



GASTRO PROTECTIVE, ANTIAPOPTOTIC AND ANTI-INFLAMMATORY EFFECT OF RUTIN ON ETHANOL-INDUCED GASTRITIS IN RATS

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ABSTRACT

The present study was undertaken to evaluate the potential protective and therapeutic effect of rutin (RTN) administration on ethanol-induced gastritis in rats. Fourty male albino rats were divided into four groups. Group I :(Control group): received no drugs. Group II :(Gastritis non-treated group): administered with a single oral dose of 1 ml/rat of absolute ethanol for gastritis induction. Group III :(Gastritis + RTN protected group): received RTN (200 mg/kg body weight/day) orally for 14 days prior ethanol administration. Group IV :(Gastritis + RTN treated group) received RTN as in group III and the treatment was continued for 7 days later. Blood samples for serum separation and gastric tissue were collected at the 15th. and 22th. days from the onset of RTN administration for determination of serum nitric oxide (NO) and sialic acid (SA), gastric tissue Glutathione peroxidase (GPx), Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GR), reduced glutathione (GSH), L- Malondialdehyde (L-MDA), vitamin C, DNA-fragmentation and myeloperoxidase (MPO). The obtained results showed that, ethanol induced gastritis caused a significant decrease in serum NO, SA, and gastric tissue GSH, vitamin C concentrations and GPX, SOD, GR and CAT activities. However, MPO activity and DNA-fragmentation were significantly increased. Administration of RTN was able to mitigate gastritis induced by ethanol through increasing of NO, SA, GSH, vitamin C concentrations, GPx, SOD, GR, CAT activities in addition to decreasing DNA-fragmentation and MPO in gastric tissue. These results suggest that, RTN may be successful in the therapeutic of gastritis by its radical scavenging and antiapoptotic activity, inhibited neutrophil accumulation and regenerating endogenous antioxidant mechanisms.

Keywords: Rutin; Ethanol; Gastritis; Apoptosis; Myeloperoxidase; Antioxidant enzymes.

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1. INTRODUCTION

Gastritis is an inflammation, irritation, or erosion that occurs when the endogenous defensive mechanisms of mucosal barrier cannot properly protect the organ. Usually, exposure to exceed acid and pepsin causes

insult on the gastrointestinal wall (Khazaei and Salehi, 2006), for more than a century, peptic ulcer disease has been a major cause of morbidity and mortality (Umamaheswari *et al.*, 2007). The activated neutrophils will increase the production of pro-oxidative and pro-inflammatory enzymes and free radicals which lead to oxidative burst (Chatterjee *et al.*, 2007).

Administration of absolute ethanol into the gastric lumen induced gross lesions in the glandular part of the stomach (Natale *et al.*, 2001). Intragastrically administered Ethanol rapidly penetrates the gastrointestinal mucosa, causing membrane damage, exfoliation of cells and gastritis. The increase in mucosal permeability together with the release of vasoactive products from mast cells, macrophages and blood cells may lead to vascular injury, necrosis and ulcer formation. Thus, generation of free radicals by the metabolism of arachidonic acid, platelets, macrophages, and smooth muscle cells has been suggested as one of the mechanisms responsible for gastro duodenal injury (Pihan *et al.*, 1987). The effects of alcohol on the gastric mucosa are dose-dependent, and the damage appears as early as 30 minutes after ingestion and reaches a peak at about 60 minutes (Knoll *et al.*, 1998).

Oxidative stress and depletion of anti-oxidants have been considered a crucial step in alcohol-induced gastritis and so they have been widely investigated in a number of studies (Arafa and Sayed-Ahmed, 2003). Overproduction of reactive oxygen species (ROS) has been concerned as one of the major pathogenic factors that directly results in oxidative damage, including lipid peroxidation, protein oxidation, and DNA damage, which can lead to cell death (Ali and Harty, 2009).

Rutin (a quercetin-rhamnoglucoside) is a glycosylated conjugate of quercetin (quercetin- 3-rutinoside) is one of the most common native flavonoids occurring mainly in glycosidic forms (Kamalakkannan and Prince, 2006). Also, it is a powerful antioxidant (La-Casa *et al.*, 2000) with anti-inflammatory activity (Han, 2009). Moreover, a series of complexes of this ligand display an enhancement of free radical scavenger ability (Kostyuk *et al.*, 2007). Accordingly, the purpose of the present study

was to investigate the effect of RTN against ethanol-induced gastritis in rats. Also, to determine whether RTN when administered to gastritis in rats would attenuate the oxidative stress in gastric tissue, beneficial for the prevention and treatment of gastritis complications and provide therapeutic alternatives for repairing gastric mucosal damage.

2. MATERIALS AND METHODS

2.1. Experimental animals:

Fourty white male albino rats of 12-16 weeks old and weighting 200 - 250 gm were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was supplied *ad-libitum*. The animals were left 14 days for acclimatization before the beginning of the experiment.

2.2. Rutin:

Rutin is pale yellow crystalline powder (purity ~99%) was manufactured by Sigma Chemical Co. (St. Louis, Mo, USA) and purchased from Schnelldorf, Germany through the Egyptian International Center for Import Cairo, Egypt. RTN was dissolved with in propylene glycol solution and administered to rats in daily oral dose of 200 mg/kg body weight for 21 days (Abdel-Raheem, 2010).

2.3. Ethanol-induced gastritis:

Rats were fasted for 18 hours and allowed free access of water prior to the administration of ethanol for gastritis induction. All the rats except those of the control group orally administered with absolute ethanol at a dose of 1ml/rat (Şehirli *et al.*, 2008).

2. 4. Experimental design:

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Rats were randomly divided into four main groups, placed in individual cages and classified as follow:

Group I (normal control group): received no drugs. This group was divided into 2 subgroups:

Subgroup (a):

Included 6 rats sacrificed at the 15th day of the experiment, served as the normal control rats for early gastritis group.

Subgroup (b):

Included 6 rats sacrificed at the 22th day of the experiment, served as the normal control rats for non-treated late gastritis group.

Group II (gastritis non-treated group):

Included 12 rats, administrated once orally with 1ml/rat absolute ethanol for induction of gastritis. This group was divided into 2 subgroups:

Subgroup (a):

Consisted of 6 rats, served as early gastritis non-treated group, for comparison with RTN protected group. This group received absolute ethanol at a dose of (1ml/rat) on empty stomach and the rats were sacrificed one hour later of ethanol administration.

Subgroup (b):

Consisted of 6 rats served as late gastritis non-treated group, for comparison with RTN treated group. This group received absolute ethanol at a dose of (1ml/rat) on empty stomach and the rats were left free and sacrificed 7 days later of ethanol administration.

Group III (RTN protected group):

Comprised 8 male rats received RTN (200 mg/kg body weight/day) orally for 14 days prior absolute ethanol administration. One hour after the administration of ethanol the animals were sacrificed.

Group IV (RTN treated group):

Included 8 male rats received RTN orally(200 mg/kg body weight) for 14 days before ethanol administration and the treatment were continued with RTN for 7 days later.

2. 5. *Sampling:*

Blood samples and tissue specimens (gastric tissues) were collected one hour after administration of ethanol in normal control group (subgroup a), gastritis non-treated group (subgroup a) and RTN protected gastritis group at the 15th day from the onset of treatment with RTN. Also, blood samples and tissue specimens (gastric tissues) were collected after 21days from the onset of treatment with RTN in normal control group (subgroup b), gastritis non-treated group subgroup (b) and RTN treated gastritis group.

2. 5. 1. *Blood samples:*

Blood samples for serum separation were collected by ocular vein puncture at the end of each experimental period in dry, clean, and screw capped tubes and serum were separated by centrifugation at 2500 r.p.m for 15 minutes .The clean, clear serum was separated by automatic pipette and received in dry sterile samples tube and kept in a deep freeze at -20oC until used for subsequent biochemical analysis. All sera were analyzed for NO and SA.

2. 5. 2. *Tissue samples (gastric tissue):*

After 14 and 21 days of RTN administration the rats were sacrificed by cervical decapitation. The stomach was quickly removed, and opened along the greater curvature using a scrapper, cleaned by rinsing with cold saline and stored at -20°C for subsequent biochemical analyses.

Stomach tissue preparation:

Briefly, gastric tissues were cut, weighted and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold

0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant were used for the determination of the following parameters: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), glutathione reductase (GR), L-Malondialdehyde (L-MDA), reduced glutathione (GSH), vitamin C, myeloperoxidase (MPO) and DNA fragmentation.

2. 6. Biochemical analysis:

Serum NO, SA, and, gastric tissue vitamin C, L-MDA, GPx, SOD, CAT, GR, GSH, DNA fragmentation, and MPO were determined according to the methods described by Vodovotz, (1996); Human Sialic acid (SA) Elisa kit (Cat. no. CSB- E09605h); Mesbah et al., (2004); Rat Vitamin C, VC ELISA kit (Cat .No.E0913r); Gross et al., (1967); Kakkar et al., (1984); Luck, (1974); David and Richard, (1983); Moron et al., (1979); Shi et al., (1996) and Rat Myeloperoxidase ELISA kit (Kamiya Biomedical Company, Cat .No. KT- 60345), respectively.

2.7. Statistical analysis:

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science (SPSS, 13.0 software, 2009). Values of $P < 0.05$ were considered to be significant.

3. RESULTS

3.1. Effect of rutin pretreatment on some serum and gastric tissue parameters of ethanol-induced gastritis in rats:

The obtained data demonstrated in table (1) revealed that, a significant decrease in

serum NO and SA levels and gastric tissue GSH, vitamin C concentrations , GPx, SOD, GR and CAT activities with a significant increase in DNA fragmentation, L-MDA concentration and MPO activity were observed in ethanol-induced gastritis group in protective period when compared with normal control group. Pretreatment with RTN in gastritis –induced rats resulted in a non-significant increase in NO level, SOD and GR activities and a significant increase in SA, vitamin C and GSH levels, GPx and CAT activities with significant decrease in DNA fragmentation and L-MDA concentration in addition to a non significant decrease in MPO activity were observed in protective period when compared with control gastritis non-treated group.

3.2. Effect of rutin treatment on some serum and gastric tissue parameters of ethanol-induced gastritis in rats:

The obtained data presented in table (2) revealed that, a significant decrease in serum NO, SA and gastric tissue GSH and vitamin C concentrations and in GPx, SOD, GR and CAT activities with a significant increase in DNA fragmentation, L-MDA concentration and MPO activity were observed in gastritis -induced rats in treatment period when compared with normal control group. Administration of RTN to ethanol–induced gastritis in rats resulted in a significant increase in NO and SA levels, GPx activity with a non-significant increase in gastric tissue GSH and vitamin C levels, SOD, GR and CAT activities and a significant decrease in DNA fragmentation and MDA in addition to a non-significant decrease in MPO activity were observed in treatment period when compared with control gastritis non- treated group.

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Table 1: Effect of pretreatment with rutin on some serum and gastric tissue parameters of ethanol-induced gastritis in rats.

Parameters	Experimental groups at protective period		
	Control Normal group	Gastritis induced group	Gastritis + RTN protected group
NO (mmol/L)	148.94 ± 2.60 ^a	76.06 ± 2.19 ^c	84.27 ± 3.21 ^c
SA (mg/ ml)	55.87 ± 1.63 ^a	12.64 ± 0.93 ^d	28.28 ± 2.02 ^c
GPx (ng/ g.tissue)	51.84 ± 0.91 ^a	22.32 ± 0.68 ^e	30.63 ± 1.07 ^d
SOD (u/g.tissue)	21.18 ± 0.40 ^a	12.80 ± 1.00 ^d	14.92 ± 1.03 ^{cd}
CAT(mmol/g.tissue)	66.15 ± 1.00 ^a	30.00 ± 0.92 ^d	46.67 ± 1.05 ^c
GR (ng /g tissue)	3.66 ± 0.13 ^a	1.83 ± 0.07 ^c	2.19 ± 0.13 ^{bc}
L-MDA (mmol/g.tissue)	57.09 ± 0.55 ^d	111.61 ± 0.72 ^a	74.54 ± 7.88 ^{bc}
GSH (ng/g.tissue)	9.59 ± 0.79 ^a	1.95 ± 0.54 ^d	6.58 ± 0.75 ^b
Vitamin C (ng / g tissue)	40.38 ± 1.00 ^a	16.45 ± 0.73 ^d	23.74 ± 1.50 ^c
DNA-fragmentation (cells/well. tissue)	96.25 ± 2.63 ^c	1390.00 ± 4.22 ^a	1017.31 ± 42.65 ^b
MPO (ng/g.tissue)	5.78 ± 0.14 ^c	14.32 ± 0.68 ^a	12.74 ± 0.52 ^a

Data are presented as (Mean ± S.E). S.E = Standard error.

Mean values with different superscript letters in the same row are significantly different at ($P < 0.05$).

Table 2: Effect of rutin treatment on some serum and gastric tissue parameters of ethanol-induced gastritis in rats.

Parameters	Experimental groups at treatment period		
	Control Normal group	Gastritis induced group	Gastritis + RTN treated group
NO (mmol/L)	163.38 ± 2.01 ^a	55.63 ± 2.12 ^c	102.05 ± 13.99 ^b
SA (mg/ml)	51.14 ± 1.20 ^a	20.80 ± 0.66 ^d	27.69 ± 2.16 ^c
GPx (ng/ g.tissue)	40.88 ± 0.97 ^a	26.27 ± 0.84 ^d	29.88 ± 0.90 ^c
SOD (U/g.tissue)	24.49 ± 0.84 ^a	12.06 ± 0.28 ^d	13.28 ± 0.95 ^d
CAT(mmol/g.tissue)	63.46 ± 1.55 ^a	35.36 ± 1.01 ^d	43.85 ± 1.62 ^{cd}
GR (ng /g tissue)	3.83 ± 0.16 ^a	1.40 ± 0.11 ^b	2.11 ± 0.30 ^b
L-MDA (mmol/g.tissue)	71.26 ± 1.07 ^c	128.39 ± 1.30 ^a	94.95 ± 6.01 ^b
GSH (ng/g.tissue)	10.98 ± 1.02 ^a	3.78 ± 0.39 ^c	5.46 ± 0.66 ^c
Vitamin C (ng /g. tissue)	38.82 ± 0.47 ^a	20.00 ± 0.66 ^c	23.66 ± 1.80 ^c
DNA-fragmentation (cells/well tissue)	17.50 ± 1.36 ^d	1015.00 ± 2.13 ^a	835.38 ± 58.16 ^b
MPO (ng/g.tissue)	3.12 ± 0.14 ^d	12.76 ± 0.77 ^a	11.41 ± 0.58 ^a

Data are presented as (Mean ± S.E). S.E = Standard error.

Mean values with different superscript letters in the same row are significantly different at ($P < 0.05$).

4. DISCUSSION

The ethanol model is widely used to assess the protective and healing activity of many drugs in ulcer studies (Jong-il *et al.*, 2010). Due to its ability to reduce endogenous NO level and blood flow in gastric mucosa, which leads to a serious hemorrhagic necrosis and consequently depletes gastric mucus constituents (Kryger *et al.*, 2000), resulting in an increased flow of Na⁺ and K⁺, elevated pepsin secretion, loss of H⁺ ions and histamine into the lumen (Sandor, 1987).

A significant decrease in serum NO and SA concentrations were observed in ethanol-induced gastritis group in both protective and treatment period. Lower nitrites level in alcoholics than in control group might result from endothelium dysfunction, or decreased NOS reaction on stimuli (Enomoto *et al.*, 2000), or NO consumption in free radicals reactions with peroxynitrites (ONOO-) overproduction (Banan *et al.*, 2000), Potentially formed during NO reaction with free radicals overproduced during ethanol metabolism (Zima *et al.*, 2001). The levels of sialic acid were found to be reduced in ethanol treated gastritis. The decrease in the glycoprotein moieties in the gastric mucosa may be attributed to the decreased activity of defense mechanisms as a result of damage to the gastric mucosa (Amudhan and Begum, 2008).

A non-significant increase in serum nitric oxide concentration was observed in RTN protected gastritis group. Meanwhile, a significant increase in serum nitric oxide concentration was observed in RTN treated late gastritis groups compared with the gastritis non treated groups. These results are nearly similar to those reported by (Olszanecki *et al.*, 2002) who reported that, treatment with RTN increased the level of NO concentration in gastric mucosal tissues by enhancing the activity of cNOS and inhibiting the activity of iNOS. Nitric oxide has an

important role in maintaining gastric mucosal integrity. Inhibition of gastric NO formation decreases gastric blood flow, deprives the tissue of oxygen, and increases mucosal vulnerability to intragastric administration of irritants that mildly damage the gastric mucosa. The cumulative data thus show that endogenous NO is an essential protective factor in the pathogenesis of gastric injury induced by agents such as ethanol and iodoacetamide (Karmeli *et al.*, 1996). On another hand, continuously production of NO and superoxide anion is likely during inflammation and pathological conditions. They react together to form peroxynitrite. The scavenging effect on superoxide anion by NO may be a mechanism by which tissues of host are protected from the deleterious effects of superoxide and superoxide derived reactive oxygen species (Beckman *et al.*, 1990).

Sialic acid is the generic term given to a family of acetylated derivatives of neuraminic acid which occur mainly at terminal positions of glycoprotein and glycolipid oligosaccharide side-chains. In the present study RTN may be help in protection of gastric mucosa from ethanol-induced gastritis due to its positive effect on increasing serum sialic acid concentration in protective and treatment period. Mucus secretion is a crucial factor in the protection of gastric mucosa from the gastric lesions and has been regarded as an important defensive factor in the gastric mucus barrier. A decrease in the synthesis of sulphated mucus glycoprotein has been implicated in the etiology of gastritis (Younan *et al.*, 1982). Further, a recent report indicated that the glycosidic linkage of sialic acid is a potential target for superoxide and other related ROS (Eguchi *et al.*, 2005). Mucin acts as a sacrificial scavenger for ·OH and its protective function is exerted by the direct reaction with its sialic acids (Ogasawara *et al.*, 2007).

Effect of Rutin on ethanol-induced gastritis in rats

A significant decrease in gastric tissue GSH, vitamin C concentrations, GPx, SOD, GR and CAT activities were observed in ethanol-induced gastritis rats group in protective and treatment periods when compared with normal control group. Similarly, Hussein et al, (2014) reported that, a significant decrease in gastric tissue GPX, SOD and CAT activities and vitamin C level were observed in protective and treatment period in ethanol-induced ulcerated rats group. Vitamin C is a water-soluble antioxidant present in the circulation and tissues (Kucharz, 1992). It scavenges and destroys the free radicals in combination with glutathione. The observed decreased in these antioxidants in gastritis rats may be due to increased utilization in scavenging the free radicals (Prakash *et al.*, 2008). The release of oxygen-derived free radicals (ROS) has drawn attention as a possible pathogenic factor of gastric mucosal injury associated with ethanol consumption (Smith *et al.*, 1996). To scavenge ROS, gastric cell have several enzymatic and non-enzymatic antioxidants including CAT, SOD, GPx, and endogenous GSH, but excessive generation of ROS enhance lipid peroxidation and depletes these antioxidants enzymes. Superoxide produced by peroxidase in the stomach tissues might damage cell membranes and cause gastritis by increasing MDA level (Cadirci *et al.*, 2007). SOD is considered as the first line of defense against the deleterious effects of oxygen radicals in the cells and it scavenges ROS by catalyzing the dismutation of superoxide to H₂O₂ (Okado-Matsumoto and Fridovich, 2001). There is evidence to indicate that ethanol significantly depresses SOD activities (Rukkumani *et al.*, 2004).

Ethanol inhibited SOD and thus superoxide radicals could not convert to H₂O₂. The inhibition of SOD activity may result in an increased flux of superoxide in cellular compartments which may be the

reason for the increased lipid peroxidative indices. In this context, the present results is nearly similar with that reported by Megala and Geetha, (2010). Moreover, ethanol decreased the gene expression and the activity of SOD in the gastric mucosa, suggesting that the suppression of key mucosal antioxidant enzyme, along with the elevation of lipid peroxidation, play an important role in the pathogenesis of these lesions (Brzozowski *et al.*, 1998). In the present study, SOD activity decreased significantly in the ethanol treated rats group, which might be due to an excessive formation of superoxide anions. These excessive superoxide anions might inactivate SOD and decrease its activity. In the absence of adequate SOD activity, superoxide anions are not dismuted into H₂O₂, which is the substrate for the H₂O₂ scavenging enzymes CAT and GPx. These result in inactivation of the H₂O₂ scavenging enzymes CAT and GPx, leading to a decrease in their activities (Panda *et al.*, 2012). The decrease in GPx activity may attenuate the radical scavenging function (Oh *et al.*, 1998), GR accelerating the conversion of GSSG to GSH and enhancing the detoxification of reactive metabolites by conjugation with GSH (Panda *et al.*, 2012). Moreover, glutathione peroxidase plays a primary role in minimizing oxidative damage. (GPx), an enzyme with selenium and Glutathione-s-transferase (GST) works together with glutathione in the decomposition of H₂O₂ or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione (Bruce et al., 1982). Reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme (Hodgson and Fridovich, 1975). Also, decline in the GPX activity may be due to over production of free radical induced cells damage. It is now known that, when there is an imbalance between free radical production and antioxidant defenses, 'oxidative stress' occurs resulting in deregulation of cellular functions

(Bandyopadhyay et al., 1999). On the other hand, the decrease in GR activity may be due to the over production of free radical and hydrogen peroxide. GSH is required to maintain the normal reduced state and to counteract the deleterious effects of oxidative stress.

Pretreatment with RTN in gastritis-induced rats resulted in a non-significant increase in SOD and GR activities and significant increase in vitamin C and GSH levels, GPx and CAT activities in protective period. However, administration of RTN to ethanol-induced gastritis in rats exhibited significant increase in gastric tissue GPx activity with a non-significant increase in GSH and vitamin C levels, SOD, GR and CAT activities in treatment period when compared with control gastritis non-treated group. Antioxidant effects of RTN is based on that it increased the levels of non-enzymic antioxidants, decreased oxidative stress resulted in the restoration of glutathione levels in the plasma. Glutathione through its significant reducing power contributes to the recycling of other antioxidants such as vitamin C and vitamin E, that have become oxidized (Kidd, 1997). Vitamin C is an excellent hydrophilic antioxidant in plasma, because it disappears faster than other antioxidant when plasma is exposed to reactive oxygen species (Fri et al., 1989). GSH is required to maintain the normal reduced state and to counteract the deleterious effects of oxidative stress. During the reduction of hydrogen peroxide, GSH is oxidized to GSSG. When GSSG levels are enhanced, the GSH-reductase activity was activated to convert GSSG in GSH (Cui et al., 2011). The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues (Kojo, 2004). Meanwhile, after administration of RTN the concentration of GSH increased and superoxide radicals decreased. Therefore, GR activity increased. In the present study,

administration of RTN associated with increased SOD activity in gastric tissue. The enzymatic antioxidant defense systems are the natural protectors against lipid peroxidation. They include superoxide dismutase and glutathione peroxidase (Koppenol, 1981). Superoxide dismutase is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O₂ and to the less reactive species H₂O₂. Peroxide can be destroyed by CAT or GPX reactions (Teixeira et al., 1998). First, SOD converts the superoxide anion to hydrogen peroxide in a cellular antioxidant reaction. Thereafter, GSH-Px detoxify hydrogen peroxide produced (Jaeschke, 1995). It has been suggested that RTN can reduce oxidized GSH and increase the GSH status, which in turn exhibits increased free radical scavenging property, so RTN indirectly influences the activity of SOD thereby preventing the deleterious effect of superoxide radical formed, That causes activation of SOD, GPx and CAT (Akondi et al., 2011). Glutathione and glutathione-related enzymes play a key role in protecting the cells against the damaging effects of reactive oxygen species. Intracellular GSH can act as a reductant, reducing hydrogen peroxide and lipid hydroperoxides directly to H₂O, a reaction catalyzed by GSH-Px. Depletion of intracellular GSH, under conditions of continuous intracellular oxidative stress, leads to oxidation and damage of lipids, proteins and DNA by the reactive oxygen species (Nordberg and Arner, 2001). Glutathione has a very important role in protecting against oxygen-free radical damage by scavenging of hydroxyl radicals. RTN restored the reduced glutathione levels that were depleted by ethanol in gastric mucosa. This results was supported by Bishnoi et al., (2007) who reported that RTN increases the glutathione level that was reduced after haloperidol treatment.

Effect of Rutin on ethanol-induced gastritis in rats

A significant increase in gastric tissue L-MDA level, DNA fragmentation and MPO activity were observed in gastritis -induced rats in protective and treatment period when compared with normal control group. Similarly, Hussein et al., (2014) reported that, a marked increase in gastric tissue L-MDA, DNA-fragmentation and MPO were observed in ethanol induced gastric mucosal damage in rats. Malondialdehyde is the final product of lipid peroxidation and is used to determine lipid peroxidation levels (Johansen, et al., 2005). There is consensus that the deleterious effects of ethanol on gastric mucosa are consequence of enhanced lipid peroxidation. The presence of oxygen free radicals that cause lipid peroxidation have been reported in the pathogenesis of gastric mucosal lesions induced by ulcer inducing agents such indomethacin, alcohol and aspirin in rats (Takeuchi et al., 1986). Free oxygen radicals initiate lipid peroxidation by removing one hydrogen atom from polyunsaturated fatty acids with the subsequent formation of hydro peroxides. As a result of these reactions, the membrane fluidity and membrane integrity of cells are impaired, leading to disintegration of cells and cell death. These subcellular structures that are released into the extracellular environment trigger several inflammatory events and further worsen the ongoing damage (Eşrefoğlu et al., 2006). RTN administration to gastritis induced rats group resulted in significant decrease in gastric tissue L-MDA concentration when compared with gastritis non treated group. Free radicals react with lipids in cell membranes and form lipid peroxides and this changes the integrity of cells. RTN, being an anti-lipoperoxidant agent, inhibits formation of lipid peroxides (Nègre-Salvayre et al., 1991). Also, Annapurna et al., (2009) reported that, the two natural bioflavonoids, Rutin and quercetin, showed to decreased MDA levels and increased antioxidant enzyme levels in cardiac ischemia reperfusion injury. The mechanism of

reperfusion injury induced oxidative stress is similar in cardiac reperfusion injury and testicular ischemia reperfusion injury (Annapurna et al., 2009).

The present study indicates that EtOH exposure increases the apoptotic DNA fragmentation ratio of the gastric mucosa, which seems to be responsible of severe injury. Furthermore, it was investigated that ROS may cause DNA-fragmentation (Bagchi *et al.*, 1999). Under normal physiological conditions, the balance between gastric epithelial cell proliferation and death is of great importance in maintaining gastric mucosal integrity. Since, the balance between cell apoptosis and cell proliferation has important role to keep the gastric mucosa healthy (Kalia *et al.*, 2000). Since, the gastric epithelial cells proliferate in the lower part of the glandular neck and migrate up the crypt towards the surface and then are shed into the lumen by apoptosis (Ohkura et al., 2003). Disturbance of this balance could result in either cell loss, leading to mucosal damage and gastritis, or cell accumulation, leading to cancer development (Kohda *et al.*, 1999). The gastric mucosal hemorrhage evoked by extra amounts of alcohol is initiated by the microcirculatory damage of the gastrointestinal mucosa, namely a disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting. It has been shown that EtOH dramatically increases the low level of spontaneous apoptosis in gastric tissues which normally occurs to protect against the survival and expansion of genetically damaged cells (Chattopadhyay *et al.*, 2004). RTN administration to gastritis induced rats resulted in significant decrease in gastric tissue DNA fragmentation percentage when compared with gastritis non treated group. RTN treatment, in the present study, suppressed the high percentage of DNA fragmentation in the gastric mucosa, while gastric epithelial integrity was

maintained. Similarly, Fang et al., (2008) reported that, treatment with RTN significantly reduced apoptotic cell death caused by oxidative stress (H_2O_2) through a significant reduction in pro-apoptotic gene (Bax) expression and increased anti-apoptotic gene (Bcl-2) expression in H9c2 cells treated with H_2O_2 in vitro. Also, Shakeerabanu et al., (2001) provide evidence that, quercetin enhances the healing of gastric tissue damage by increasing PH, decreasing of (acidity, acid output, pepsin concentration), as well as by its antiapoptotic activity.

Myeloperoxidase is an essential enzyme for normal neutrophil function, released into extracellular fluid as a response to various stimulatory substances. MPO activity is considered as an index for the evaluation of neutrophil infiltration. In the present study, a significant increase in MPO activity was observed in ethanol-induced gastritis group. The elevated activity of MPO in the gastric mucosa indicates oxidative injury induced by ethanol involves the contribution of neutrophil accumulation (Amudhan and Begum 2008). The increase in enzyme activity level may be associated with increase in the levels of neutrophil infiltration and H_2O_2 in the gastric damaged tissues administered with ethanol (Megala and Geetha 2010). Also, one mechanism in the pathogenesis of mucosal lesions provoked by ethanol may be circulating neutrophils (Kvietys et al., 1990). The leukocytes might create gastritis through various mechanisms, such as the production of reactive oxygen metabolites or the release of proteases and lipid mediators (Zimmerman and Granger 1994). Moreover, activated neutrophils produce many enzymes and free radicals that damage the gastric mucosa, neutrophil is considered as an aggressive factor in gastritis (Fukumura et al., 1995).

The gastric injury induced by EtOH involves toxic oxygen metabolites. Since one

of the sources of oxygen radicals in gastritis induced by EtOH in rats seems to be the neutrophils Vazquez-Ramirez et al., (2006). In the present study, the role of neutrophils was assessed by tissue-associated MPO activity, demonstrating a significant elevation in both periods of gastritis. RTN administration to gastritis induced rats group resulted in a non-significant decrease in gastric tissue MPO activity in protective and treatment periods when compared with gastritis non treated group. The obtained results are nearly similar to those reported by Pincemail et al., (1988) who recorded that, RTN inhibits neutrophil infiltration as demonstrated from reduction of human myeloperoxidase activity both with purified enzyme and in stimulated human neutrophils. Moreover, RTN was reported to enhance the activity of cNOS leading to increase in the nitric oxide levels in gastric mucosal tissues. cNOS-derived nitric oxide improves the mucosal blood flow and tissue perfusion leading to attenuation of neutrophil infiltration (Olszanecki et al., 2002). Furthermore, RTN administration was previously shown to offer gastric mucosal protection against ethanol-induced gastric lesions by a neutrophil-dependent mechanism (La Casa et al., 2000).

In conclusion, the present study demonstrated that, RTN possesses significantly gastroprotection and treatment effects against gastritis and oxidative damage in gastric tissue induced by ethanol in rats. Since, RTN was able to ameliorate serum biochemical parameters, enzymatic and non-enzymatic antioxidant defense system, mucus secretion and prevent DNA fragmentation in gastric tissue. Based on the data of the current study, the effect of RTN against ethanol-induced gastric lesions can be attributed to the inhibitory effects on neutrophil infiltration, and its reduction of inflammatory markers as well as its antiapoptotic effect. We recommended that, administration of diet rich

in the antioxidant flavonoid RTN is very important for protection of different body tissue, especially gastric tissue, against

oxidative stress or even inflammation or erosion.

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التأثير الوقائي للمعدة والمضاد لموت الخلايا والالتهابات للروتين في التهاب المعدة المحدث بالكحول الايثيلي في الفئران

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الملخص العربي

في هذه الدراسة تم تقييم التأثير الوقائي والعلاجي للروتين على التغيرات في مستوى أكسيد النيتريك وحمض السياليك، فيتامين ج ، الإنزيمات المضادة للأكسدة ، تركيز إل-مالون داي ألدهيد، انزيم الميلوبيروكسيداز، الجلوتاثيون المختزل وتفتيت الدى ان ايه في دم وأنسجة الجرذان المستحدث فيها التهاب المعدة بالكحول الايثيلي . هذا وقد أستخدم لأجراء هذه الدراسة عدد40 من ذكور الجرذان البيضاء أعمارهم تتراوح من 12-16 أسبوع و أوزانها من 200-250 جرام وقد قسمت إلي اربعة مجموعات وتم توزيعها كالتالي: المجموعة الأولى: (المجموعة الضابطة): اشتملت على 12 فأر لم تعطى أي أدوية واستخدمت كمجموعة ضابطة للمجموعات الأخرى. المجموعة الثانية: (المجموعة المحدث بها التهاب المعدة): تكونت من 12 فأر تم اعطاؤها الكحول الايثيلي عن طريق الفم بتركيز 100% بمعدل (1 مل / فأر). المجموعة الثالثة: (مجموعة الروتين الوقائية والمحدث بها التهاب المعدة): اشتملت على 8 فأر تم تجريعها الروتين يومياً عن طريق الفم بجرعة مقدراها (200 مللى جرام/ كيلوجرام من وزن الجسم) لمدة 14 ايام وفى اليوم الخامس عشر تم تجريعها الكحول الايثيلي عن طريق الفم بتركيز 100% بمعدل (1 مل / فأر). المجموعة الرابعة (مجموعة الروتين العلاجية والمحدث بها التهاب المعدة): اشتملت على8 فأر تم تجريعها الروتين يومياً كما فى المجموعة الثالثة واستمر العلاج بالروتين بنفس الجرعة السابقة لمدة 7 أيام. هذا وقد تم تجميع عينات الدم والأنسجة فى اليوم الخامس عشر فى المجموعة الوقائية بعد ساعة من تجريعها بالكحول الايثيلي واليوم الثاني والعشرون فى المجموعة العلاجية من بداية التجربة. وقد أسفرت نتائج التحليل البيوكيميائى عن وجود انخفاض معنوي فى كلا من أكسيد النيتريك وحمض السياليك بالمصل بالإضافة إلى مستوى فيتامين ج والجلوتاثيون المختزل وأيضاً نقص معنوي فى نشاط الجلوتاثيون بيروكسيداز و سوبر أكسيد ديسميوتيز و الكتاليز وانزيم الجلوتاثيون ريدكتاز فى نسيج المعدة مع حدوث زيادة معنوية فى نشاط الميلوبيروكسيداز و تركيز إل-مالون داي ألدهيد وتجزئة الحمض النووي دى ان ايه فى المجموعة المحدث بها التهاب المعدة. كما أوضحت النتائج أن مجموعتي الجرذان المحدث بها التهاب المعدة والتي تم وقايتها وعلاجها بالروتين عن وجود زيادة فى كلا من أكسيد النيتريك وحمض السيالك بالدم بالإضافة الى فيتامين ج والجلوتاثيون المختزل وسوبر أكسيد ديسميوتيز والكتاليز والجلوتاثيون بيروكسيداز و الجلوتاثيون ريدكتاز فى نسيج المعدة فى حين انخفض مستوى الميلوبيروكسيداز و تركيز إل-مالون داي ألدهيد وتجزئة الحمض النووي دى ان ايه. وأوضحت الدراسة أن استخدام الروتين كان له دور فعال فى حماية الغشاء المبطن للمعدة من الالتهاب والتآكل المحدث باستخدام الكحول الايثيلي وأدى استخدامه كذلك الى الحفاظ على نسب القياسات البيوكيميائية فى الدم والأنسجة لما يقارب النسب الطبيعية ، و يرجع ذلك إلى نشاط الروتين الوقائي والمضاد للأكسدة والالتهابات والمضاد لتكسير الدى ان ايه والموت الخلوي للخلايا. لذلك توصى الدراسة بضرورة استغلال تلك المزايا الهائلة للروتين كماده وقائية مضادة للأكسدة و إدخاله كماده فعالة فى صناعة العقاقير الطبية المستخدمة فى وقاية و علاج المعدة من الالتهابات والتآكل.

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