlier studies have shown that treatment with diadzein decreases the rise in TNF-α, IL-6, and CRP levels in various experiments.[32-34]These data suggest that diadzein-induced reductions in blood levels of TNF-α, IL-6, and CRP play a role in diadzein's preventive and therapeutic effects in acute pancreatitis.

Lipids, like AP, are one of the most common targets for free radical destruction. By eliminating one hydrogen atom from polyunsaturated fatty acids and forming hydroperoxides, the latter causes lipid peroxidation. As a result of the disruptions in cellular fluidity and membrane integrity, cells disintegrate and die necrotically. As a result, subcellular components released into the extracellular fluid will trigger a cascade of inflammatory processes, worsening the damage already present.[35,36]When compared to the L-arginine-treated pancreatitis group, the MDA level in the diadzein-treated group was substantially lower. These findings point to an antioxidative mechanism that protects against free radical-induced damage by inhibiting lipid peroxidation.

L-arginine supplementation has been linked to an imbalance in oxidative and antioxidant defense capability. In this study, pancreatitis caused by L-arginine resulted in an increase in oxidative stress due to lower levels of SOD, GSH, and CAT.[22,37] Diadzein treatment reduced reactive oxygen species by increasing SOD, GSH, and CAT levels, which is consistent with earlier studies in which diadzein contributes to *in vivo* antioxidant actions.[38,39]

Results indicated that injection of L-arginine generated cytoplasmic vacuolation inside acinar cells, as well as fat necrosis in the adipose tissue surrounding the pancreas.[37] Pretreatment with diadzein reduced L-arginine-induced inflammatory processes in the pancreas and pancreatic damage. These findings demonstrated that diadzein treatment reduces the degree of inflammation and stimulates the natural regeneration of pancreatic tissue, as evidenced by histological analysis.

CONCLUSION

The current investigation found that diadzein therapy significantly reduced the severity of L-arginine-induced acute pancreatitis. The anti-inflammatory and strong antioxidative properties of diadzein are responsible for the improvement. Histopathological investigations have also confirmed the protective effect. As a result, diadzein can be regarded a possible option for reducing oxidative stress-induced acute pancreatitis, which is a major clinical issue with L-arginine intoxication, as well as its clinical potential for novel therapeutic target supplementing in AP.

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