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Research Article

General Preventive Effect of Flavonoids Isolated from Plants at Experimental Acute Pancreatitis

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Abstract

With the L-arginine model of experimental pancreatitis (EP), hyperproteinemia, hyperlipidemia, hyperglycemia, hypercholesterolemia, a sharp increase in the activity of digestive hydrolases (α-amylase, lipase, protease complex, active phosphatase) occur. The introduction of flavonoids before the onset of the disease reduced the number of organic substrates and the secretion of hydrolytic enzymes. Therefore, the dysfunction of acinar cells in the pancreas is reduced under the influence of acute pancreatitis. Dihydroquercetin (DHQ) and Rutins (Rt) showed a more effective antipancreatic effect than Pulicaron (Pl) and Tamiflazid (Tm). During EP, the activity of α-glucosidases (maltase, sucrase) decreased in the mucous membrane of the small intestine, while the activity of β-galactosidase (lactase) did not change. The introduction of Rt, DHQ, Pl, and Tm before EP induction led to the fact that the activity of maltase and sucrase approached normal levels in the mucous membrane and cavity of the small intestine, i.e., in the chyme. The rate of glucose transfer from the small intestine into the blood from the carbohydrate polymer, dimer, and monomers was different and was expressed at the highest level in disaccharides (maltose and sucrose). In EP, the transition of glucose from solutions of starch, maltose, sucrose, lactose and glucose into the blood decreases depending on the time of incubation in the small intestine. The introduction of flavonoids before the induction of EP increases the transport of glucose into the blood from almost all substrates. Rt and DHQ have a more stimulating effect on the transport of glucose from carbohydrate substrates into the blood, in contrast to Pl and Tm. With EP, occurs edema, infiltration and inflammation, and vacuole degeneration in the pancreatic acini. The constriction of blood vessels observed during the prophylactic effect of Rt, DHQ, and Tm prevented interstitial tissue edema, vacuolization, and general structural tissue disorders. In contrast to Rt, DHQ, and Tm, the prophylactic effect of Pl on the histostructure of the pancreas was not expressed at all.

Keywords: Experimental Acute Pancreatitis; L-Arginine, Dihydroquercetin; Rutin; Pulicaron; Thamiflaside; A- Amylase; Acute Pancreatit; Glucose; Histology; Pancreatic Tissue

Introduction

Acute pancreatitis (AP) is a common disease and can lead to disability. There is significant level of morbidity and mortality associated with AP, and it places a considerable burden on the healthcare system [1]. In accordance with the world statistics, the incidence of acute pancreatitis worldwide ranges from 5 to 80 people per 100,000 population, and the highest rate according to 2019 statistics is in India (618,862.3), China (493,765.4) and the United States (228699.2). In men, the frequency of AP, and chronic pancreatitis (CP) is 10-30% higher than in women. The most common age of the disease in the world is 60-64 years for women and 44-49 years for men [1]. Acute pancreatitis is a common and potentially fatal disease of the gastrointestinal tract [2,3]. This disease causes 275,000 hospitalizations in the United States annually and costs $2.5 billion in healthcare costs [4]. Approximately 20% of patients with disorders of the digestive tract develop moderate to severe acute pancreatitis, of which 20 to 40% are fataL [5-7]. There are also differences between different age groups, and it has been found that the incidence rate is significantly higher among the population older than 70 years. Indeed, aging is one of the important factors that increase susceptibility to acute pancreatitis. The incidence of acute pancreatitis caused by gallstones increases dramatically with age in both men and women [8]. In addition, Floyd., *et al.* (2009) found that increased use of drugs such as azathioprine was also associated with a higher incidence of acute pancreatitis in the elderly [9].

The pervasiveness and growth of pancreatic diseases throughout the world have created difficulties in their clinical management. Pancreatitis is an acute inflammatory process caused by the "self-destruction" of pancreatic tissue under the influence of its own enzymes. Basically, there are 3 main types of pancreatitis: acute pancreatitis (AP), recurrent AP and CP (chronic pancreatitis). Chronic pancreatitis in particular is an irreversible disease that causes the destruction of healthy pancreatic tissue and the development of fibrous scar tissue. A gradual decrease in exocrine and

endocrine functions is accompanied by such clinical manifestations as steatorrhea, abdominal pain, and diabetes. Loss of pancreatic endocrine islet cells occurs later in the disease process as endocrine cells proliferate in the pancreatic parenchyma. Patients may develop type 3 diabetes (pancreatogenic) complicated by reduced glucagon secretion and therefore an increased risk of hypoglycemia [10]. CP is usually caused by untreated AP. AP is a disease characterized by activation of digestive proteases, inflammatory infiltration of macrophages and neutrophils, and pancreatic tissue necrosis, which increases the risk of death with progression to severe acute pancreatitis (SAP) [11]. AP is characterized by inappropriate trypsinogen activation, inflammation, cellular infiltration, and destruction of secretory cells [12]. The most common causes of AP are cholelithiasis and alcohol. Sometimes AP is caused by various viral infections, such as the mumps virus. Acute but mild pancreatitis has also been reported in patients with acute viral hepatitis A, hepatitis B and, more recently, hepatitis E [13]. An animal model of EP has mainly been administered with cerruline (50 mg/kg every 6 h) [14] or high dose L-arginine (500 mg/100 g twice 12 h apart) [15]. Oxidative stress is an important regulator of the mechanism of AP occurrence. Reactive oxygen species (reactive oxygen species, ROS) activate cascade inflammatory reactions, increasing the accumulation of inflammatory cells and tissue damage in AP. The hallmark of the inflammatory response in pancreatitis is the induction of cytokine expression, which includes a range of signalling molecules, including oxidation-sensitive transcription factors such as nuclear factor kappa B (NF-κB) (NF-DB), activating protein-1 (AP-1), transducer signal and is regulated by a signal transducer and transcription activator, a signal protein, and mitogen-activated protein kinases. ROS and pro-inflammatory cytokines are important in AP. These reactions and interacting processes enhance the inflammatory cascade in the inflammatory process. In acute pancreatitis, the concentration of α-2-macroglobulin in the blood plasma decreases [16]. Trypsin is the main enzyme that activates pancreatic zymogens [17]. In the acinar cells of the pancreas, the presence of a physiological concentration of Ca^{2+} maintains the regulation of fluid and enzyme secretion, and an excessive concentration of Ca^{2+} induced by pathological agents cause destructive processes leading to an inflammatory process. Ca^{2+} signaling in periacinar stellate cells may also play a role in the development of AP [18]. In 1989-2012 endoscopic and surgical interventions in patients with pancreatitis were unsuccessful. At present, it has been possible to improve the structure of β-cells by pancreatectomy and auto transplantation of endocrine islets [19]. But widely used pharmacological preparations for the prevention and treatment of pancreatitis have not been created. Currently, there is no specific method for treating the disease, which indicates the need to study its pathogenesis and develop new therapeutic strategies. Due to unpredictable symptoms, the inflammatory process and the relatively hidden anatomical structure in the retro peritoneum, studies of the human pancreas remain challenging. In this regard, for the past 100 years, research on the pathogenesis of this disease has mainly been carried out in animal models, so we decided to test flavonoids isolated from local plants in animal models of AP and chose the following flavonoids [20].

Rutin (Rt) is a flavonoid with the structure 3',4',5,7-tetrahydroxy-flavone-3-rutinoside (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside [21].) and a molecular weight of 610518 g/mol. Synonyms: quercetin-3-O-rutonoside, rutoside, sophorin, vitamin P [22,23]. More than 70 plant species are known to contain Rt. Buckwheat (*Fagopyrum esculentum Moench*) from the Polygonaceae family is considered the main source of natural Rt [24]. Rt is highly soluble in organic solvents DMSO (dimethyl sulfoxide) and dimethylformamide. The solubility of Rt in the solvents is 25-30 mg/ml. Neutralizes 1,1-diphenyl-2-picrylhydrazyl radicals and diammonium salts (3-ethylbenzothiazoline-6-sulfonic acid) [25]. When Rt is used at a concentration of 163.79 μmol, it inhibits iron autoxidation in cell-free assays [26]. The better anticarcinogenic and immune effects of Rt may manifest as better pharmacological properties. It was studied the modulating effect on cytochrome P450 and phase II enzymes in human hepatocellular carcinoma cells and found that Rt inhibited the proliferation of human hepatocellular carcinoma cell lines in a dose-dependent manner [27]. Rt (100 mg/kg) was found to have a significant antioxidant effect in experimental streptozotocin.

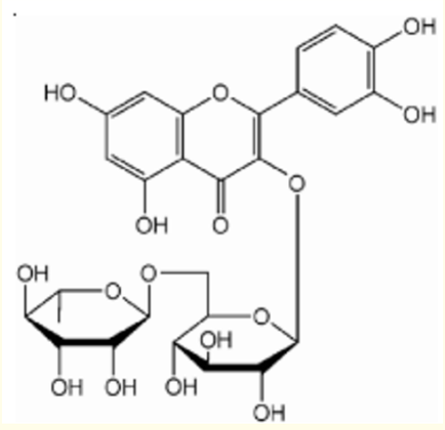

Figure 1: Structural formula of Rutin [20].

Induced diabetes after oral administration for 45 days [28]. The use of colon-coated Rt pellets for the treatment of experimental colitis in rats was evaluated *in vitro* and *in vivo*. They found that Rt at 10mg/kg could correct inflammation in the colon. Flavonoids like Rt and quercetin possess many biochemical effects like inhibition of enzymes, regulatory role on different hormones and pharmacological activities like antimicrobial, antioxidant, anticancer, antihepatotoxic, protection of cardio vascular system [29]. As a result, the colon-to-body weight ratio is reduced and the activity of the myeloperoxidase enzyme is significantly suppressed. Based on these results, the authors concluded that the use of Rt as a therapeutic agent in the treatment of inflammatory bowel disease (IBD) has many advantages and may provide a promising drug without side effects for lifelong therapy of this debilitating illness is shown [30]. Rt reduces the activity of *Staphylococcus aureus*, *Staphylococcus glurance* and *Eschericia coli* [31].

Rt is a naturally occurring flavonoids responsible for its numerous pharmacological actions anti-inflammatory, antioxidant and anti-hemorrhoidal activity with their respective mechanisms. The role of Rt in inhibiting the expression of IL-1β, IL-6, TNF-α, and nitrite formation in a croton oil-induced hemorrhoidal model has already been reported earlier [32,33]. Administration of relevant doses of Rt is remarkably neuroprotective in rats against 6-Hydroxydopamine induced neurotoxicity. Rutin acts as a memory enhancer and an anti-oxidant Rutin treatment protects behavioral changes, and significantly attenuated oxidative damage and improved mitochondrial complexes enzyme activities in different regions (striatum, cortex and hippocampus) of rat brain against 6-OHDA induced neurotoxicity. Intracerebroventricular administration of 6-Hydroxydopamine is known to produce hypoactivity that resembles juvenile onset and advanced Parkinson's disease in rats. The results show that Rt treatment is effective in various behavioural models, thus it could be used as an effective therapeutic agent in the management of Parkinson's disease and related conditions. We attempted to investigate the neuroprotective effect of Rt in animal model of Parkinson's disease, and thus it shows the effect of Rt on 6-hydroxydopamine onduced memory impairment in Parkinson's disease in Rodents [34]. Rt exerts an anti-inflammatory effect by inhibiting the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase in the skin of UV-β irradiated mice [35]. Rt has multifunctional therapeutic properties in cognitive impairment associated with chronic cerebral hypoperfusion, such as dementia and Alzheimer's disease [36].

Dihydroquercetin (DHQ) or taxifolin (3,5,7,3,4-pentahydroxyflavanone) is a flavonoid commonly isolated from onion, milk thistle, French maritime pine bark, and Douglas fir bark. It occurs in plants of various families, but in large quantities (up to 4.5%) isolated only from Siberian larch (*Larix sibirica*) and Dahurian larch (*L. gmelinii*). Therefore, they are the main raw material base for the industrial production of DHQ [37].

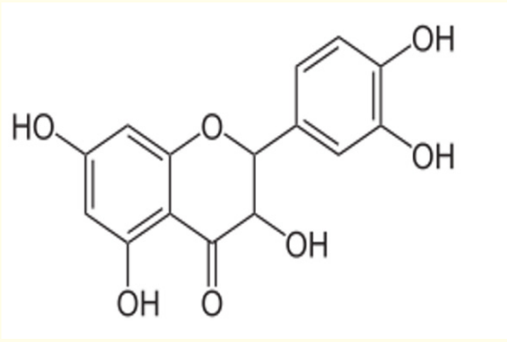

Figure 2: Structural formula of Dihydroquercetin [38].

According to a number of scientific studies, DHQ exhibits antioxidant, capillary-protective, anti-inflammatory, gastric and hepatoprotective, radioprotective, hypolipidemic and diuretic activity [39-43].

DHQ is usually a white crystalline pentahydroxyflavanone, also known as taxifolin [44]. DHQ exhibits various biological activities such as anti-cancer, antioxidant [45], and antiviral activities, and also plays an important role in cardiovascular and liver diseases [46,47]. Therefore, dihydroquercetin can be developed as health foods and pharmaceuticals. The use of DHQ in the complex treatment of a new coronavirus infection COVID-19 is currently being discussed [48].

In general, DHQ is a compound with a short half-life. It is biotransformed rapidly. Experiments carried out on mice have shown that after intravenous administration at doses of 3-15 mg/kg, the half-life can vary from 16 minutes [49] to 2.24 ± 0.42 hours [50]. After oral administration of DHQ nanodispersion (15 mg/kg), the elimination half-life is 4.83 ± 2.54 hours; after introduction into the mixture, its half-life was 6.03 ± 1.42 hours [51].

DHQ is well studied as a biologically harmless drug [52-56], there are no data in the literature on the toxic effect in overdose in humans. According to the national standard of Russia [57], DHQ belongs to the 4th hazard class (substances of low hazard) in terms of the degree of impact on humans. DHQ belongs to the 6th risk class (low risk) according to the degree of risk of causing harm to health [58]. The safety of DHQ is currently more undisputable. This was confirmed in 2017 in a scientific opinion by the European Food Safety Authority based on experimental data [59]. The mean semi-lethal dose (LD50) could not be obtained with intragastric administration of DHQ to mice and rats. Animals did not die even after oral administration of 10-15 g/kg for seven days. In a six-month study of chronic toxicity of DHQ in rats and dogs (including pregnant women) at a dose of 150-1500 mg/kg, no toxic effect was observed. DHQ did not show immunotoxicity, embryotoxicity and mutagenicity. Also, DHQ is not phototoxic and photostable (unlike quercetin), that is, it is resistant to sunlight (including UV radiation) [60], but this issue requires additional research, since DHQ is a polymorphic compound and can exist both in crystalline and in amorphous forms [61]. DHQ can penetrate the cell membrane. However, extracting it from natural raw materials in a bioavailable form and preserving its biological activity is a very difficult task. The very low bioavailability of DHQ (bioavailability) in pharmacy retail chains significantly limits its use in clinical practice [62-65]. After oral administration, the absolute bioavailability of 99% pure DHQ in the mixture was 0.49%; After the preparation of nanodispersed preparations of DHQ, its permeability increased to 0.75% [66]. Data published in 2009 gave an absolute biopermeability of less than 0.17% for 98% pure DHQ in solution [67]. Due to its low water solubility, DHQ can accumulate in the liver. From this point of view, the most important factor affecting the bioavailability of SCC is its water solubility, which is mainly due to its crystalline form and structure [68]. Various experiments are being carried out to increase the biopermeability of DHQ to the cell, in particular, it has been found that it is possible to increase the biopermeability of DHQ by 1.59 times and prolong its absorption compared to a

pure, poorly soluble substance using liposomal solutions containing it [69]. Encapsulation of DHQ in β-cyclodextrin (glucose oligomer) made it possible to increase its intragastric biopermeability, since the solubility of the resulting nanocomplex improved, and DHQ in an aqueous solution was separated from β-cyclodextrin within several hours [70]. The solubility of DHQ in complexes with γ-cyclodextrin can be increased by 18.5-19.8 times, the dissolution rate by 2.8 times, biopermeability by 3.7 times when using an emulsion solvent in combination with lyophilization [71]. Special attention should be paid to the research conducted recently at the First Moscow State Medical University named after I.M. Sechenov [72,73]. Their results showed that crystal engineering aimed at activating the self-assembly of microtubules from cylindrical crystalline nanoparticles is the most promising way to increase the solubility of DHQ. Such single crystals are a pseudopolymorphic modification of a commercially available crystalline substance. The solubility of the original crystalline substance ranges from 0.0001 - 0.001 g/ml. The solubility of microtubules can be 100-1000 times or more [74]. Crystal technology allows you to significantly optimize and change the physico-chemical properties of flavonoids [75].

Pulicaron (Pl) is isolated from a perennial plant *Pulicaria gnaphaloides* (family *Asteracea*), widespread in Central Asia. Grows on dry stony and gravelly slopes, on dry layers of gravel, on peaks. Flowering in July-August, fruiting in August-September. The topsoil is used as raw material. The plant *P. gnaphaloides* has been widely used in traditional folk medicine since ancient times, especially as an antidote for mushroom poisoning. The chemical composition of plants was studied by scientists of the S. Yu. Yunusov Institute of the Chemistry of Plant Substances [76]. The Pl consists of several flavonoids, the hypotensive, antispasmodic, sedative effects of some of them have been studied. Pl contains 5 flavonoids listed below.

If we take 100%, isoquercetin 40%, quercetin 30%, hyperoside

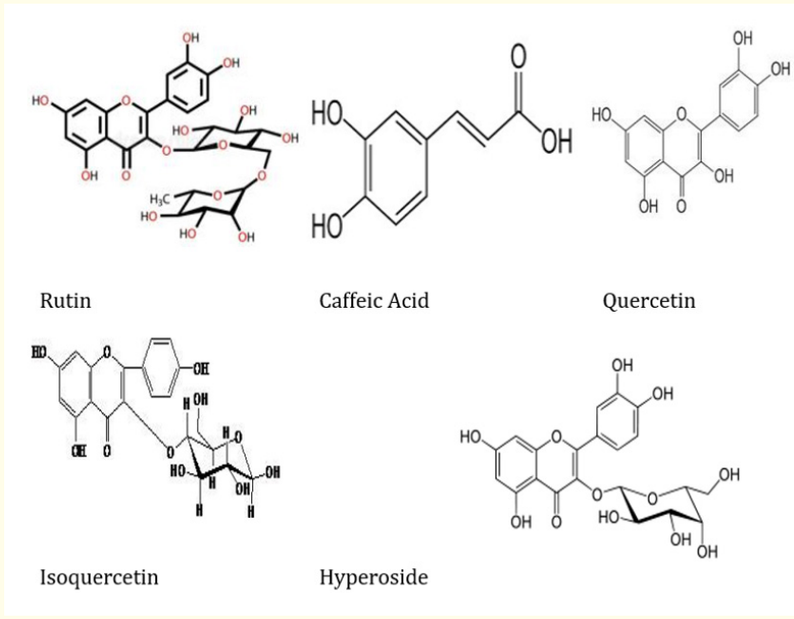

Figure 3: Formula of flavonoids included in Pulicaron [77].

10%, Rt 10%, caffeic acid 8%, quercetin 2%, that is, 4:3:1:1:0.8:0.2 is. These 5 flavonoids are highly potent flavonoids and their antioxidant and anti-inflammatory properties have been well studied [78].

Thamiflaside (Tm) is a glycoside flavonoid derived from the plant *Thalictrum minus*, belonging to the *Ranunculaceae* family. *Thalictrum minus* (Dwarf meadow rue) is a herbaceous flowering plant, often growing in forest edges and meadows. This plant is also grown as an ornamental flower. A new flavonoid diglycoside Tm has been isolated from the aerial part of *Thalictrum minus*. There is little information about the chemical composition and structure of the flavonoids of this plant species; in the course of research, alkaloids [79]. and cycloartan glycosides [80]. were isolated from this plant species for the first time. *T.minus* plants were collected in the Surkhandarya region of Uzbekistan (Kyzylkum, Peter the Great Ridge) at the Institute of Chemistry of Plant Matter of the Academy of Sciences of the Republic of Uzbekistan [81]. The structure of the new compound, which we called thamiflaside, was established as apigenin 7-O-α-L-2‴-methoxyrhamnopyranosyl-(1→6)-β-D-glucopyranoside. Compound was a light-yellow powder of formula $C_{28}H_{32}O_{14}$, m/z 593.300 [82].

Materials and Methods

Materials

Flavonoids isolated from plants were provided by the Laboratory of Coumarins and Terpenoids of the Institute of Chemistry of Plant Substance (Academy of sciences of the Republic of Uzbekistan). And all of them were isolated from plants growing in Uzbeki-

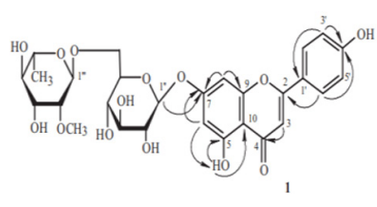

Figure 4: *Thalictrum minus* [83], and the formula of the thamiflaside flavonoid [84], thamiflaside, isolated from it *Thalictrum minus*. The flavonoid thamiflaside was recently purified (in 2020), so its pharmacological properties are almost completely known [85].

stan. Reagents purchased from "Human" (Germany) were used in the experiments. Male rats weighing 180 ± 20 g were used in the experiments. The experimental animals were fed with standard vivarium food, which included wheat, pistachios, milk and dairy products, meat products, wheat bread, herbs, vegetables, table salt, and compound feed. The intake of food and water by the rat was not limited.

The rats were kept at room temperature 22-24°C, humidity 40-60% and natural light conditions, 5 rats in plastic cages 50x30x28 cm in size.

Methods

During the study, all experimental rats were divided into four groups, six rats in each group.

As a "positive" control, rats of the 1st group were used, which, instead of various pharmacological agents, were injected with saline in an equivalent volume and at the appropriate time and methods.

Rats of the 2nd group were used as a "negative" control; they were intraperitoneally injected twice with a solution of L-arginine prepared in physiological saline at a dose of 500 mg/100 g/12 d. The model of experimental pancreatitis induced by L-arginine described above is widely used in scientific research [86-88].

Rats of the 3rd group were intragastrically injected with Rt (purity 99.0%), dissolved in a solution of dimethyl sulfoxide (1% DMSO) (50 mg/kg/24 h) for 2 days before the induction of pancreatitis with L-arginine.

Rats of groups 4, 5, and 6 were intragastrically injected with DHQ, Pl, and Tm dissolved in physiological saline (50 mg/kg/24 s) for 2 days before AP.

All rats were given food and water ad libitum for 2 days to stabilize their digestive parameters before drug administration to the group of rats. 2 or 3 nights after administration of L-arginine, tail blood was taken from the animals and the glucose level was determined to determine the degree of development of hyperglycemia. Experiments were continued on animals when the amount of α-amylase in the blood serum exceeded the control values by 2-3 times.

Determination of the activity of certain organic substances and pancreatic enzymes in blood serum and urine

During decapitation, rat blood was collected into heparin-treated tubes and centrifuged at 1500 rpm.

The concentration of glucose in the obtained serum was determined by the glucose oxidase method using the human reagent kits (Germany). The resulting glucose reacts with phenol and 4-aminophenazones in the presence of hydrogen peroxidase to form a red-violet product whose absorbance can be measured. Due to the concentration of color as the concentration of glucose increases, the amount of glucose is proportional to the optical density of the solution and was determined by the photoelectrocalorimetric method.

The total protein in the blood serum was determined using a set of reagents from the company "Human" (Germany). The reaction is based on the formation of a blue-colored complex of copper ions with proteins soluble in an alkaline medium. The resulting color was proportional to the protein concentration and was measured by the photoelectrocalorimetric method (chemical analyzer Rayto RT1904C, semi-automatic (China)). Urine samples were taken from rats and poured 5 µl into special plates. Urinalysis was performed on a URIT-660 microplate (China).

Preparation of the pancreatic preparation

For the study, a piece of the pancreas was taken from each group of rats, placed in a 10% formalin solution, and the preparation was fixed for a day. After keeping the pieces of the pancreas in the fixative for three days, they were washed with running tap water. After washing the particles, they were gradually concentrated in ethanol solutions of increasing degree from 40°C (50, 70, 80, 90, 96) to 100°C, i.e., in absolute alcohol. The particles were kept in alcoholic solutions of various concentrations for a day, dehydrated, and concentrated.

Paraffin was used in the compacted preparation to solidify the sections. The tissues embedded in paraffin were cut out with a thickness of 5–6 µm (Thermo FS HM 340E microtome, USA) and staining with a solution of hematoxylin-eosin (hematoxylin No. 551062 - BioVitrum LLC, Russia). The preparations were photographed using a LEICA computer light microscope (Germany) (DM750 x 10) and micrographs of individual parts were taken.

Results

Preventive action of some flavonoids in acute pancreatitis

In this section, flavonoids were administered to rats before induction of experimental pancreatitis, and their preventive effect on several parameters (biochemical parameters of blood and urine, volumetric hydrolysis of carbohydrates, membrane hydrolysis of carbohydrates, absorption of glucose from carbohydrates in the small intestine, histological changes in the pancreas). In addition, the influence of flavonoids on the activity of disaccharidases in the mucous membrane of the small intestine *in vitro* was considered.

Preventive effect of flavonoids on the parameters of biological fluids in EP

The effect of flavonoids on the biochemical parameters of blood is presented in table 1. As can be seen from the table, a double injection of L-arginine into the abdominal cavity increases the amount of total blood serum proteins by 2.7 times, and the amount of cholesterol by 7.7 times; increase in glucose levels - 2.9 times and triglycerides - 11.9 times.

Group of animals	Total protein (g/l)	Cholesterol (mmol/l)	Glucose (mmol/l)	Triglycerides (mmol/l)
Cont	61,12 ± 1,8	2,00 ± 0,09	3,27 ± 0,12	0,75 ± 0,04
AP	166,49 ± 6,22	15,40 ± 0,18	9,87 ± 0,26	8,96 ± 0,22
P_1	<0,001	<0,001	<0,001	<0,001
AP+Rt	82,84 ± 1,56	3,58 ± 0,14	3,72 ± 0,09	1,12 ± 0,15
P_1	<0,001	<0,001	>0,11	<0,001
P_2	<0,001	<0,001	<0,001	<0,001
AP+DHQ	76,54 ± 0,53	7,50 ± 0,15	4,94 ± 0,24	3,11 ± 0,17
P_1	<0,001	<0,001	<0,001	<0,001
P_2	<0,001	<0,001	<0,001	<0,001
AP+ Pl	94,12 ± 4,48	11,19 ± 0,35	4,67 ± 0,46	3,72 ± 0,22
P_1	<0,001	<0,001	<0,001	<0,001
P_2	<0,001	<0,001		<0,001
AP+Tm	109,38 ± 7,10	8,64 ± 0,56	4,43 ± 0,64	4,75 ± 0,46
P_1	<0,001	<0,001	<0,01	<0,001
P_2	<0,001	<0,001	<0,001	<0,001

Table 1: Influence of flavonoid administration before acute pancreatitis on some biochemical parameters of blood (M ± m, n = 6).

Note: *Cont and AP-positive control and AP, AP + Rt, AP + DHQ, AP + Pl, AP + Tm values in rats treated with Rt: Rutin; DHQ: Dihydroquercetin; Pl: Pulicaron and Tm: Thamiflaside; respectively, prior to induction of arginine pancreatitis.

Pretreatment with almost all flavonoids reduced the increase in total protein, cholesterol, glucose, and triglycerides, markers of pancreatitis, but these markers did not decrease to control levels.

With the introduction of Rt, DHQ, Pl and Tm before the induction of pancreatitis, the total protein content was 35.54%, respectively; 25.23%; 53.99% and 78.96% were higher than the positive control values.

Also, the introduction of flavonoids before the induction of pancreatitis significantly reduced (by 7.7 times) the increase in cholesterol observed in the disease, but its amount remained high compared to the positive control. The increase in cholesterol when administered to animals Rt, DHQ 5.00%; It was 459.50% and 332.00%.

The preventive effect of flavonoids was also expressed in a decrease in the level of hyperglycemia in pancreatitis, if the increase in glucose levels caused by pancreatitis was 201.83% compared with the positive control. When Rt was administered before the onset of the disease, its amount remained normal. The preventive effect of flavonoids was expressed in the reduction of hyperglycemia associated with pancreatitis. However, the introduction of DHQ, Pl and Tm before the onset of pancreatitis did not increase the amount of glucose to the level of the disease, but the amount of glucose under the influence of DHQ, Pl and Tm was 51.07%, respectively; 42.81% and 35.47% were higher than the positive control values.

This trend was also noted in the amount of triglycerides in the blood. That is, the introduction of flavonoids before the onset of the disease reduced the increase in triglycerides. This restorative effect was especially pronounced for Rt and DHQ.

With the introduction of flavonoids before experimental pancreatitis, the results of the suppression of the activity of digestive enzymes are presented in table 2.

As shown in table 2, digestive hydrolase activity was dramatically increased in experimental pancreatitis. Such an increase was observed at the level of α-amylase activity by 3.7 times, lipase activity by 3.1 times, protease complex activity by 4.7 times and active phosphatase activity by 3.3 times.

Intragastric administration of flavonoids before the onset of the disease caused a decrease in the activity of digestive hydrolases. The activity of almost all hydrolytic enzymes was lower than in the negative control under the influence of flavonoids, and higher than in the positive control. Only α-amylase activity was reduced to full positive control values with DHQ and Tm.

The preventive effect of flavonoids on some biochemical parameters of urine is shown in figure 5. It can be seen that during AP,

Group of	α-Amylase (U/l/sec)	Triglyceride lipase (μmol/l/hour)	Protease Complex mg/ml/min	Alkaline phosphatase (U/l)
Cont	163,09 ± 1,70	28,66 ± 1,0	170,1 ± 6,99	268,83 ± 1,14
AP	607,95 ± 3,53	176,41 ± 13,48	799,62 ± 28,68	881,59 ± 8,38
P_1	<0,001	<0,001	<0,001	<0,001
AP+Rt	126,45 ± 1,33	31,08 ± 2,14	145,8 ± 3,99	352,24 ± 14,75
P_1	<0,001	<0,001	<0,01	<0,001
P_2	<0,001	<0,001	<0,001	<0,00
AP+DHQ	162,23 ± 4,46	10,00 ± 0,22	298,38 ± 5,58	325,53 ± 3,36
P_1	>0,5	<0,001	<0,001	<0,001
P_2	<0,001	<0,001	<0,001	<0,001
AP+ Pl	336,06 ± 9,70	38,40 ± 3,02	503,55 ± 13,35	476,85 ± 9,29
P_1	<0,001	<0,001	<0,001	<0,001
P_2	<0,001	<0,001	<0,001	<0,001
AP+Tm	267,82 ± 8,34	48,28 ± 6,44	211,53 ± 17,15	134,35 ± 6,75
P_1	>0,5	<0,001	<0,05	<0,001
P_2	<0,001	<0,001	<0,001	<0,001

Table 2: Effect of flavonoid administration before acute pancreatitis on the activity of digestive enzymes in the blood (M ± m; n = 6).

Note: *Cont and AP–positive control and Acute pancreatit, AP+Rt, AP+DHQ, AP+ Pl, AP+Tm values in rats treated with Rt: Rutin, DHQ: Dihydroquercetin, Pl: Pulicaron and Tm: Thamiflaside, respectively, prior to induction of arginine pancreatitis.

the amount of total proteins in the urine increased by 8.03 times, the amount of glucose - by 3.4 times, and the activity of α-amylase - by 3.8 times. Biochemical indicators of urine, which are a sign of pancreatitis. The content of total protein of total Rt, DHQ, Pl and Tm in urine was 29.74%, respectively, when administered before the induction of pancreatitis; 43.49%; 14.03% and 32.07% decreased compared to the negative control values. (Figure 3.1 A). In particular, the introduction of flavonoids before the induction of pancreatitis significantly reduced (by 3.4 times) the increase in the amount of glucose in the urine during the disease, but its content remained high compared to the positive control. Before the induction of pancreatitis, when Rt, DHQ, Pl and Tm were administered to animals, the increase in glucose was 39.76% compared to the negative control, respectively; 44.78%; It decreased by 8.61% and 18.84%. (Figure 5B).

With the introduction of Rt, DHQ, Pl and Tm before the induction of pancreatitis, the amount of α-amylase was 6.42%, respectively; 16.04%; 102.14% and 51.87% remained above the positive control values. (Figure 5C). However, their values were significantly lower than those of the negative control.

Thus, intragastric administration of flavonoids before L-arginine pancreatitis in rats leads to a decrease in the activity of organic substrates and digestive hydrolases in blood serum and urine. A decrease in the number of organic substrates and secretion of pancreatic hydrolases under the influence of flavonoids indicates a decrease in the level of decay of acinar cells, which is usually observed with AP in the pancreas. The antipancreatic effect of DHQ and Rt was more effective than that of Pl and Tm.

Preventive effect of flavonoids in AP on the histology of pancreatic tissue

Changes in the pancreas in acute pancreatitis are shown in figure 6.

The overall histological structure of the gland was not disturbed in rats in the positive control group. There are no dystrophic changes in the shape of typical exocrinocytes and parenchymal cells with eosinophilic and secretory granules in the apical part of the acinus. It was not observed a vacuolar dystrophy of exocrinocytes. The acini of the exocrine part of the pancreatic tissue are of the same size. The shape of the pancreatic acinus is predominantly round, the epithelial cells are arranged in a row. The intermediate tissue of the gland is poorly developed and consists of connective tissue.

In animals treated with Experimental Pancreatit(EP), necrobiotic changes were noted in acinar cells and vacuolization of the cytoplasm. In animals with experimental pancreatitis, edema, infiltration and inflammation occurred in the pancreatic tissue. Histological examination 48 hours after the administration of L-arginine revealed a progressive infiltration of polymorphonuclear leukocytes, lymphocytes and macrophages into the interstitial and pancreatic tissues. It can be seen that foci of necrosis began in

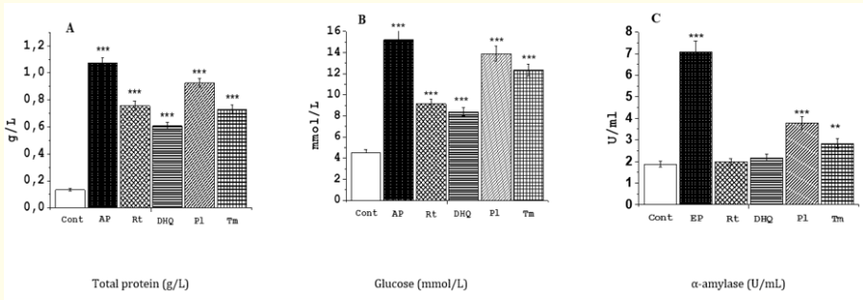

Figure 5: Effect of flavonoid administration before acute pancreatitis on total urine protein (A), urine glucose (B), and urine α-amylase activity (C) (M ± m, n = 6).

Note: *Cont and AP–positive control and Acute pancreatit, AP + Rt, AP + DHQ, AP + Pl, AP + Tm values in rats treated with Rt: Rutin; DHQ: Dihydroquercetin; Pl: Pulicaron and Tm: Thamiflaside, respectively, prior to induction of arginine pancreatitis.

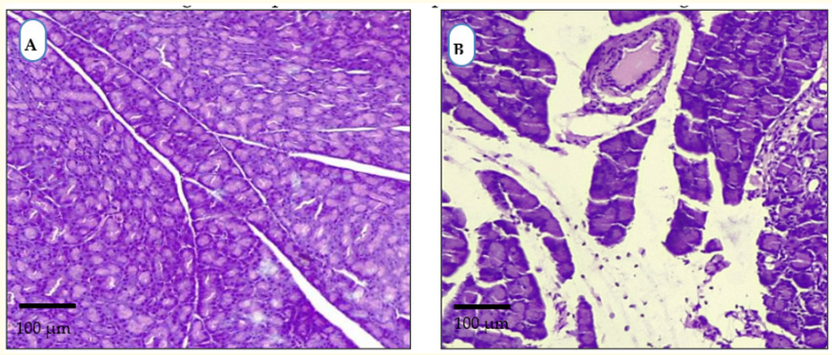

Figure 6: Histological view of the pancreas of rats in AP A)-Control. B) - a fragment of the pancreas of animals with AP. dye - hemotoxylin-eosin; Microscope Leica DM750 x 10.

acinar cells, necrosis destroyed most of the parenchyma, caused a violation of the architecture of the pancreas, and damage to acinar cells. A complete loss of structural homogeneity of exocrine acini was noted in the gland. Epithelial cells in the gland are stained darker than normal, it was found that the size of their cytoplasm is increased, and dystrophy develops in some exocrinocytes. (Figure 6). At this stage of the study, it was found that the acinar cells of the pancreas are gradually destroyed, and the pancreatic ducts are dilated and filled with mucus. Intragastric administration of flavonoids before EP induction significantly protected L-arginine-induced histological gland damage (Figure 7). There was no difference in pathological damage scores between the Rt and DHQ groups (p = 0.174). The pathological damage scores of the Pl and Tm -groups were higher than those of the corresponding Rt, DHQ - groups. Figure 7D shows that there was no obvious pathological damage in the DHQ- groups; the pancreatic lobule was intact, the demarcation was clear, and there was almost no inflammatory cell infiltration, necrosis, or hemorrhage. After 24 h, patchy necrosis was observed in group Pl, in which inflammatory infiltration was still predominant. Vacuolar dystrophy is decreased of exocrinocytes observed at AP. Almost all flavonoids prevented the regeneration of parenchymal cells and the expansion of the pancreatic ducts. This effect of histological prophylaxis of the pancreas was observed in all flavonoids, except for Pl. Under the prophylactic effect of Rt, DHQ, and Tm, changes in the cell structure and a sharp decrease in the number of cells observed at AP were not observed, however, in animals treated with Pl, karyolysis and pycnosis were noted in most cases. nuclei, and part of the nuclear chromatin condensed along the periphery and stained black

Discussion

All flavonoids that used in the experiments - Rt dihydroquercetin, Pl and Tm had a preventive effect on the development of acute pancreatitis. Active phosphatase showed a decrease in enzyme activity, which was manifested by a decrease in all its studied symptoms, however, the preventive effect for dihydroquercetin and tamilflazid was more pronounced than for other flavonoids. Perhaps the similarity of the effects of the studied drugs is due to the analogy of their structure. It is known that in pancreatitis, primarily due to lipid peroxidation, the structure of cell membranes is destroyed. Flavonoids are based on their antioxidant properties, membranotropic action [89,90]. In our opinion, prevention of flavonoids used in experimental acute pancreatitis is associated with the preserva-

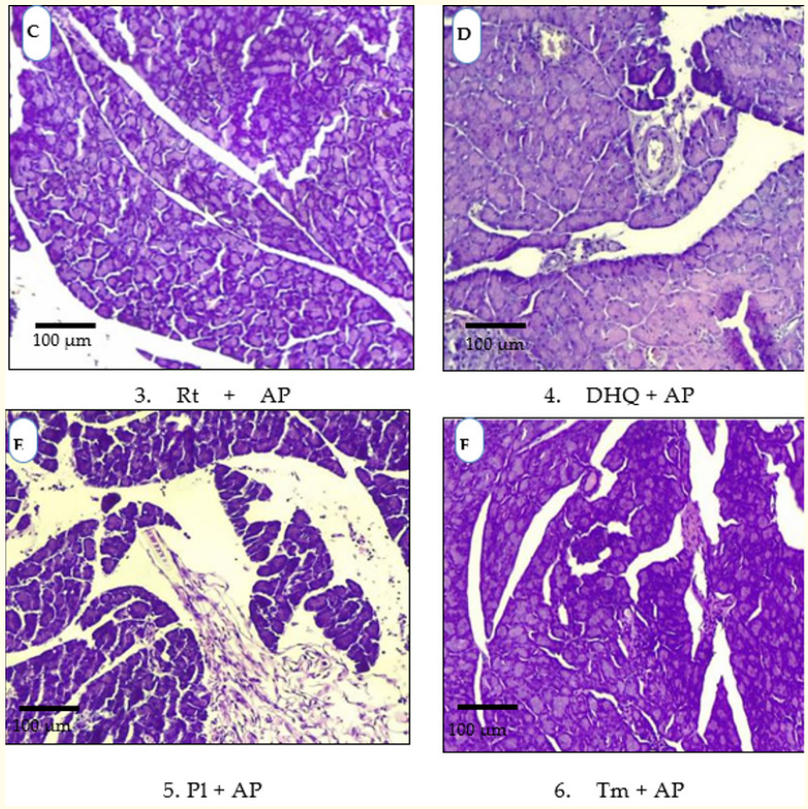

Figure 7: Histological view of the pancreas of rats in AP, dye - hemotoxylin-eosin; Microscope Leica DM750 x 10.
Note: *Cont and AP-positive control and Acute pancreatit, AP) + Rt, AP+DHQ, AP+ Pl, AP + Tm values in rats treated with Rt: Rutin; DHQ: Dihydroquercetin; Pl: Pulicaron and Tm: Thamiflaside, respectively, prior to induction of arginine pancreatitis.

tion of lipids in the membranes of pancreatic cells and the structure of the acinar system, as a rule, by preventing the breakdown of lipids in the pancreatic membranes.

Conclusions

From the obtained results, it can be seen that EP inhibits each stage of carbohydrate absorption (initial hydrolysis, final hydrolysis, absorption), but before the induction of EP, the introduction of some flavonoids into the blood serum and hyperproteinemia, hyperlipidemia, hyperglycemia, hypercholesterolemia and digestive hydrolases (α-amylase, lipase, protease complex and active phosphatase) reduces a sharp increase in activity. In EP, the main element of diagnosis is an increase in serum amylase or lipase [91]. As a "gold standard" in acute pancreatitis, the sensitivity of blood plasma amylase is 81-95% [92]. However, this depends on the definition of "abnormal" and the thresholds chosen. In most guidelines, an amylase concentration of 2 to 4 times the upper limit of normal is acceptable for diagnostic accuracy. before reporting illness [93,94]. The administration of flavonoids reduces the amount of organic substrates and the secretion of hydrolytic enzymes. Consequently, the dysfunction of pancreatic acinar cells is reduced under the influence of EP. The antipancreatic effect of DHQ and Rt was more effective than that of Pl and Tm.

Pancreatic α-amylase (EC 3.2.1.1) is synthesized by pancreatic acinar cells and secreted into the duodenum as the main component of pancreatic fluid [95]. The breakdown of starch to glucose catalyzes the first step of hydrolysis and is therefore a key enzyme in energy production [96]. It is an endotype enzyme that cleaves α-1,4-glycosidic random bonds of starch with maltose or maltooligosaccharides [97]. Under the influence of AP, the activity of α-amylase in the pancreatic tissue increases 3 times compared to the upper limit of the normal indicator, and decreases in the chyme [98]. Under the preventive action of Rt, DHQ, Pl and Tm, the activity of pancreatic α-amylase in the pancreatic tissue and intestinal chyme normalized. The therapeutic effect of AP rutin induced by L-arginine is presented in the literature [99,100]. Flavonoids, in particular, can regulate the processes of incretion and secretion of the pancreas.

In animals with experimental pancreatitis, the edema, infiltration, and inflammation observed in the pancreatic tissue were significantly reduced by the prophylactic action of Rt, DHQ, and Tm, but did not disappear completely. The preventive effect of Rt, DHQ and Tm was also expressed in the narrowing of dilated vessels and a decrease in the swelling of the interstitial tissue. In addition, under the influence of flavonoids, vacuolar degeneration in exocrino-

cytes decreased. Unlike Rt, DHQ, and Tm, no preventive effect of Pl was observed at all.

Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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