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Effects of Rutin and Injection of Various Antioxidants on Experimental Acute Pancreatitis

Kayumov Khasan Yusupovich[1*], Kuchkarova Lyubov Salijanovna[1] , Safarova O'g'iloy Sirochovna[2]

[1]Department of Human and Animals Physiology, National University of Uzbekistan, Mirzo Ulugbek, Tashkent, Uzbekistan
[2]Department of Pediatric, Tashkent Pediatric Medical Institute, Tashkent, Uzbekistan

Review Article

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***For Correspondence:**
Kayumov Khasan Yusupovich, Department of Human and Animals Physiology, National University of Uzbekistan, Mirzo Ulugbek, Tashkent, Uzbekistan
E-mail:
qayumovhasan642@gmail.com

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ABSTRACT

In this review article, recent advances in the understanding of the pathogenesis of pancreatitis are discussed and the paradigm shift underway to develop phytoceuticals and antioxidants to treat it is introduced. Despite the promise of studies evaluating the effects of antioxidants/phytoceuticals in pancreatitis, translation to the clinic has thus far been disappointing. However, it is expected that continued research will provide solid evidence to justify the use of antioxidative phytoceuticals in the treatment of pancreatitis. The aim of this study is to investigate the effect of antioxidants, dihydroquercetin, rutin, and various combinations of the two flavonoids on α-amylase and protease activities and the ability of the flavonoids to inhibit some pro-oxidants-induced lipid peroxidation in rat pancreas.

INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disorder of pancreas which is characterized by severe epigastric pain and increased/irregular secretion of pancreatic enzymes, associated with involvement of multiple systems. The pancreatic acinar cells (pacs) in the exocrine pancreas secrete zymogens (inactive digestive enzymes) namely prolipase, trypsinogen and amylase into the pancreatic ducts. Usually, these zymogens are activated in duodenum. When the premature activation of these zymogens occurs in the pacs, it results in the digestion of pancreas itself. This self-digestive condition leads to inflammation, oedema, haemorrhage and necrosis which underlies the

inimical pathology of AP. AP is exacerbated by the release of pro-inflammatory mediators such as tumour necrosis factor-α (TNF-α), interleukin (IL)-6 and IL-1β by the injured pacs further aggravating the forward inflammatory responses as shown. Acute pancreatitis (AP) is an inflammatory disease that can progress to severe acute pancreatitis (SAP), which increases the risk of death. AP is characterized by inappropriate activation of trypsinogen, infiltration of inflammatory cells, and destruction of secretory cells. Other contributing factors may include calcium (Ca^{2+}) overload, mitochondrial dysfunction, impaired autophagy, and endoplasmic reticulum (ER) stress. In addition, exosomes are also associated with pathophysiological processes of many human diseases and may play a biological role in AP. However, the pathogenic mechanism has not been fully elucidated and needs to be further explored to inform treatment. Acute pancreatitis (AP) is considered one of the most common gastrointestinal disorders; the annual worldwide incidence for AP is 4.9-73.4 cases/100,000 people and the total mortality rate is 4-8%, increasing to 33% in patients with infected necrosis. Although gallstones and alcohol consumption are the most common causes of AP, hypertriglyceridemia, drugs, endoscopic retrograde cholangiopancreatography (ERCP), trauma, obesity, diabetes, and infection are also well-known triggers of local and systemic inflammation [1,2]. As a model, it has been shown that large doses of L-arginine induce acute pancreatitis[3]. A single dose of 500 mg/kg L-arginine is known to induce necrotizing pancreatitis in rats and it is found that such dose can selectively induce pancreatic acinar cell damage without any morphological changes in the islets of Langerhans [4,3]. L-arginine-induced AP model is highly reproducible and produces selective, dose-dependent acinar cell necrosis [5].

L-arginine is the precursor for the endogenous synthesis of nitric oxide (NO). NO is a highly reactive radical gas and an important messenger molecule that is involved in functions such as neurotransmission, inflammation, and regulation of gene expression. Additionally NO is a powerful vasodilator and can increase blood flow [6]. The mechanism by which L-arginine causes AP is still unknown but it has been proposed that oxygen/nitric oxide, and inflammatory cytokines may be involved in the development of the disease.

Aim

This study investigated the beneficial effects of rutin and antioxidants on experimental AP induced by L-arginine administration in rate.

MATERIALS AND METHODS

Animals and animal's procedures

Male Wistar rats, weighing 160-210 g, aged 8-12 weeks obtained from the central animal house of Academy of Sciences of Uzbekistan Institute of Bioorganic Chemistry were used in this study. Animals were randomly divided into twelve groups of six in each and experiments performed after 12 hour fasting. Rat submitted to AP induction were treated with dihydroquercetin, rutin, chresariol, cinnarizine, *Ferula foetida* resin (10, 15, or 25 mg kg^{-1}, p.o.) Or vehicle (saline) after 24, 36, 48, and 60 hr of AP induction. Abdominal hyperalgesia, serum enzymes, , pancreatic inflammatory parameters and antioxidant enzyme activities contents were measured 72 h after induction. Animal care and experiments were performed in accordance with the Animal Research: Reporting of *in vivo* Experiments (ARRIVE) guidelines [7].

Drugs administration: Drugs were administered orally using a ball tipped stainless steel gavage attached to a syringe. Dihydroquercetin, rutin, chresariol, cinnarizine, *F. Foetida* resin was dissolved in L-arginine was dissolved in normal saline. Five experimental groups were established: Group I: saline as normal control, Group II: rutin (20 mg/kg), Group III L-arginine (500 mg/kg), Group IV: L-arginine (500 mg/kg) and rutin (20 mg/kg). Group V: dihydroquercetin (15 mg/kg). Group VI: L-arginine (500 mg/kg) and dihydroquercetin (15 mg/kg). Group VII: chresariol (15 mg/kg). Group VIII: L-arginine (500 mg/kg) and chresariol (15 mg/kg). Group X: cinnarizine (25 mg/kg). Group XI: L-arginine (500 mg/kg) and cinnarizine (10 mg/kg). Group X: *F. Foetida* resin (25 mg/kg). Group XII: L-arginine (500 mg/kg) and *F. Foetida* (10 mg/kg). Animals in all groups received their medication on a daily basis except those.

Serum analysis: Animals in all groups were scarified in an ether chamber after 24 h from the last application of the treatments. Blood samples were taken by intracardiac puncture and collected into heparinized tubes. Samples were centrifuged at 3000 rpm for 10 min. The serum amylase and lipase were determined by routine colorimetric methods using commercial kits (Human diagnostic worldwide, Germany) and expressed as U/L.

And we conducted a literature search for published manuscripts on AP up to April 2021 in pubmed, sciencedirect, Web of Science, and Scopus databases and employed the following search terms:"acute pancreatitis","pancreatitis","diagnostic criteria","pathogenetic mechanism","rutin treatment of the disease", "injection of antioxidants on acute pancreatitis", and "rutin". Qualitative and quantitative data were extracted by interpreting each paper in cycles to avoid missing potentially valuable data (Figures 1-5).

RESULTS

Rate submitted to L-arginine injections developed abdominal hyperalgesia and increased serum amylase and triglyceridlipase contents. Rutin and treatment significantly impaired all the parameters that were altered by AP induction. DHQ and rutin Pretreatment Reduced the Mortality of acute pancreatic rate.

The present study aimed to investigate the potential of large dose of L-arginine (as a model) to induce acute pancreatitis in rats and the protective effect of simvastatin. In accordance with the previous reports [8], L-arginine induced acute pancreatitis as evidenced by a dramatic increase in plasma triglyceridlipase levels, and to a lesser degree in amylase levels in L-arginine treated group. It has been shown early that lipase might be more specific biochemical marker than amylase for diagnosis of acute pancreatitis [9]. The increase in lipase levels was reversed by pre-administration of simvastatin indicating that simvastatin might inhibit some signaling pathway involved in the development of AP (Figures 6-8).

Figure 1. α - Amylase (U/L) specific. In this picture Abbreviated names of animals given Acute Pancreatit-AP; digidrokversitin-DGK, rutin Rt, chresariol -Cr, cinnarizine -Cn, *Ferula foetida resin* -Ffr .The effect of L-arginine (500 mg/Kg) and/or antioxidants (mg/Kg) in pancreas α -amylase activity for 5 days. (M ± m n=6).

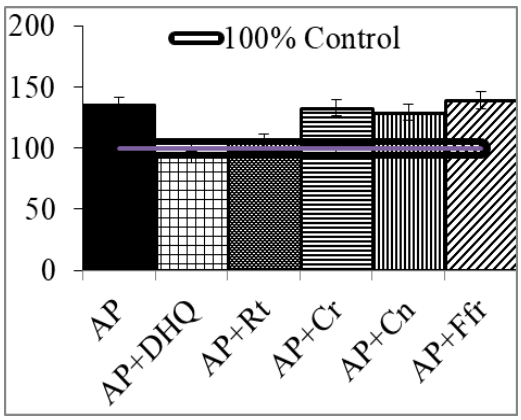


Figure 2. α - Amylase (U/L) Total. In this picture Abbreviated names of animals given Acute Pancreatit-AP; digidrokversitin-DGK, rutin Rt, chresariol -Cr, cinnarizine -Cn, *Ferula foetida resin* -Ffr .The effect of L-arginine (500 mg/Kg) and/or antioxidants (mg/Kg) in pancreas α -amylase activity for 5 days. (M ± m n=6).

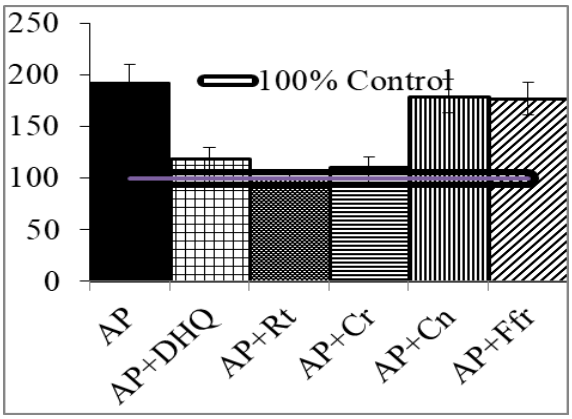


Figure 3. α - Amylase (U/L) in small intestine chymus.

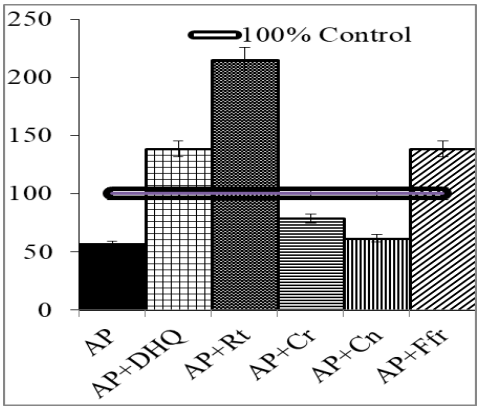


Figure 4. Triglyceridlipase(specific).

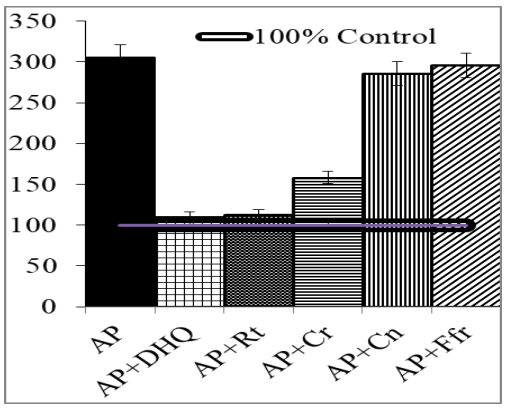


Figure 5. α - Amylase (U/L) on plasma.

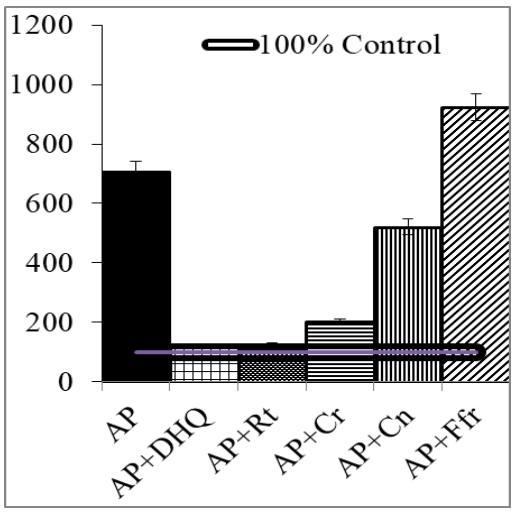


Figure 6. Triglyceridlipase (Total). The effect of L-arginine (500 mg/Kg) and/or antioxidants (mg/Kg) in pancreas. Triglyceridlipase activity for 5 days. (M ± m n=6).

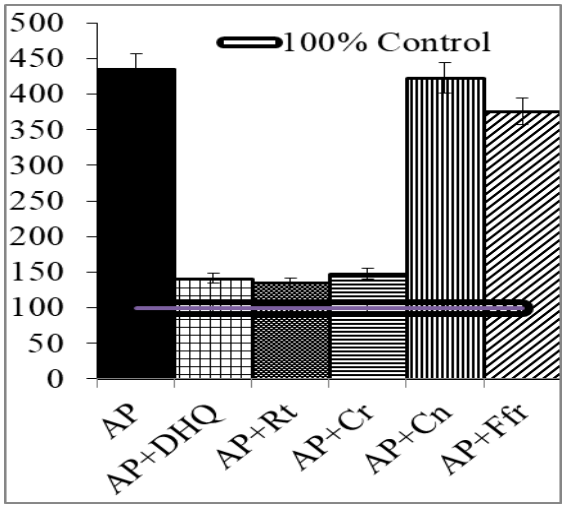


Figure 7. Triglyceridlipase (in small intestine chymus).

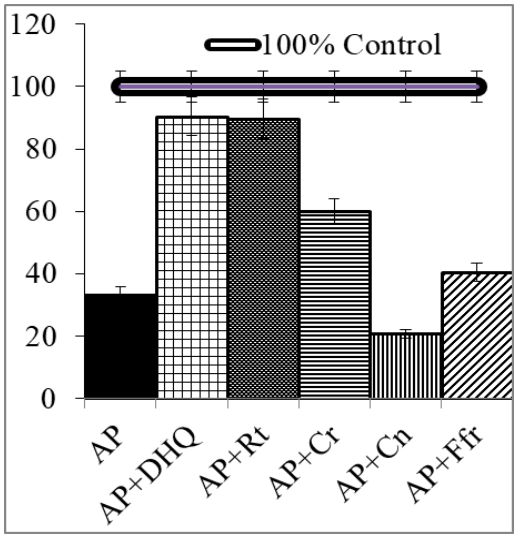
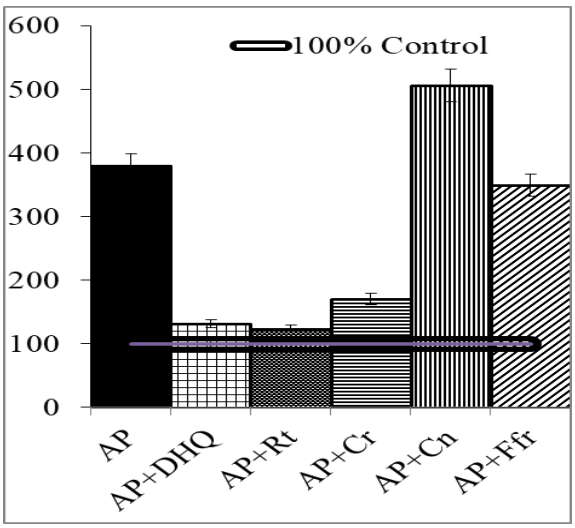


Figure 8. Triglyceridlipase (on plasma).



DISCUSSION

It is well known that antioxidants are potent scavengers of free radicals and serve as inhibitors of oxidant stress related pathologies. A large number of synthetic and natural antioxidants have been demonstrated to induce beneficial effects on human health and disease prevention. However, the structure-activity relationship, bioavailability and therapeutic efficacy of the antioxidants differ extensively. Proanthocyanidins consist of a group of polyhydroxyl-flavan-3-ol (or flavan-3,4-diol) oligomers and polymers linked by carbon-carbon bonds between flavanol subunits. They are the most abundant natural phenolic components [10,11],including phenoldienones, epicatechin, epigallocatechin, epigallocatechin gallate, ferulic acid, caffeic acid, p-coumaric acid, kaempferol, quercetin, and myricetin derived from common dietary foods such as grapes, cranberries and almonds, as well as chocolate and cacao beans [12,13]. These compounds have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress both *in vitro* and *in vivo* [4].Following AP, lipids are one of the main targets for free radicals damage. The later will induce lipid peroxidation by removing one hydrogen atom from polyunsaturated fatty acids and form hydroperoxides. As a result, perturbations in cellular fluidity and membrane integrity lead to disintegration of cells and necrotic cell death. Consequently, subcellular structures released into the extracellular media will induce several inflammatory events and further worsen the ongoing damage [14,15]. The MDA level in the simvastatin-treated pancreatitis group was found to be significantly lower compared to the L-arginine-treated group ($p<0.05$). These results suggest a protective effect against free radical-induced damage through inhibition of lipid peroxidation.

Dihydroquercetin

Being the most powerful of the currently known natural antioxidants, Dihydroquercetin (DHQ, known also as Taxifolin), protect the most important component of the cell–DNA from metabolic products. Dihydroquercetin (DHQ; 3, 5, 7, 3′, 4′-pentahydroxy-flavanone) [16] is an operative flavonoid, abundantly found in olive oil, grapes, in citrus fruits, and onions with molecular weight of 304.25 [17]. It is water-alcohol and polar solvents soluble. There are two forms of DHQ, the trans- and cis-form. The trans-DHQ oxidizes more actively, providing hydrogen atoms to form the oxidation product quercetin [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4- one.DHQ shows (Figures 6-8) a tremendous variety of pharmacological and biochemical consequences, including hepatoprotective, anti-diabetic, cardioprotective, antitumor, and aneuroprotective effects [16]. It attenuates oxidative stress by upregulating Nuclear factor erythroid 2-related factor 2 (Nrf2)-associated antioxidant genes, including Heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase-1 (NQO1), and glutamate-cysteine ligase modifier subunits.We recently reported that administration of DHQ could inhibit oxidative stress in Concanavalin A (cona)-induced liver injury *via* upregulation of HO-1 through the AMPK/Nrf2 signaling pathway in macrophages/Kupffer cell. This finding indicates that Nrf2 and HO-1 were both involved in the mechanism by which DHQ protects against oxidative stress. Dihydroquercetin (DHQ) is a flavonoid compound known for its anti-oxidant effects. Oxidative stress plays a dominant role in regulating the pathways associated with systemic inflammatory immune activation during endotoxemia. Whether and how DHQ regulates inflammatory responses in endotoxemia remains elusive. Here we show DHQ pretreatment effectively reduced the Ten-day mortality in bacterial endotoxin lipopolyssacharide (LPS)-challenged mice, suppressing LPS-induced inflammatory responses reflected by impaired production of tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) in the serum of mice. In Raw 264.7 cells, DHQ pretreatment significantly inhibited the transcriptional upregulation of TNF-α, interferon-γ (IFN-γ), interleukin-10 (IL-10) and toll-

like receptor 4 (TLR-4) after LPS stimulation. Additionally, knockdown of heme oxygenase-1 (HO-1), one of the most important DHQ induced antioxidant genes, cancelled the inhibition of DHQ treatment on LPS induced TNF-α, IFN-γ production. Nuclear factor erythroid 2-related factor 2 (Nrf2) expression and AMP-activated protein kinase (AMPK) phosphorylation were both enhanced by DHQ in Raw 264.7 cells, indicating a DHQ induced AMPK/Nrf2/HO-1 signal axis. In conclusion, DHQ pretreatment could protect mice against the inflammation and mortality associated with endotoxemia [18].

The potent anti-inflammatory effects of DHQ prompted us to further investigate the molecular mechanisms underpinning its activity in cultured macrophages.

However, to the best of our knowledge, the anti-inflammatory effects of DHQ have not been thoroughly investigated.

Rutin

Rutin (3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rhamnoglucoside), is a flavonol, abundantly found in plants, such as passion flower, buckwheat, tea, and apple. It is a vital nutritional component of food stuff [19] and Amalaki fruits (*Emblica officinalis*) contain large amounts of quercetin ramnoglycoside. Rutin was found to be less absorbed than other quercetin glucosides (rutin was ~ 80% less than other available quercetin glucosides) [20,21]. Herein, it is associated with the conversion of rutin to various compounds (e.g., 3, 4-dihydroxyphenylacetic acid, 3, 4-dihydroxytoluene) by the intestinal microflora in the colon. Rutin, also called as rutoside, quercetin-3-rutinoside, and sophorin is a citrus flavonoid glycoside found in buckwheat [22]. The name 'rutin' comes from the plant *Ruta graveolens*, which also contains rutin. Chemically it is a glycoside comprising of flavonolic aglycone quercetin along with disaccharide rutinose. It has demonstrated a number of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities [18, 23, 24, 25, 26, and 27]. In *in-vitro* studies, the 3, 4-dihydroxytoluene rutin metabolite showed anti-inflammatory effects in LPS, disabling NF-k B signaling and stimulating RAW 264.7 macrophages. Therefore, this metabolite can be used as a potential adjuvant against local and systemic inflammation in pancreatitis. In the acute pancreatitis model under the influence of L-arginine using the anti-necrosis apoptosis method in animal studies, rutin reduced pancreatic injury (decreased necrosis, edema, and infiltration and activity of pancreatic enzymes), as well as enhanced apoptosis. (Increase in the number of apoptotic cells in the pancreas). Paradoxically, rutin can antagonize factors involved in other types of cell death, such as pyroptosis. For example, rutin treatment reduces the expression of caspase-1 and pyrine domain (PYD), a spot-like protein (ASC) related to apoptosis. Rutin also plays an important role in oxidative stress processes. In the L-arginine model of acute pancreatitis, in addition to relieving abdominal hyperalgesia (abnormally high sensitivity to pain), rutin reduced oxidative stress (reflected in improved 3- nitrotyrosine levels) and inhibited lipid peroxidation (a decrease in MPO).

Antidiabetic effects

Streptozotocin is a toxic chemical known to deplete levels of insulin by destroying pancreatic islets. Streptozotocin selectively assaults pancreatic β-cells by generating free radicals of oxygen and nitrogen monoxide along with reducing levels of NAD and NADP. Excessive production of glucose and its decreased utilization by tissues serve as the fundamental bases of hyperglycemia [28].In a study, chronic administration of rutin in streptozotocin-induced diabetic rats caused a decrement in plasma glucose, augmentation in insulin levels, and restitution of glycogen content and glycolytic enzymes. Significant rejuvenation of pancreatic islets along with diminished fatty infiltrate was observed in rutin-treated diabetic rats.

CONCLUSION

Based on current literature, it is clear that there is a need for more specific pharmacological therapeutic measures for treatment of AP. Rutin treatment exerted a protective effect on L-arginine-induced AP by mechanisms involving the reduction of oxidative stress, which suggests that this flavonoid has a potential for future approaches designed for the management of AP.

It can be concluded from the above (Figures 2-5), that pancreatitis and diabetes are related diseases. Experimental observations on rats have shown that simultaneous attempts have been made to identify a local herbal remedy that corrects both pancreatitis and diabetes. When studying the effect of corrective drugs from several tested drugs (dihydroquercetin, rutin, chresariol, cinnarizine, *Ferula foetida resin*), a positive effect of dihydroquersitin and rutin on both the secretion and excretion of the pancreas have been determined.This means that in combination with various surgical and therapeutic agents, it is possible to use drugs derived from local plants, including flavonoids, in the treatment of pancreatitis. The advantage of these is that not only drugs are antioxidants, but also they can have a general healing effect on the body, in addition to pancreatic pathologies. This study, in combination with our findings, suggests that uncovering this intersection may illuminate the relationship between anti-inflammatory responses and may thus contribute to development of new therapeutic strategies for inflammatory diseases.

The present study demonstrated that dihydroquercetin and rutin treatment provided a significant protective effect against L-arginine induced acute pancreatitis. Therefore, dihydroquercetin and rutin can be considered a potential candidate to minimize acute pancreatitis induced by oxidative stress which is a major clinical problem with L-arginine intoxication.

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