Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

RUTIN MODULATES ETHANOL AND HIGH FAT DIET - INDUCED INFLAMMATORY CHANGES IN PANCREAS – A DOSE RESPONSE STUDY IN RATS

RAVIKUMAR ARUNA, ARUMUGAM GEETHA*, PERIYANAYAGAM SUGUNA

Department of Biochemistry, Bharathi Women's College – Affiliated to University of Madras, Chennai 600 108, Tamilnadu, India.
Email: drgeetha08@yahoo.in, geethav21@yahoo.co.in.

Received: 07 May 2012, Revised and Accepted: 19 Jun 2012

ABSTRACT

The effect of rutin at different concentrations on ethanol (EtOH) and high fat diet (HFD) induced changes in pancreas were investigated in rats. Male albino Wistar rats were divided into 7 groups. Group 1 and 2 served as control to receive normal diet containing 5% fat. Group 3-7 rats were administered orally with EtOH, 8g/kg body weight /day and also fed with high fat diet containing 25% fat for 90 days. In addition, group 4-7 received rutin of 25, 50, 100 and 200mg/kg body weight respectively at the last 60days of the experimental period. Group 2 rats received 200mg/kg body weight of rutin similarly for 60days. Serum lipase, amylase, cytokine IL-1β, thiobarbituric acid reacting substances, oxidative stress index, myeloperoxidase activity and antioxidant status in pancreas were assessed. We observed a significant decrease in the activity of serum amylase, lipase, IL-1β and oxidative stress index in animals received rutin in addition to EtOH and HFD. Rutin supplementation along with EtOH and HFD was found to maintain the activity of myeloperoxidase and antioxidants in pancreas. The optimum protective effect was observed in rats received 100mg/kg body weight of rutin and the histopathological observations added more evidence for our results.

Keywords: Antioxidants, Ethanol, High fat diet, Inflammation, Myeloperoxidase, Pancreas and Rutin.

INTRODUCTION

Pancreatitis is inflammation of the pancreas. It occurs when the enzymes that digest food are activated in the pancreas itself instead of in small intestine. Acute pancreatitis is marked by the sudden onset of right upper quadrant or epigastric pain, whereas chronic pancreatitis is characterized by a recurring or persistent abdominal pain with or without steatorrhea or diabetes mellitus [1, 2]. Chronic pancreatitis is a fibro-inflammatory disease of the pancreas characterized by irreversible morphologic changes that typically cause pain and/or loss of function [3, 4]. Pain occurs in 80% to 90% of patients, and is considered the most important factor affecting quality of life [5].

Eighty percent of pancreatitis is caused by alcohol and gallstones. Alcohol consumption is a leading cause of both acute and chronic pancreatitis, followed by gallstones and autoimmune diseases etc [6]. It is generally believed that pancreatitis is caused by the self-digestion of pancreatic acinar cells following the conversion of the inactive trypsinogen to the active trypsin[7].

Experimentally, ethanol (EtOH) consumption along with high fat diet (HFD) is proved to develop chronic pancreatitis in rats[8]. EtOH metabolism also takes place in pancreas where acetaldehyde is produced as a major metabolite which is highly toxic to acinar cells to cause injury [9, 10]. As a response to tissue injury, cytokines are produced to minimize the damage. But the excess cytokines can induce inflammation in normal cells. HFD has been shown to aggravate the alcohol induced inflammatory changes [11].

Many supportive allopathic medicines have been currently used to alleviate the complications associated with inflammation in pancreas[12]. The ever increasing mortality due to pancreatitis demands the identification and evaluation of more and more plant based medicines which have fewer side effects.

Rutin is a flavonol glycoside composed of quercetin and disaccharide rutinose and richly present in the fruits of *Emblica officinalis*, black tea and apple skin peels [13, 14]. Rutin has been shown to have anti inflammatory and vasoactive properties [15, 16]. It has also been reported that rutin is a potent scavenger of hydroxyl and superoxide radicals [17, 18] and also prevents lipid peroxidation in pathological conditions like diabetes and cancer [19, 20].

The present study is an attempt to evaluate whether rutin could modulate the changes induced in the pancreas of rats administered with EtOH and HFD. The dose responsive protective effect of rutin on pancreas has also been studied.

MATERIALS AND METHODS

Chemicals and reagents

Rutin was obtained from Santa Cruz Biotechnology, Inc (US and Canada). ELISA kit for IL-1β was purchased from Abcam. All other chemicals and solvents used for the analyses were of analytical grade.

Experimental protocol

Male albino rats (Wistar) weighing 175-200g were maintained on 12 hours light and 12 hours dark cycle at 22°C. All animals were individually housed and fed *ab libitum* standard rat chow obtained from Hindustan Lever Ltd, Bangalore, India. during the acclimation period. Subsequently rats were randomly assigned into 7 groups and fed *ab libitum* for 90 days either a standard rat chow for group 1 and 2 or HFD along with EtOH (8g/kg body weight/day) for group 3-7. In addition group 4 – 7 rats were administrated with 25, 50, 100, 200mg/kg rutin respectively for the last 60 days of the experimental period. Group 2 rats were administrated with 200mg/kg rutin along with normal standard diet. Table 1 shows the detailed composition of normal and HFD.

Table 1: Composition of diet

	Normal Diet %	High Fat Diet %
Carbohydrate	67.3	13
Protein	19.3	17
Fat	4.3	25
Mineral mix	0.95	1.17
Calcium phosphate	1.23	1.51
Calcium carbonate	0.52	0.64
Potassium citrate	1.56	1.92
Vitamin mix	0.95	1.17
Choline bitartate	0.19	0.23

Rutin preparation

Rutin was dissolved in distilled water and mixed thoroughly. Rutin was administrated orally by intragastric intubation.

The work protocol was submitted and approved by The Animal Care Ethical Committee. After the experimental period of 90 days, rats were fasted overnight and anesthetized by intramuscular injection of ketamine hydrochloride (30mg/kg body weight) and killed by cervical decapitation. Blood was collected with/without anticoagulant and plasma / serum separated were stored until analysis. In order to rule out the day- to- day variation in results, one animal from each group was killed per day.

Tissue homogenate preparation

Immediately after the animal sacrifice, pancreas was removed carefully washed and homogenized in 0.1 M Tris HCl buffer pH-7.4 and centrifuged at low speed to remove any cell debris. The supernatant was used for the determination of glutathione, protein, lipid peroxides and anti-oxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx).

Biochemical Investigations

Determination of serum lipase activity

Lipase activity in serum was measured by the method of Lowry and Tinsley[21]. The lipolysis reaction was initiated with the addition of serum in 25ml olive oil/triton X - 100 emulsions as substrate. 0.3ml subsamples of reaction mixture were taken at predetermined time intervals and used for the assay of liberated free fatty acids spectrophotometrically at 715 nm. The activity of enzyme was expressed as IU/L.

Determination of serum amylase activity

Amylase activity was determined by the method of Gomori [22]. The method was based on the activity of enzyme on substrate starch and the measurement of maltose liberated by using luqol's iodine solution. The colour intensity was measured spectrophotometrically at 640nm and the enzyme activity was expressed as IU/L.

Determination of myeloperoxidase activity

Myeloperoxidase (MPO) activity in the pancreatic tissue was measured according to the method of Bradley *et al.,* [23]. Pre-weighed tissue was homogenized (1:10 w/v) in 0.5% hexadecyltrimethyl ammonium bromide in 50mM potassium phosphate buffer (pH 6.0) before sonication in an ice bath for 20 sec. Three freeze / thaw cycles were performed followed by sonication (20 sec in icebath). The samples were centrifuged at 17000 g (5 min, 4°C) and the enzyme activity was assayed by mixing 0.1 ml of supernatant and 2.9ml of 10mM potassium phosphate buffer (pH 6) containing 0.167g/L o-dianisidine dihydrochoride and 0.0005% hydrogen peroxide. The change in absorbance at 460nm was measured for 4 min using UV visible spectrophotometer. The enzyme activity was expressed as units/mg protein.

Estimation of lipid peroxides and oxidative stress index (OSI)

The level of lipid peroxides in plasma and pancreas was determined by measuring thiobarbituric acid-reacting substances (TBARS) [24]. The value was expressed as nmol/ml plasma and nmol/100 mg tissue protein.

The peroxide content was measured in plasma by using FOX 2 method [25] with minor modifications. The FOX 2 test system is based on oxidation of ferrous ion to ferric ion by various types of peroxides contained within samples, to produce a coloured ferric-xylenol orange complex whose absorbance was measured at 560 nm. Total antioxidant capacity (TAC) was determined by the method of Miller *et al.,* [26]. The decolorization of the assay mixture containing 2, 2'-azino bis 3- ethyl benzo- thiazoline- 6- sulfonate (ABTS) and the sample was monitored by measuring the absorbance at 734 nm and the % inhibition was calculated and plotted as a function of concentration of antioxidants and of trolox for the standard reference data. The ratio of total peroxides to total antioxidant capacity was calculated as OSI.

Estimation of glutathione and antioxidant enzymes

Glutathione (GSH) level was determined by the method of Moron *et al.,* [27]. Aliquots of plasma or homogenate were mixed with equal volume of ice cold 5% TCA and the precipitated proteins were removed by centrifugation. The supernatant was added to equal volume of 0.2 M phosphate buffer, pH 8.0 and measured at 412 nm. Glutathione peroxidase (GPx) was assayed by the method of Flohe and Gunzler [28]. The activity of GPx was expressed as nM of glutathione oxidized / min / mg protein.

Superoxide dismutase (SOD) activity was measured according to method the of Kakker *et al.,,* [29]. The inhibition of reduction of nitroblue tetrazolium to blue coloured formazan in the presence of phenazine methosulfate and NADH was measured at 560 nm using n- butanol as blank. The enzyme activity was expressed as units/mg protein. Decomposition of H_2O_2 in the presence of catalase (CAT) was kinetically measured at 240nm [30]. CAT activity was defined as the amount of enzyme required to decompose 1μM of H_2O_2 / min. The enzyme activity was expressed as μM of H_2O_2 consumed/ min/ mg protein.

Assay procedure for IL 1β

The assay was performed according to manufacturer's instructions (ab100767). Standards or serum samples were pipette into the wells precoated with IL -1β antibody and IL-1β present in a sample is bound to the well by the immobilized antibody. The wells were washed and biotinylated anti-Rat IL-1β antibody was added. The unbound biotinylated antibody was washed and added HRP-conjugated streptavidin to the wells. The wells were washed again and TMB substrate solution was added to the wells. The intensity of the colour developed was proportional to the amount of IL-1β present in the sample. The stop solution changes the colour from blue to yellow, and the intensity of the colour was measured at 450 nm. The activity of IL-1β was expressed as pg/ml.

Estimation of protein

Protein concentration was determined in the tissue homogenate by the method of Bradford [31]. In tissue homogenate the protein level was used to calculate the enzyme activity.

Histopathology

For histopathology examination, the pancreatic tissues were excised and rinsed with ice-cold saline solution (0.9% sodium chloride) to remove blood and debris of adhering tissues. The tissues were then fixed in 10% formalin for 24h. The fixative was removed by washing through running tap water and after dehydration through a graded series of alcohols, the tissue were cleaned in methyl benzoate and embedded in paraffin wax. Sections were cut into 5μM thickness and stained with hematoxylin and eosin. After dehydration and cleaning, the sections were mounted and observed under light microscope for details. Histology score was determined for each slide and the average score has been presented in the result section.

Statistical Analyses

Data were analyzed by using a commercially available statistics software package (SPSS for window V.7.5). Student's t test was performed and results were presented as mean ± S.E.M.

RESULTS

The biochemical analyses revealed the following results:

Effect of rutin on body weight and food consumption

The initial and final body weight, the net weight gain and the average food consumption of control and experimental rats are shown in table 2. The net weight gain was significantly low in EtOH and HFD fed rats (group 3) when compared to control rats (group 1). Rutin administration along with EtOH and HFD showed improvement in body weight gain.

Table 2: Weight gain and food consumption by experimental rats

Groups	Net weight gain (g)[Final- Initial]	Food Consumption by rats (g)
Control	42±4.2	10.33±1.23
Rutin control	39±5.66 NS	10.02±1.5 NS
EtOH+HFD	27±3.38*	7.6±0.874*
EtOH+HFD + Rutin 25mg/kg body wt.	30±3.9NS	8.7±1.175#
EtOH+HFD + Rutin 50mg/kg body wt.	35±3.85@	9.01±1.17a
EtOH+HFD + Rutin 100mg/kg body wt.	38±5.32@	9.6±1.01@
EtOH+HFD + Rutin 200mg/kg body wt.	39±5.27*	9.7±1.41$

Values are expressed as mean ± SD for six animals in each group. Control Vs EtOH+HFD, EtOH+HFD & Rutin Vs EtOH+HFD, Control Vs Rutin. *p<0.001, @p<0.002, $p<0.01, ap<0.025, #p<0.05, NS – Non significant.

The effect was found to be dose dependent up to 100mg/kg body weight and 200mg/kg body weight of rutin received animals did not show further improvement in the weight gain. The food consumption was also found to be decreased in group 3 rats which received EtOH and HFD alone when compared to group 1 control rats. Co-administration of 100mg /kg body weight of rutin was found to maintain the level of average food consumption in rats.

Effect of rutin on serum lipase and amylase

The activities of serum lipase and amylase, the marker enzymes of pancreatic functions are presented in table 3. Serum amylase and lipase activities were increased significantly in EtOH and HFD received animals (group 3) when compared to group 1 rats. The rats (group 4-7) co-administrated with rutin showed decrease in the level of serum amylase and lipase in a dose dependent manner and an optimum decrease was observed in rats received 100mg/kg body weight of rutin.

Effect of rutin on IL-1β and MPO

Table 4 shows the level of serum IL-1 β and MPO in the pancreas of experimental animals. In EtOH and HFD fed rats (group 3) the level of IL-1 β was found to be increased in serum. Rats co-administered with rutin (group 4-7) showed a decrease in the level of IL-1 β and a well pronounced decrease was observed in rats received 100mg/Kg body weight of rutin.

The activity of MPO in pancreas was significantly elevated in group 3 rats and the level was significantly maintained in rats received 100 and 200mg/Kg body weight of rutin.

Table 3: Activity levels of serum amylase and lipase in experimental animals

Groups	Lipase (IU/L)	Amylase (IU/L)
Control	121±12.71	1455±160.05
Rutin control	119.4±16.72NS	1400±168 NS
EtOH+HFD	178.72±22.34*	2300±333.5*
EtOH+HFD + Rutin 25mg/kg body wt.	161.88±17.81#	2100±241.5#
EtOH+HFD + Rutin 50mg/kg body wt.	152.72±20.62$	1875±187.5*
EtOH+HFD + Rutin 100mg/kg body wt.	124.89±18.2*	1550±201.5$
EtOH+HFD + Rutin 200mg/kg body wt.	123.01±12.30*	1480±222*

Values are expressed as mean ± SD for six animals in each group. Control Vs EtOH+HFD, EtOH+HFD + Rutin Vs EtOH+HFD, Control Vs Rutin. *p<0.001, $p<0.01, #p<0.05, NS – Non significant.

Table 4: Activity levels of serum IL – 1β and myeloperoxidase in the pancreas of experimental animals

Groups	IL - 1β (pg/ml)	Myeloperoxidase (Units/mg protein)
Control	12.2 ± 1.281	1.94± 0.19
Rutin control	11.3 ± 1.356NS	1.72 ± 0.26NS
EtOH+HFD	23.4 ± 3.276*	3.50 ± 0.46*
EtOH+HFD + rutin 25mg/kg body wt.	18.4 ± 2.3*	3.07 ± 0.35$
EtOH+HFD + rutin 50mg/kg body wt.	14.6 ± 2.19*	2.50 ± 0.26*
EtOH+HFD + rutin 100mg/kg body wt.	13.3 ± 1.796*	2.05 ± 0.246*
EtOH+HFD + rutin 200mg/kg body wt.	13.2 ± 1.45*	2.02 ± 0.27*

Values are expressed as mean ± SD for six animals in each group. Control Vs EtOH+HFD, EtOH+HFD + Rutin Vs EtOH+HFD, Control Vs Rutin. *p<0.001, $p<0.01, NS – Non significant.

Table 5: Various concentration of Rutin on the levels of TBARS, peroxide content, TAC and oxidative stress index in experimental animals

Groups	TBARS		Peroxide (mmol /l)	TAC (mmol trolex eq./l)	OSI
	Plasma (nmol/ml)	Tissue (nmol/100mg protein)			
Control	0.15±0.021	3.01±.0.32	179.1±20.6	345.6±41.47	0.51±0.064
Rutin control	0.16±0.02NS	2.78±0.31 NS	180±26.1 NS	335±43.55 NS	0.53±0.056 NS
EtOH+HFD	0.42±0.063*	5.07±0.66*	278.03±34.75*	201.04±30.16*	1.14±.0.17*
EtOH+HFD + rutin 25mg/kg body wt.	0.40±0.054 NS	4.48±0.63NS	255.32±28.1 NS	278.03±38.92@	1.05±0.142NS
EtOH+HFD + rutin 50mg/kg body wt.	0.30±0.035!	3.72±0.45*	201.78±27.2*	364.52±41.92*	0.64±0.064*
EtOH+HFD +100mg/kg body wt.	0.25±0.036@	3.02±0.30*	187.62±19.7*	320±32*	0.58±0.087*
EtOH+HFD + rutin 200 mg/kg body wt.	0.24±0.024*	2.92±0.44!	185.71±22.9*	324.02±35.64*	0.55±0.077*

Values are expressed as mean ± SD for six animals in each group. Control Vs EtOH+HFD, EtOH+HFD + Rutin Vs EtOH+HFD, Control Vs Rutin. *p<0.001, @p<0.002, !p<0.005, NS – Non significant.

Effect of rutin on oxidative stress

The levels of TBARS, total peroxide, total antioxidant capacity (TAC) and OSI are given in table 5.

Supplementing rutin to EtOH and HFD fed rats significantly decreased the level of TBARS, peroxide concentration and OSI. TAC was found to be decreased in group 3 rats when compared to the rats co-administrated with rutin at different concentration. TBARS concentration in pancreas was found to be decreased significantly by rutin administration at the concentration of 100 and 200 mg/kg body weight of rutin.

Effect of rutin on antioxidants in pancreas

The activities of SOD, CAT and GPx in the pancreas of control and EtOH-HFD received rats with/without rutin administration are presented in table 6. EtOH and HFD received rats showed significantly low level of enzymatic antioxidants and reduced glutathione in pancreas. Rutin co-administration in these rats was found to maintain the level of antioxidants in a dose dependent manner with the optimum effect at 100mg/kg body weight of rutin.

Effect of rutin on the histology of pancreas

The histopathological scores in the pancreas of control and experimental rats are shown in Table 7. The pancreas of control rats showed normal architecture without steatosis, inflammation and necrosis. Rats received EtOH and HFD showed significant inflammatory and necrotic changes with extensive fibrosis. Rutin co-administration showed significant reduction in steatosis and inflammation in a dose dependent manner. The protective effect of rutin on pancreas was optimum in rats received 100mg/kg body weight of rutin.

Table 6: Activity levels of antioxidant enzymes and glutathione in animals administrated with various concentration of Rutin

Groups	GPx (nM of GSH oxidized /min/ mg protein)	SOD (Units/mg protein)	CAT (µ mol H_2O_2 consumed /min mg protein)	GSH Tissue (mg /g protein)
Control	1.56±0.18	13.68 ± 1.50	109.5 ± 15.88	13.57 ± 1.42
Rutin control	1.49±0.19^{NS}	12.78 ± 1.53^{NS}	104.8 ± 13.62^{NS}	12.55 ± 1.76^{NS}
EtOH+HFD	0.51±0.05*	8.52 ± 1.24*	69 ± 6.9*	7.07 ± 0.06*
EtOH+HFD + rutin 25mg/kg body wt.	0.72± 0.1008*	9.07 ± 1.36^{NS}	80.42 ± 12.06$^{\#}$	8.90 ± 1.07$^{!}$
EtOH+HFD+ rutin 50mg/kg body wt.	0.98± 0.12*	10.78 ± 1.46$^{!}$	90.37 ± 10.39$^{@}$	10.21 ± 1.48$^{@}$
EtOH+HFD+ rutin 100mg/kg body wt.	1.25± 0.125*	11.88 ± 1.49*	103 ± 10.82*	12.41 ± 1.37*
EtOH+HFD + rutin 200mg/kg body wt.	1.27± 0.18*	12.09 ± 1.27*	104 ± 14.04*	12.92 ± 1.68*

Values are expressed as mean ± SD for six animals in each group. Control Vs EtOH+HFD, EtOH+HFD + Rutin Vs EtOH+HFD, Control Vs Rutin. $^{*}p<0.001$, $^{@}p<0.002$, $^{!}p<0.005$, $^{\#}p<0.05$, NS – Non significant.

Table 7: Pathological scores in the pancreas

Groups	Steatosis	Inflammation	Acinar necrosis	Total score
Control	0	0	0	0
Rutin control	0	0	0	0
EtOH+HFD	2.7±0.27	1.1 ± 0.16	1.4 ± 0.15	5.2 ± 0.68
EtOH+HFD + Rutin 25mg/kg body wt.	2.0 ± 0.25*	0.7 ± 0.08*	0.7 ± 0.095*	3.1 ± 0.31*
EtOH+HFD + Rutin 50mg/kg body wt.	1.2 ± 0.17*	0.5 ± 0.05*	0.4 ± 0.05*	2.1 ± 0.24*
EtOH+HFD + Rutin 100mg/kg body wt.	0.3 ± 0.03*	0.2 ± 0.026*	2.0 ± 0.3$^{@}$	0.7 ± 0.098*
EtOH+HFD + Rutin 200mg/kg body wt.	0.2 ± 0.03*	0.2 ± 0.024*	0.15± 0.015*	0.65 ± 0.08*

Values are expressed as mean ± SD for six animals in each group. Control Vs EtOH+HFD, EtOH+HFD + Rutin Vs EtOH+HFD, Control Vs Rutin. $^{*}p<0.001$, $^{@}p<0.002$.

DISCUSSION

Herbal medicines are given prior importance for their safety, efficacy and compatibility with the human body. Polyphenolic pigments such as flavonoids are widely present in plants for human consumption as dietary components or as therapeutic compounds. Rutin, a flavonoid derivative has shown beneficial effects on pancreas when the glandular organ is insulted by EtOH-HFD.

The average food consumption by rats from different experimental groups and their weight gain showed that there is a significant weight reduction in EtOH- HFD fed rats. The food consumption is also found to be low in those animals but rutin supplementation was found to improve the net weight gain and also the food consumption in a dose dependent manner. Ethanol treatment has been reported to reduce the net weight gain both in human subjects as well as in experimental animals [32].

Ethanol is metabolized in liver as well as in pancreas and forms toxic metabolites such as acetaldehyde which can cause injury to the cells of the organs. This might result in reducing the metabolic functions of liver that synthesize anabolic proteins for weight maintenance. Pancreatic injury may result in reduced supply of proteolytic enzymes for digestion in intestine. This might result in reduced absorption of nutrients and thereby reduce energy formation. Reduced food consumption might also be due to the functional disturbance in gastrointestinal tract. Rutin supplementation was found to improve food consumption as well as to maintain net weight gain showing its modulatory effect on EtOH- HFD induced changes in rats.

Medicinal plants such as *E.officinalis* have been consumed for its rich polyflavonoid content. One of the predominant flavonoid present in plant is rutin[33] which is well known for its healing effect on various pathological diseases including liver, stomach and intestinal disorders[34].

The role of rutin as pancreato protective agent is well proved through its effect on reducing the serum lipase and amylase activity in a dose dependent manner. EtOH- HFD group of animals have shown increased level of lipase and amylase due to cell damage in pancreas. Rutin administration is found to reduce the cell damage as well as the release of enzymes into blood circulation. Serum amylase and lipase are generally measured to determine the functional efficiency of pancreas. These enzymes are synthesized by pancreatic cells which when subjected to injury release the enzymes to blood circulation and elevating the enzyme level in serum [35, 36]. Rutin

supplemented rats showed reduced activity of the enzymes in serum showing the protective activity on the pancreas. The optimum effect was found in 100mg/kg body weight of rutin supplemented rats. Many therapeutic agents which protects pancreas from injury has been found to reduce the activity of serum lipase and amylase[37].

Interleukins are inflammatory cytokines which are produced in response to cell injury to alleviate the cell damage but when they are produced in excess they can even damage the normal cell to promote tissue injury. Interleukin 1β is known as pro- inflammatory cytokines expressed at the site of chronic inflammation in a variety of diseases including cancer, infectious diseases and myocarditisis[38, 39].

IL- 1β is the cytokine involved in promoting inflammation and acts as marker for tissue inflammation[40]. We could find significant increase in the serum level of IL- 1β in EtOH – HFD fed rats when compared to control rats. Rutin supplementation is found to reduce the level of interleukin significantly in dose dependent manner and the optimum effect is observed in 100mg/kg body weight fed rats. IL- 1β has been reported to be involved in the pathogenesis of obesity and diabetes [40 -43].

IL- 1β binds to the receptors IL- 1 R1 / IL- 1R1 ACP heterodimer which then initiates the signaling cascade resulting in the translocation of the transcription factor nuclear factor kappa β into the nucleus, where it induces the transcription of pro and anti inflammatory gene including inducible nitric oxide synthatase (iNOS), IL- 6 IL- 1Ra and COX 2 catalyses the conversion of arachidonic acid (AA) to prostaglandin H2 [44–46]. Rutin was found to reduce the serum concentration of IL- 1β showing its potent anti-inflammatory nature.

Rutin was found to minimize the level of TBARS formation and peroxides both in serum and pancreas. Among the doses studied, 100mg/kg body weight of rutin was found to produce optimum free radicals reducing effect. Reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and peroxynitrite are involved in promoting inflammation in cells [47]. The deleterious effect of these radical acts as stimuli for pro inflammatory cytokines production [48]. EtOH- HFD feeding was found to enhance the free radicals formation which might be accounted for the inflammation and the high level of IL-1β in blood. This effect shows that rutin ameliorates inflammation probably by reducing the free radical formation.

Among the promising medicinal plants *E.officinalis* is a wonder plant with rich traditional background. For its antioxidant content, the fruit pulp has been consumed for various ailments in which ROS are involved. Rutin has been reported to inhibit the iron dependent microsomal lipid peroxidation in *invitro*. The various medicinal properties of *E.officinalis* were accounted for the presence of rutin in the fruit pulp [49].

Antioxidants *invivo* can be either enzymatic or non enzymatic, counteracts with the formation of ROS and thus protect the cells from the harmful effect of free radicals. Rutin was found to reduce the level of glutathione and enzymatic GPx, SOD and CAT. These enzymes are effective in quenching and clearing the toxic free radicals [50, 51]. The results of the study consistently show the antioxidant nature of rutin. It was also found to maintain the level of antioxidants *invivo*.

EtOH-HFD induced depletion in the level of glutathione and other enzymatic antioxidants were counteracted by rutin supplementation. Glutathione a prime antioxidant that can scavenge free radicals and prevent the formation of hydrogen peroxide was found to be maintained significantly by rutin supplementation. The decreased concentration of pancreatic GSH was observed in our study in EtOH- HFD fed rats might be due to its elevated consumption or reduced formation. Many pancreatic disorders are associated with depleted GSH level in various organs. Enzymes GPx, CAT and SOD were active in clearing the free radicals and their depletion are also observed in many pathological conditions affecting pancreas [52, 53]. Our results are in accordance with the other reports which showed that rutin scavenge free radicals and also play an important role in maintaining endogenous antioxidant status *invivo.* The histopathological study revealed that in EtOH and HFD fed rats there was marked inflammation, necrosis and steatosis in pancreas. Rutin supplementation along with EtOH and HFD was found to reduce the abnormal changes in tissue architecture.

The present study showed that rutin supplementation at the dose of 100mg/kg body weight to the rats fed with EtOH-HFD markedly reduces the pancreatic damage. The histopathological studies further confirm that rutin provides optimum protection to the pancreas at the dose of 100mg/kg body weight in rats fed with EtOH-HFD.

CONCLUSION

The present study is an experimental proof for the potent pancreato protective nature of rutin. Rutin modulates the abnormal changes induced by simultaneous administration of EtOH- HFD by minimizing amylase and lipase release from pancreas into circulation, myeloperoxidase activity, cytokine production and by maintaining the antioxidant status in the glandular organ. Among the four doses of rutin studied, 100mg/kg body weight was found to be optimum and most effective in modulating the changes during EtOH and HFD induced pancreatic injury. However, further study on the effect of rutin on the activity of other cytokines and caspase -1 and their regulation by inflammasomes is needed to evaluate the mechanism of action of rutin.

REFERENCES

1. Harper SJ, Cheslyn-Curtis S. Acute pancreatitis. Ann Clin Biochem 2011; 48: 23-37.
2. Braganza JM, Lee SH, MCCloy RF, McMahon MJ, Chronic Pancreatitis. Lancet 2011; 377: 1184-1197.
3. Etemad B, White comb DC. Chronic Pancreatitis: diagnosis, classification, and new genetic developments. Gastroenterology 2001; 120: 682-707.
4. S. Pradeep kumar, D. Prathibha, R. Parthibarajan, C. Rubina Reichal. Novel colon specific drug delivery system: A Review. Int J Pharm Pharm Sci, 2011; 4: 22-29.
5. Drewes AM, Krarup AL, Detlefsen S Malmstrom ML, Dimcevski G, Funch-Jensen p. Pain in chronic Pancreatitis the role of neuropathic pain mechanisms. Gut 2008; 57: 1616-1627.
6. Apte MV,Pirola RC, Wilson JS, Mechanisms of alcoholic Pancreatitis. J Gastroenterol Hepatol 2010; 1826-1826.
7. Gaisano HY, Gorelick FS. New insights into the mechanisms of Pancreatitis. Gastroenterology 2009; 136: 2040-2044.
8. H. Tsukamoto, S. J. Towner, G. S. Yu, and S. W. French, Potentiation of ethanol-induced pancreatic injury by dietary fat. Induction of chronic pancreatitis by alcohol in rats. Am J Pathol 1988; 131: 246–257.
9. Majumdar AP, Haiman MJ, Zylbert BA, Billy HT, Vesenka GD, Geokas MC. Acetaldehyde inhibition of protein synthesis in isolated rat pancreatic acini. Toxicol Appl Pharmacol 1986; 83: 86-94.
10. Geokas MC, Lieber CS, French S, Halsted CH. Ethanol, the liver, and the gastrointestinal tract. Ann Intern Med 1981; 95: 198-211.
11. Sarles H: An international survey on nutrition and pancreatitis. Digestion 1973; 9: 389-403.
12. Motoo Y., Su S.B., Xie M.J., Taga H., Sawabu N. Effect of herbal medicine Saiko-keishi-to (TJ-10) on rat spontaneous chronic pancreatitis. Int J Pancreatol 2000; 27: 123-129.
13. Mariana K.A. Araruna, Samara A. Brito, Maria F.B. Morais-Braga, Karla K.A. Santos, Teogenes M. Souza, Tiago R. Leite, Jose G.M. Costa, and Henrique D.M. Coutinho Evaluation of antibiotic & antibiotic modifying activity of pilocarpine & rutin Indian J Med Res 2012; 135: 252–254.
14. Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. Int J Food Sci Nutr 2005; 56: 287-291.
15. Rotelli AE, Guardia T,Juarej AO, Rocha NE, Pelzer LE. Comparative study of flavonoids in experimental models of inflammation. Pharmacol Res 2003; 48: 601-606.
16. Lindahl M,Tagesson C. Flavonoids as phopholipase A_2 inhibitors : importance of their structure for selective inhibition of group II phospholipase A2. Inflammation 1997; 21: 347-356.

17. Oomah BD, Mazza G. Flavonoids and antioxidative in buckwheat. J Agri Food chem., 1996; 1746-1750.
18. Metodiewa D, Kochman A, Karolczak S. evidence for antiradical and antioxidant properties of four biologically active N,N-diethylaminoethyl ethers of flavonone of oximes : a comparison with natural Polyphenolic flavonoid (rutin) action. Biochem Mol Biol Int 1997; 41: 1067-1075.
19. Lee CC, Hsu WH, Shen SR, Cheng YH, Wu SC. Fagopyrum tataricum (Buckwheat) Improved High-Glucose-Induced Insulin Resistance in Mouse Hepatocytes and Diabetes in Fructose-Rich Diet-Induced Mice. Exp Diabetes Res. 2012; Article ID 375673: 10 pages.
20. Pierre F, Taché S, Petit CR, Van der Meer R, Corpet DE. Meat and cancer: haemoglobin and haemin in a low-calcium diet promote colorectal carcinogenesis at the aberrant crypt stage in rats. Carcinogenesis 2003; 24:1683-1690.
21. Lowry R.R., Tinsley I.J. Rapid colorimetric determination of free fatty acids. J. Am. Oil Chem. Soc 1976; 53: 470-472.
22. Gomori G. Asssay of serum amylase with small amounts of serum. Am.J.Clin.Path 1957; 27: 714-716.
23. Bradley P.P., Priebat D.A.,Christensen R.D., Royhstein G. Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982; 78: 206-209.
24. Draper H.H., Hadley M. Malondialdehyde determination as index of lipid peroxide. Methods Enzymol 1990; 186: 421-431.
25. Miyazawa T. Determination of phosphorlipid hydroperoxides in human blood plasma by a chemiluminescence- HPLC assay. Free Radiac Biol Med 1989; 7: 209-217.
26. Miller N.J., Rice Evans C.A., Davis M.J., Gopinathan V., Milner A. A noval method for measuring antioxidant capacity status in premature neonates. Clin Sci 1993; 84: 407-412.
27. Moran M.S., Depierre J.W., Mannervik B. Levels of, glutathione, glutathione reductase and glutathione S- transferase activities in rat lung and liver. Biochem Biophys Acta. 1979; 582: 67-78.
28. Flohe L., Gunzler W., A. Assays of glutathione peroxidase. Methods Enzymol 1984; 105: 114-121.
29. Kakkar P., Das B., Viswanathan P.N., A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys 1984; 21: 130-132.
30. Aebi H. Catalase *in vitro* Methods Enzymol 1984; 105: 121-126.
31. Bradford, M.M., A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal. Biochem 1976; 72: 248–254.
32. Giger U., Stanga Z., DeLegge M.H.Management of chronic pancreatitis. Nutr Clin Pract 2004; 19: 37-49.
33. Krishnaveni M, Mirunalini S. Therapeutic potential of Phyllanthus emblica (amla): the ayurvedic wonder. J Basic Clin Physiol Pharmacol 2010; 21: 93-105.
34. Chatterjee A, Chattopadhyay S, Bandyopadhyay S.K. Biphasic Effect of Phyllanthus emblica L. Extract on NSAID-Induced Ulcer: An Antioxidative Trail Weaved with Immunomodulatory Effect. Evid Based Complement Alternat Med 2011; 2011:146808.
35. Lugea A, Jun Gong, Nguyen J, Nieto J, French S.W, Pandol S.J. Cholinergic Mediation of Alcohol-Induced Experimental Pancreatitis. Alcohol Clin Exp Res 2010; 34: 1768–1781.
36. Kono H.K., Nakagami M., Rusyn I., Connor H.D., Stefanovic H., Brenner D.A., Mason M.P., Arteel G.E., Thurman R.G. Development of an animal model of chronic alcohol-induced pancreatitis in the rat. Am J Physiol Gastrointest Liver Physiol 2001; 280:1178-1186.
37. Campo GM, Avenoso A, Campo S, Nastasi G, Traina P, D'Ascola A, Calatroni A. Chondroitin-4-sulphate reduced oxidative injury in caerulein-induced pancreatitis in mice: the involvement of NF-kappaB translocation and apoptosis activation. Exp Biol Med 2008; 233: 741-752.
38. Gallo O, Gori AM, Attanasio M, Martini F, Giusti B, Boddi M, Gallina E, Fini O, Abbate R. Interleukin-1 beta and interleukin-6 release by peripheral blood monocytes in head and neck cancer.Br J Cancer 1993; 68: 465-468.
39. Galea J, Armstrong J, Gadsdon P, Holden H, Francis SE, Holt CM.Larsen C.M., Faulenba M., Vaag A., Vølund A., Ehses J.A., Interleukin-1 beta in coronary arteries of patients with ischemic heart disease. Arterioscler Thromb Vasc Biol 1996; 68:b1000-1006.
40. Prakash Chandra Gupta. Biological and pharmacological properties of Terminalia chebula retz. (Haritaki) – An overview. Int J Pharm Pharm Sci, 2011; 4: 62-68.
41. Seifert B., Mandrup-Poulsen T.D., Donath M.Y. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 2007; 356: 1517–1526.
42. Osborn O., Brownell S.E., Sanchez-Alavez M., Salomon D., Gram H., Bartfai T.O. Treatment with an Interleukin 1 β antibody improves glycemic control in diet induced obesity. Cytokine 2008; 44: 141-148.
43. Sauter N.S., Schulthess F.T., Galasso R., Lawrence W., Castellani, Maedler K. The anti-inflammatory cytokine IL-1Ra protects from high fat diet-induced hyperglycemia. Endocrinology 2008; 149: 2208-2018.
44. Luheshi GN. Cytokines and fever. Mechanisms and sites of action. Ann N Y Acad Sci 1998; 29: 83–89.
45. Mengshol J.A., Vincenti M.P., Coon C.I, Barchowsky A., Brinckerhoff C.E., Brinckerhoff C.E. Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-Jun N-terminal kinase, and nuclear factor kappaB: differential regulation of collagenase 1 and collagenase 3.Arthritis Rheum 2000; 43: 801–11.
46. Vincenti M.P., Brinckerhoff C.E. Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1β. Arthritis Res 2001; 3: 381–388.
47. K. Hemalatha, Dr.K.Girija. Synthesis, Characterization, preliminary QSAR studies and invitro antioxidant activity of some novel 2,3- disubstituted quinazolinone derivative. Int J Pharm Pharm Sci, 2011; 4: 99-103.
48. de Beaux AC, Ross JA, Maingay JP, Fearon KC, Carter DC. Proinflammatory cytokine release by peripheral blood mononuclear cells from patients with acute pancreatitis. Br J Surg. 1996; 83: 1071-1075.
49. Ihantola-Vormisto A, Summanen J, Kankaanranta H, Vuorela H, Asmawi ZM, Moilanen E. Anti-inflammatory activity of extracts from leaves of Phyllanthus emblica. Planta Med 1997; 63: 518-524.
50. Kusano C., Ferrari B. Total Antioxidant Capacity: a biomarker in biomedical and nutritional studies. J Cell and Mol Bio 2008; **7**: 1-15.
51. Biruk Sintayehu B., Asres K., Raghavendra Y. Radical scavenging activities of the leaf extracts and a flavonoid glycoside isolated from Cineraria abyssinica Sch. Bip. Exa. Rich Journal of Applied Pharmaceutical Science 2012; 02: 44-49.
52. Das D., Mukherjee S., Das A.S., Mukherjee M., Mitra C. Aqueous extract of black tea (Camellia sinensis) prevents EtOH + cholecystokinin-induced pancreatitis in a rat model. Life Sci 2006; 78: 2194–2203.
53. Gül M., Eşrefoğlu M., Öztürk F., Ateş B., Otlu A. The Beneficial Effects of Pentoxifylline on Caerulein-Induced Acute Pancreatitis in Rats. Digest Dis Sci 2009; 54: 555-563.