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Evaluation of Gastroprotective Effect and Anti-Inflammatory Role of Resveratrol against Gastric Mucosal Alterations in Experimental Model of Gastritis in Rats

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ABSTRACT

Resveratrol is a phytoalexin polyphenol exhibiting antioxidant and anti-inflammatory properties. The present study was designed to evaluate the potential protective and therapeutic effects of resveratrol (RSV) on ethanol-induced gastritis in rats. Thirty two male rats divided into four groups. Normal control group: received no drugs, gastritis-induced group: administered with a single oral dose of 1 ml/rat of absolute ethanol, gastritis + RSV protected: received RSV orally (10 mg/kg b. wt/day) for 2 days prior ethanol administration and gastritis + RSV treated: received RSV as in group III and the treatment was continued for 7 days later. Blood samples and gastric tissue specimens were collected for determination of serum and gastric tissue parameters. The obtained results showed a significant decrease in serum nitric oxide (NO), sialic acid (SA), and gastric tissue GSH, vitamin C concentrations and GPX, SOD, GR and CAT activities in gastritis induced rats. However, myeloperoxidase (MPO) and cyclooxygenase II (COX-2) activities, NF-KB p65, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1beta (IL-1β), L- Malondialdehyde (L-MDA) and DNA-fragmentation were significantly increased. Administration of RSV was able to mitigate gastritis induced by ethanol through increasing of NO, SA, GSH, vitamin C concentrations, enzymatic antioxidants status in addition to decreasing NF-KB p65, Pro-inflammatory cytokines, L-MDA, DNA-fragmentation, MPO and COX-2. Additionally, various pathological changes in gastric tissues were observed in gastritis induced rats. Interestingly, the severity of these alterations was reduced in resveratrol protected and resveratrol treated groups with variable degree. The results suggest that, RSV may be effective in the therapeutic of gastritis by its radical scavenging and antiapoptotic activity, inhibited neutrophil accumulation, restoring endogenous antioxidant mechanisms and attenuate the severity of histopathological alterations in the gastric mucosa.

Keywords: Resveratrol; Gastritis; inflammatory markers; oxidative stress; antioxidant status, histopathology.

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 [1], for more than a century, peptic ulcer disease has been a major cause of morbidity and mortality[2]. The activated neutrophils will increase the production of pro-oxidative and pro-inflammatory enzymes and free radicals which lead to oxidative burst [3].

The activated neutrophils will increase the production of pro-oxidative and pro-inflammatory enzymes and free radicals which lead to oxidative burst [3]. On the other hand, cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) play important roles

in the pathogenesis of acute gastric lesions induced by ethanol [4].

Administration of absolute ethanol into the gastric lumen induced gross lesions in the glandular part of the stomach[5]. 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[6]. 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[7]. The direct contact of alcohol with gastric mucosa can induce numerous metabolic and functional changes. These alterations may lead to marked mucosal damage, which can result in a broad spectrum of acute and chronic diseases, such as gastrointestinal bleeding and ulcers [8].

The oxygen derived free radicals play a key role in tissue damage during pathogenesis of various disorders of the digestive tract caused by physical, chemical and psychological factors that lead to gastric ulceration in human and experimental animals[9]. Oral administration of ethanol in rats causes severe gastric mucosal damage by disruption its barrier and provokes rapid, strong microvascular events in mucosal capillaries [10]. 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 [11]. 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 [12].

Resveratrol (3,5,4′-trihydroxystilbene) is a phytoalexin phenolic non-flavonoid compound [13]. Also, RSV is reported as one of the most potent anti-inflammatory and antioxidant against ROS and oxidative stress. During oxidative stress, RSV displayed direct antioxidant effects by scavenging free radicals [14]. Accordingly, the purpose of the present study was to investigate the effect of RSV in protecting against ethanol-induced gastritis in rats and to elucidate the underlying mechanisms. Also, to determine whether RES when administered to gastritis induced in rats would attenuate the oxidative stress and gastric tissue damage, 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:

Thirty two 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. Resveratrol:

Resveratrol is white powder with yellow casts (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.

RSV was freshly prepared in 0.9% saline solution, and administered to rats at a dose of (10 mg/kg body weight/day) for 9 successive days [15].

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 a single dose of 1ml/rat absolute ethanol [16].

2.4. Experimental design:

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

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

Subgroup (a).
Included 6 rats sacrificed at the 3rd day of the experiment, served as the normal control rats for early gastritis group.

Subgroup (b).
Included 6 rats sacrificed at the 10th 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 RSV 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 RSV 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 (RSV protected group).
Comprised 4 male rats received RSV (10 mg/kg body weight/day) orally for 2 days prior absolute ethanol administration. One hour after the administration of ethanol the animals were sacrificed.

Group IV (RSV treated group).
Included 4 male rats received RSV orally (10 mg/kg body weight) for 2 days before ethanol administration and the treatment were continued with RSV 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 RSV protected gastritis group at the 3rd day from the onset of treatment with RSV. Also, blood samples and tissue specimens (gastric tissues) were collected after nine days from the onset of treatment with RSV in normal control group (subgroup b), gastritis non-treated group subgroup (b) and RSV 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 -20°C until used for subsequent biochemical analysis. All sera were analyzed for the following parameters: Nitric oxide (NO), Sialic acid (SA), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and L- Malondialdehyde (L-MDA).

2.5.2. Tissue samples (gastric tissue)
After two and nine days of RSV 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. Additionally, gastric tissue specimens were taken from different parts of the stomach for histopathological examination.

2.5.2.1. Stomach tissue preparation for biochemical analysis:
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), nuclear factor kappaB p65 (NF-KB p65), interleukin-1beta (IL-1β), cyclooxygenase-II (COX-2), reduced glutathione (GSH), vitamin C, myeloperoxidase (MPO) and DNA fragmentation.

2.5.2.2. Stomach tissue preparation for Histopathological examination:
Gastric tissue specimens were taken from different parts of the stomach for histopathological examination. The specimens were preserved in 10% buffered neutral formalin. The fixed tissue were rinsed in tap water, dehydrated through graded series of alcohols, cleared in xylene and embedded in paraffin wax. 5 µm thick sections were cut and stained with hematoxylin and eosin (H&E) **[17]** and then the tissues were examined and evaluated by light microscopy.

2.6. Biochemical analysis
Serum NO, SA, TNF-α, IL-6 and gastric tissue vitamin C, L-MDA, GPx, SOD, CAT, GR, GSH, DNA fragmentation, NF-KB p65, IL-1β, COX-2 and MPO were determined according to the methods described by Vodovotz, (1996) [18]; Human Sialic acid (SA) Elisa kit (Cat. no. CSB- E09605h); Beyaert and Fiers, (1998) [19]; Chan and Perlstein, (1987) [20]; Mesbah *et al.,* (2004) [21]; Rat Vitamin C, VC ELISA kit (Cat .No.E0913r); Gross *et al*., (1967) [22]; Kakkar *et al*., (1984) [23]; Luck, (1974) [24]; David and Richard, (1983) [25]; Moron *et al*., (1979) [26]; Shi *et al*., (1996) [27]; Rat Nuclear factor-kappaB p65 (NF-kappaB p65) ELISA Kit instruction(Cat.No. MBS814487); RayBio® Rat IL-1beta ELISA Kit Protocol (Cat.No.ELR-IL1beta-001C); COX-2 ELISA Kit (Cat.No. E0699m) and Rat Myeloperoxidase ELISA kit (Kamiya Biomedical Company, Cat .No. K60345), 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 AND DISCUSSION

3.1. Effect of resveratrol pretreatment on some serum and gastric tissue parameters of ethanol-induced gastritis in rats
The obtained data demonstrated in table (1) showed a significant decrease in serum NO and SA levels and gastric tissue GSH, vitamin C concentrations and antioxidant enzymes(GPx, SOD, GR and CAT) activities in ethanol-induced gastritis group (early gastritis). Meanwhile, the values of serum TNF-α, IL-6, L-MDA levels and gastric tissues DNA fragmentation, NF-KB p65, IL-1β and L-MDA concentrations in addition to

MPO and COX-2 activities were significantly increased in ethanol-induced gastritis group (early gastritis)in rats when compared with normal control group. Pretreatment with RSV in gastritis–induced rats resulted in a significant increase in serum NO and SA concentrations, and in gastric tissue GPx, SOD,CAT and GR activities, GSH and vitamin C levels. Meanwhile, L-MDA, DNA fragmentation, NF-KB p65, TNF-α, IL-6, IL-1β levels, COX and MPO activities were significantly decreased in protective period when compared with early gastritis non- treated group.

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

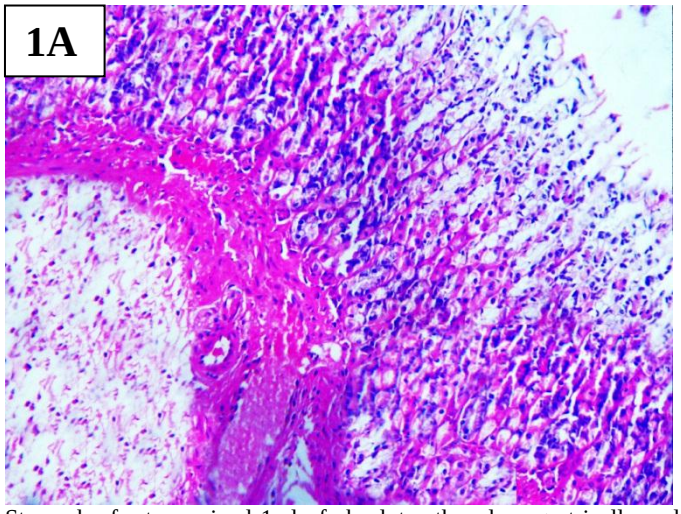
Protective period			
Experimental groups / Parameters	Control Normal group	Gastritis induced group	Gastritis + RSV protected group
NO (mmol/L)	148.94 ± 2.60^{a}	76.06 ± 2.19^{c}	138.38 ± 4.08^{a}
SA (mg/ ml)	55.87 ± 1.63^{a}	12.64 ± 0.93^{d}	43.81 ± 4.84^{b}
GPx (ng/ g.tissue)	51.84 ± 0.91^{a}	22.32 ± 0.68^{e}	33.36 ± 1.76^{b}
SOD (u/g.tissue)	21.18 ± 0.40^{a}	12.80 ± 1.00^{d}	44.66 ± 1.04^{b}
CAT(mmol/g.tissue)	66.15 ± 1.00^{a}	30.00 ± 0.92^{d}	53.93 ± 2.49^{b}
GR (ng /g tissue)	3.66 ± 0.13^{a}	1.83 ± 0.07^{c}	3.37 ± 0.14^{a}
L-MDA (mmol/g.tissue)	57.09 ± 0.55^{d}	111.61 ± 0.72^{a}	63.79 ± 1.49^{cd}
L-MDA (mmol/ml)	77.95±1.39^{d}	154.19±1.69^{a}	90.42± 3.90^{c}
GSH (ng/g.tissue)	9.59 ± 0.79^{a}	1.95 ± 0.54^{d}	7.27 ± 0.74^{b}
Vitamin C (ng / g tissue)	40.38 ± 1.00^{a}	16.45 ± 0.73^{d}	18.64 ± 1.07^{ab}
DNA-fragmentation (cells/well. tissue)	96.25 ± 2.63^{e}	1390.00 ± 4.22^{a}	225.39 ± 47.60^{d}
MPO (ng/g.tissue)	5.78 ± 0.14^{c}	14.32 ± 0.68^{a}	7.12 ± 0.41^{c}
NF-KB p65 (ng/g.tissue)	1.89±0.13^{b}	7.92±0.98^{a}	2.62 ± 0.28^{b}
TNF-α (pg/ml)	25.87±1.68^{c}	81.81±0.76^{a}	35.82 ± 3.29^{c}
IL-6 (pg/ml)	16.50±1.47^{c}	132.07±1.25^{a}	62.02±13.83^{b}
IL-1β (pg/g.tissue)	59.90±2.26^{c}	478.50±3.34^{a}	210.86±33.59^{b}
COX-2 (ng/g.tissue)	0.26±0.07^{c}	3.92±0.53^{a}	2.26±0.29^{b}

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).

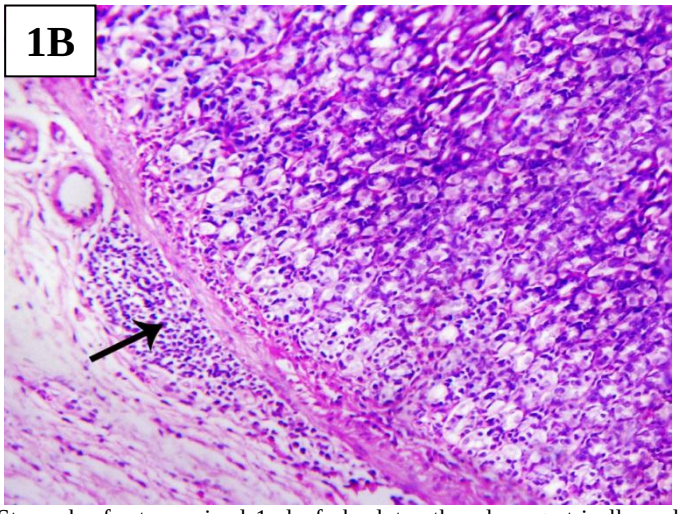
Table 2: Effect of resveratrol treatment on some serum and gastric tissue parameters of ethanol-induced gastritis in rats.

Treatment period			
Experimental groups / Parameters	Control Normal group	Gastritis induced group	Gastritis + RSV treated group
NO (mmol/L)	163.38 ± 2.01^{a}	55.63 ± 2.12^{c}	138.50 ± 9.23^{a}
SA (mg/ml)	51.14 ± 1.20^{a}	20.80 ± 0.66^{d}	46.21 ± 1.22^{a}
GPx (ng/ g.tissue)	40.88 ± 0.97^{a}	26.27 ± 0.84^{d}	36.15 ± 1.19^{a}
SOD (U/g.tissue)	24.49 ± 0.84^{a}	12.06 ± 0.28^{d}	32.30 ± 0.84^{bc}
CAT(mmol/g.tissue)	63.46 ± 1.55^{a}	35.36 ± 1.01^{d}	54.54 ± 5.04^{b}
GR (ng /g tissue)	3.83 ± 0.16^{a}	1.40 ± 0.11^{b}	2.08 ± 0.31^{b}
L-MDA (mmol/g.tissue)	71.26 ± 1.07^{c}	128.39 ± 1.30a	85.70 ± 4.40^{b}
L-MDA (mmol/ml)	66.93±1.90^{d}	167.74±1.63^{a}	83.33±5.35^{cd}
GSH (ng/g.tissue)	10.98 ± 1.02^{a}	3.78 ± 0.39^{c}	8.30 ± 0.70^{b}
Vitamin C (ng /g. tissue)	38.82 ± 0.47^{a}	20.00 ± 0.66^{c}	21.92 ± 1.17^{b}
DNA-fragmentation (cells/well tissue)	17.50 ± 1.36^{d}	1015.00 ± 2.13^{a}	136.23 ± 31.31^{d}
MPO (ng/g.tissue)	3.12 ± 0.14^{d}	12.76 ± 0.77^{a}	6.33 ± 0.97^{c}
NF-KB p65 (ng/g.tissue)	1.40±0.16^{b}	5.98±0.53^{a}	2.41±0.47^{b}
TNF-α (pg/ml)	30.98±0.26^{d}	89.80±1.60^{a}	45.60±6.21^{c}
IL-6 (pg/ml)	24.07±1.09^{b}	108.03±1.28^{a}	44.18±9.59^{b}
IL-1β (pg/g.tissue)	22.70±0.24	328.04±2.27^{a}	110.81±32.92^{c}
COX-2 (ng/g.tissue)	1.33±0.13^{c}	4.28±0.54^{a}	2.40±0.29^{b}

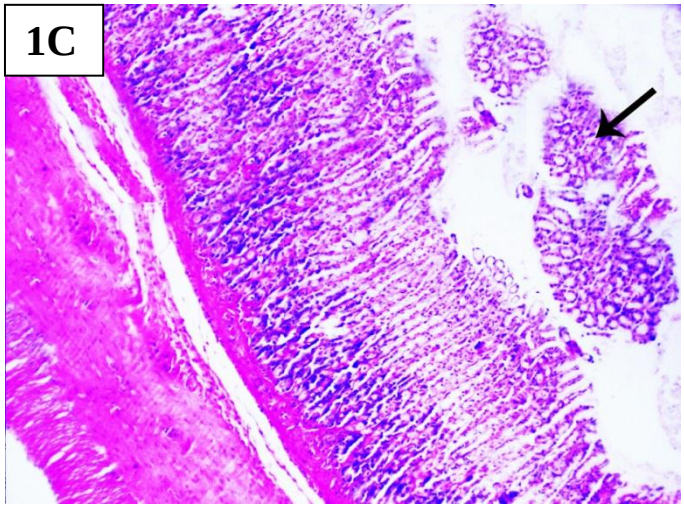
Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same row are significantly different at (P<0.05).



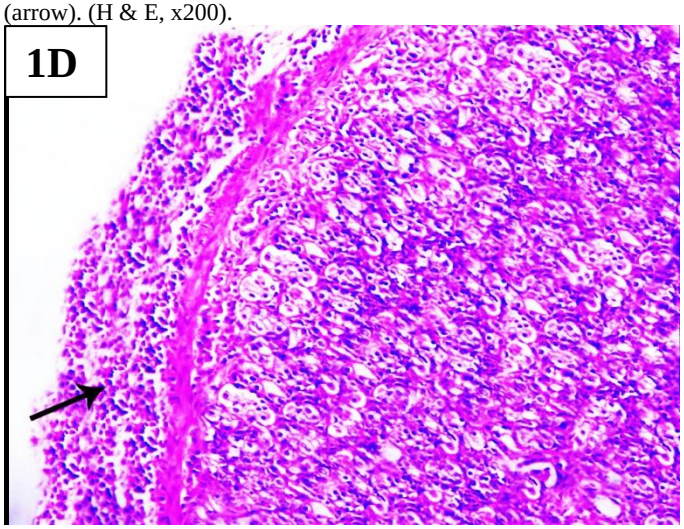
Stomach of rats received 1ml of absolute ethanol orogastrically and sacrificed after one hour of administration showing congestion of the blood vessels and few leukocytic infiltration. (H & E, x200).



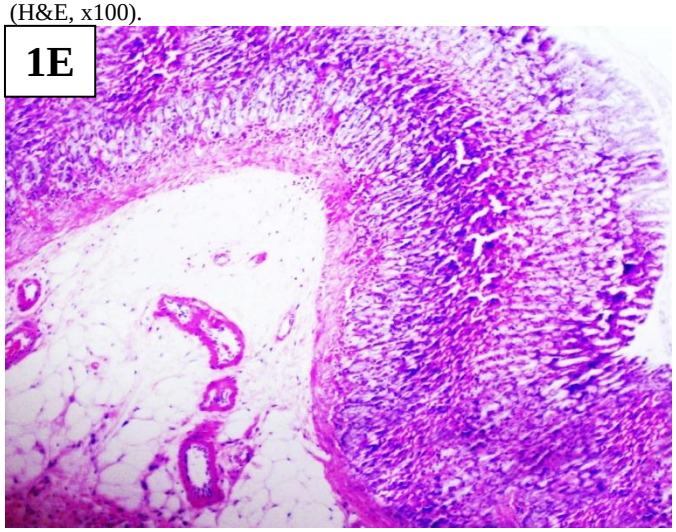
Stomach of rats received 1ml of absolute ethanol orogastrically and sacrificed after one hour of administration showing focal mononuclear cellular aggregation in the tunica muscularis of gastric mucosa (arrow). (H & E, x200).



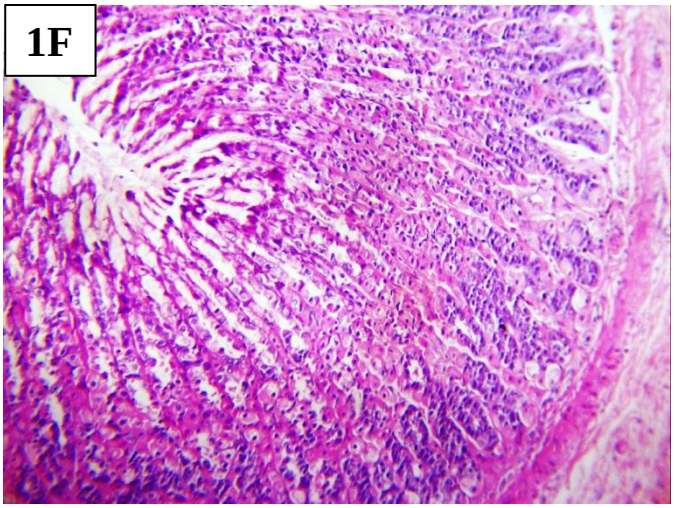
Stomach of rats received 1ml of absolute ethanol orogastrically and sacrificed after seven days of administration showing severe desquamation of the lining epithelium of gastric mucosa (arrow). (H&E, x100).



Stomach of rats received 1ml of absolute ethanol orogastrically and sacrificed after seven days of administration showing heavy leukocytic infiltration in tunica muscularis (arrow). (H&E, x200)



Stomach of rats received Resveratrol daily orogastrically for two days then received 1ml of absolute ethanol orogastrically and sacrificed after one hour of administration showing normal gastric mucosa with mild congestion of the gastric blood vessels. (H & E, x100).



Stomach of rats received Resveratrol daily orogastrically for seven days, showing nearly normal histological structure of the gastric mucousa. (H & E, x200).

3.2. Effect of resveratrol 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 in gastric tissue GSH and vitamin C concentrations, GPx, SOD, GR and CAT activities with a marked increase in serum TNF-α, IL-6, L-MDA levels and in gastric tissues DNA fragmentation, NF-KB p65, IL-1β and L-MDA concentrations as well as MPO and COX-2 activities were observed in gastritis-induced rats (late gastritis) when compared with normal control group. Administration of RSV to ethanol–induced gastritis in rats resulted in a significant increase in serum NO and SA levels and in gastric tissue GSH, vitamin C levels, GPx, SOD and CAT activities. However, RSV treatment to ethanol induced gastritis (late gastritis) in rats exhibited a significant decrease in serum TNF-α and IL-6 and in gastric tissue L-MDA, DNA fragmentation, NF-KB p65, IL-1β levels, COX-2 and MPO activities when compared with late gastritis non- treated group.

Histopathological examination:

The microscopical examination of the stomach of control rats showing normal histological structure of the glandular epithelium of the gastric mucosa of the rats.

The microscopical examination of the stomach of rats obtained after one hour from absolute ethanol (1ml/rat) administration showed severe congestion of the blood vessels and few leukocytic infiltrations (fig. 1A). Mild desquamations of the epithelial cell lining of the gastric mucosa with focal leukocytic aggregation in the tunica submucosa were seen (fig. 1B). Moreover, few leukocytic mononuclear cellular infiltrations were seen in between the glandular epithelium. Severe congestion of the blood vessels of the stomach in the tunica muscularis together with leukocytic infiltrations was also detected.

However, the histopathological examination of the stomach obtained after 7 days from ethanol administrations showing severe desquamation of the glandular epithelium lining of the gastric mucosa (fig. 1C) was also observed. The desquamated epithelium admixed with eosinophilic debris was seen in the gastric lumen. Moreover, heavy leukocytic mononuclear infiltration was also noticed in the tunica muscularis (fig. 1D).

The histopathological examination of stomach of rats received RSV (10 mg/kg body weight/day) orally for 2 days prior absolute ethanol administration for one hour revealed nearly normal gastric mucosa with the presence of few amount of eosinophilic and basophilic debris were seen in the gastric lumen. Moreover mild congestion of the gastric blood vessels was seen (fig. 1E). In the meantime, the stomach section from the same group but was taken 7-days post treatment showed nearly normal histological structure of the gastric mucosa (fig. 1F).

The ethanol model is widely used to assess the protective and healing activity of many drugs in ulcer studies [28]. 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 [29], resulting in an increased flow of Na^+ and K^+, elevated pepsin secretion, loss of H^+ ions and histamine into the lumen [30].

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 [31], or NO consumption in free radicals reactions with peroxynitrites (ONOO-) overproduction[32], Potentially formed during NO reaction with free radicals overproduced during ethanol metabolism [33]. 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 [34].

A significant increase in serum nitric oxide concentration was observed in both RESV protected and treated gastritis groups compared with the gastritis non treated group. These results are nearly similar to those reported by Liu et al., (2005) [35] who observed that, after four weeks treatment with 50mg/kg resveratrol (SPB, heart weight, ET-1 and AngII) concentrations decreased and the serum NO concentration increased suggesting that RSV appears to be able to protect against the increase in systemic blood pressure and subsequent cardiac hypertrophy in vivo and the mechanisms responsible may involve at least in part, modulation of NO, AngII and ET-1 production. 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 [36]. 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 [37].

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 RSV 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 [38]. Additionally, a recent report indicated that the glycosidic linkage of sialic acid is a potential target for superoxide and other related ROS [39]. Mucin acts as a sacrificial scavenger for $^{\cdot}OH$ and its protective function is exerted by the direct reaction with its sialic acids [40].

A significant decrease in gastric tissue GSH, vitamin C concentrations, GPx, SOD, GR and CAT activities were observed in ethanol-induced gastritis in rats group in protective and treatment periods when compared with normal control group. Similarly, Hussein et al, (2014) [41] 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 [42]. 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 [43]. The release of oxygen-derived free radicals (ROS) has drawn attention as a possible pathogenic factor of gastric mucosal injury associated with ethanol consumption [44]. 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 [45]. 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 H_2O_2 [46]. There is evidence to indicate that ethanol significantly depresses SOD activities[47].

Ethanol inhibited SOD and thus superoxide radicals could not convert to H_2O_2.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 are nearly similar with that reported by[48]. 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 [49]. 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 H_2O_2, which is the substrate for the H_2O_2 scavenging enzymes CAT and GPx. These result in inactivation of the H_2O_2 scavenging enzymes CAT and GPx, leading to a decrease in their activities [50]. The decrease in GPx activity may attenuate the radical scavenging function [51], GR accelerating the conversion of GSSG to GSH and enhancing the detoxification of reactive metabolites by conjugation with GSH[50]. 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 H_2O_2 or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione [52]. Reduced activities of GPx may result from radical–induced inactivation and glycation of the enzyme [53]. 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 [54]. 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. Studies have implicated that neutrophil infiltration into the gastric mucosa is a critical process in the pathogenesis of various gastric ulcers and the accumulation of neutrophils in the gastric mucosal tissues can be indicated by the measurement of myeloperoxidase (MPO) [55]. Cytokines often induce the activation of neutrophils [56]. The activated neutrophils will increase the production of pro-oxidative and pro-inflammatory enzymes and free radicals which lead to oxidative burst [3].

Treatment with RSV in gastritis-induced rats resulted in a non-significant increase in GR activity and significantly increased vitamin C and GSH levels, GPx, CAT and SOD activities in treatment period. However, administration of RSV to ethanol–induced gastritis in rats exhibited a significant increase in gastric tissue GPx activity, GSH and vitamin C levels, SOD, and CAT activities in treatment period when compared with control gastritis non- treated group. Antioxidant effects of RSV are 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 [57]. Vitamin C is an excellent hydrophilic antioxidant in plasma, because it disappears faster than other antioxidant when plasma is exposed to reactive oxygen species [58]. 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 [59]. The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues [60]. Meanwhile, after administration of RESV the concentration of GSH increased and superoxide radicals decreased. Therefore, GR activity increased. In the present study, administration of RSV 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 [61]. Superoxide dismutase is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. Peroxide can be destroyed by CAT or GPX reactions [62]. First, SOD converts the superoxide anion to hydrogen peroxide in a cellular antioxidant reaction. Thereafter, GSH-Px detoxify hydrogen peroxide produced [63]. It has been suggested that RSV can reduce oxidized GSH and increase the GSH status, which in turn exhibits increased free radical scavenging property, so RSV indirectly influences the activity of SOD thereby preventing the deleterious effect of superoxide radical formed, That causes activation of hepatic SOD, GPx and CAT that reduced by cadmium intoxication [64].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 H_2O, 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 [65]. Glutathione has a very important role in protecting against oxygen-free radical damage by scavenging of hydroxyl radicals. RSV restored the reduced glutathione levels that were depleted by ethanol in gastric mucosa. This results was supported by Yen et al., (2003) [66] who reported that, RSV at concentrations of 10-100 μM exerts great protection against H_2O_2 induced oxidative injury through increased GSH levels.

A significant increase in gastric tissue DNA fragmentation, tissue and serum L-MDA level 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) [41] reported that, a marked increase in gastric tissue L-MDA, DNA-fragmentation and MPO were observed in ethanol induced gastric mucosal damage in rats that could clarify the histopathological changes that demonstrated in the gastric tissues of ethanol treated rats in the current research. Malondialdehyde is the final product of lipid peroxidation and is used to determine lipid peroxidation levels [67]. Additionally, 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[68]. 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 [69].

Resveratrol administration to gastritis induced rats group resulted in significant decrease in gastric tissue and serum 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. RSV inhibited effectively the lipid peroxidation of cellular membranes, the protein oxidation as well as the DNA damage due its ability to directly scavenge various free radicals including superoxide radicals and peroxyl and hydroxyl radicals[70]. Kumar et al., (2007) [71] reported that, a significant reduction occurred in MDA levels of diabetic rats protected with RSV in relation to diabetic non protected rats.

The present study indicates that ethanol 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 [72]. 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 [73]. 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 [74] as confirmed by the histopathological results of the current work. Disturbance of this balance could result in either cell loss, leading to mucosal damage and gastritis, or cell accumulation, leading to cancer development [75]. 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 ethanol 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 [76]. RSV administration to gastritis induced rats resulted in significant decrease in gastric tissue DNA fragmentation percentage when compared with gastritis non treated group. In the present study, the microscopical examination of gastric tissue revealed marked improvement in the gastric tissue following

RSV administration in comparison to ethanol treated group. These findings could be attributed to the ability of RSV to suppress the high percentage of DNA fragmentation in the gastric mucosa, in association with maintenance of gastric epithelial integrity. Similarly, Sengottuvelan et al. (2009) [77] reported that, administration of RSV protected rat leukocytes from DMH-induced DNA damage and oxidative stress. Formation of ROS may contribute to DNA damage which is typified by the release of base oxidation products such as 8-oxo-7,8-dihydro-2-deoxyguanosine (oxo8dG) which is one of the base oxidation products resulting from C-8 hydroxylation of guanine residues. The ratio of oxo8dG to an undamaged nucleoside deoxyguanosine is a biomarker for oxidative DNA damage [78]. Also, RSV protected from potassium bromate induced DNA damage by decreasing the level of kidney oxo8Dg [79].

The histopathological examination of gastric tissue obtained from ethanol treated rats revealed variable pathological alterations in the stomach. This could be due to significant increase in Myeloperoxidase activities in the gastric mucosa that indicates oxidative injury induced by ethanol involves the contribution of neutrophil accumulation [34]. 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[48]. 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. Also, one mechanism in the pathogenesis of mucosal lesions provoked by ethanol may be circulating neutrophils[80]. The leukocytes might create gastritis through various mechanisms, such as the production of reactive oxygen metabolites or the release of proteases and lipid mediators [81]. Moreover, activated neutrophils produce many enzymes and free radicals that damage the gastric mucosa, neutrophil is considered as an aggressive factor in gastritis [82].

The gastric injury induced by ethanol involves toxic oxygen metabolites. Since one of the sources of oxygen radicals in gastritis induced by ethanol in rats seems to be the neutrophils[83]. In the present study, the role of neutrophils was assessed by tissue-associated MPO activity, demonstrating a significant elevation in both periods of gastritis. RSV administration to gastritis induced rats group resulted in a significant decrease in gastric tissue MPO activity in protective and treatment periods when compared with gastritis non treated group as confirmed by histopathological results. The obtained results are nearly similar to those reported by Brzozowski et al., (1999) [84] who observed that, Trans-resveratrol and hydroxyl stilbenes reduced MPO activity in acetic acid-induced ulcerated rat.

The nuclear transcription factor NF-kB is a key regulator of the inducible expression of many genes involved in immune and inflammatory responses in the gut. Stimuli such as oxidative stress, cytokines (IL-1, IL-6, TNF-α), bacteria and viruses can release NF-kB to allow translocation to the nucleus [85]. Chronic ethanol treatment elevates endotoxin level, and endotoxin activates kupfer's cells to produce free radicals via NADPH oxidase. The free radicals activate nuclear factor –kappa B (NF-kB), leading to an increase in production of tumor necrosis factor alpha (TNF-α), followed eventually by tissue damage[86].The imbalance between gastro toxic agents and protective mechanisms resulted in an acute inflammation. The interleukin-1 beta (IL-1β) and TNF-α are major pro-inflammatory cytokines, playing important role in production of acute inflammation[87]. This acute inflammation is accompanied by neutrophils infiltration of gastric mucosa. Neutrophils produce superoxide radical anion ($O2^-$), which belongs to group of ROS[88]. A significant decrease in gastric tissue NF-KB p65, IL-1β and serum TNF-α, IL-6 were observed in gastritis-induced rats in both protective and treatment period when compared with normal control group. Similarly, Holmes-McNary and Baldwin, (2000) [89] reported that, treatment with RSV decreased the nuclear protein expression of NF-kB p65. Also, Gonzales and Orlando, (2008) [90] suggested that, RSV expressed anti-inflammatory activity which appeared to be mediated by an inhibition of the activation of transcription factor nuclear factor-kappa B (NF-KB). Both RSV and Cur inhibited NF-KB activation, IL-6 and COX-2 expression. Moreover, Sanchez-Fidalgo et al., (2010) [91] reported that RSV treatment caused a significant decrease in the inflammatory cytokines TNF-□□ IL-1β, as well as COX-2 and iNOS activity and an increase in the anti-inflammatory immune-regulatory cytokine IL10.

Prostaglandins (PGs) are involved in a variety of physiological processes in the stomach, including acid secretion, production of mucus and mucosal blood flow [92].Cyclooxygenase (COX), the key enzyme for PG production, exists as two isozymes referred to as COX-1 and COX-2. COX-1 is constitutively expressed in normal gastric mucosa and generates PGs involved in the maintenance of essential physiological functions[93], while COX-2, characterized by a rapid inducibility in response to various pro-inflammatory stimuli, is responsible for pathological PG production at inflammatory sites [94]. A significant decrease in gastric tissue COX-2 was observed in gastritis-induced rats in both protective and treatment period when compared with normal control group. Similarly, De la Lastra and Villegas (2005) [95] reported that RSV down regulated the inflammatory response through inhibition of inducible nitric oxide synthase and cyclooxygenase-2 (COX-2) via its inhibitory effects on nuclear factor kappa B (NF-kB) or the activator protein-1 (AP-1).

4. CONCLUSION

In conclusion, the present study demonstrated that, RSV possesses significantly gastro-protection and treatment effects against gastritis and oxidative damage in gastric tissue induced by ethanol in rats. Since, RSV was able to ameliorate serum biochemical parameters, enzymatic and non-enzymatic antioxidant defense system, and mucus secretion and prevent DNA fragmentation in gastric tissue as well as improving the integrity of gastric tissue. Based on the data of the current study, the effect of RSV 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 RSV is very important for protection of different body tissue, especially gastric tissue, against oxidative stress or even inflammation or erosion.

5. REFERENCES

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