Tohoku J. Exp. Med., 2009, **218**, 251-258

Resveratrol, a Natural Antioxidant, Attenuates Intestinal Ischemia/Reperfusion Injury in Rats

Orhan Veli Ozkan,[1] Mehmet Fatih Yuzbasioglu,[2] Harun Ciralik,[3] Ergul Belge Kurutas,[4] Zafer Yonden,[5] Mehmet Aydin,[6] Ertan Bulbuloglu,[2] Ersan Semerci,[1] Mustafa Goksu,[2] Yalcin Atli,[2] Vedat Bakan[7] and Nizami Duran[8]

[1]Department of General Surgery, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey
[2]Department of General Surgery, Faculty of Medicine, Kahramanmaras Sutcuimam University, Kahramanmaras, Turkey
[3]Department of Pathology, Faculty of Medicine, Kahramanmaras Sutcuimam University, Kahramanmaras, Turkey
[4]Department of Biochemistry, Faculty of Medicine, Kahramanmaras Sutcuimam University, Kahramanmaras, Turkey
[5]Department of Biochemistry, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey
[6]Department of Physiology, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey
[7]Department of Pediatric Surgery, Faculty of Medicine, Kahramanmaras Sutcuimam University, Kahramanmaras, Turkey
[8]Department of Microbiology, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey

The intestine is highly susceptible to ischemia/reperfusion (I/R) injury. Splanchnic ischemia is the initial event that releases injurious factors, leading to systemic disorders with high morbidity and mortality. Oxidative stress mediators are believed to contribute to the intestinal I/R injury. Resveratrol, a polyphenol found in grapes, is shown to be a strong antioxidant in various tissues, with a property of an estrogen-receptor agonist. Therefore, we investigated the effects of resveratrol on oxidative injury in the intestine. Female Wistar rats were randomly allocated into four groups (n = 8, each). The sham group was only subjected to surgical procedures, while other animals were subjected to intestinal ischemia (60 min) and subsequent reperfusion (60 min). One group received resveratrol (15 mg/kg, 0.3 ml/day intraperitoneally) for both 5 days before surgery and 15 min before ischemia, while the other was treated intraperitoneally with 0.5% ethyl alcohol as vehicle (0.3 ml/day). In the I/R rat intestines, we detected severe tissure injuries ($p < 0.001$), the significant increases in the tissue levels of malondialdehyde (MDA), nitric oxide (NO), and myeloperoxidase (MPO) ($p < 0.001$), and the decrease in superoxide dismutase (SOD) activity ($p < 0.001$), compared to the sham control. Resveratrol significantly ameliorated the intestinal injury, decreased MDA, NO and MPO levels to the sham control levels, and decreased bacterial translocation in mesentery lymp nodes, liver and spleen ($p < 0.001$). Resveratrol also restored the SOD activity. These results suggest that resveratrol could protect intestinal tissue against I/R injury with its potent antioxidant properties.

Resveratrol; intestinal ischemia/reperfusion injury; antioxidants; histopathological evaluation; bacterial translocation.
Tohoku J. Exp. Med., 2009, **218** (3), 251-258.

The restoration of blood supply to ischemic tissue leads to further cellular necrosis, thus limiting the functional recovery of the injured tissue (Guneli et al. 2007; Teke et al. 2007). The tissue injury caused by the restoration of blood flow to an ischemic region is greater than the original ischemic insult, an event called ischemia/reperfusion injury (I/R injury). Increased microvascular permeability, interstitial edema, impaired vasoregulation and inflammatory cell infiltration are shown to account for the organ dysfunction accompanying I/R injury (Karabulut et al. 2006). I/R induced microvascular dysfunction is associated with some clinical entities such as small bowel transplantation, cardiopulmonary bypass, acute mesenteric ischemia, intestinal obstruction, trauma and shock (Guneli et al. 2007; Higa et al. 2007; Teke et al. 2007).

Since the gastrointestinal tract is highly sensitive to I/R injury, mesenteric I/R injury causes a considerable rate of morbidity-mortality. Intestinal I/R triggers the release of

Received April 20, 2009; revision accepted for publication May 31, 2009. doi:10.1620/tjem.218.251
*This study was presented in part as a poster presentation at the 10th European Congress of Trauma and Emergency Surgery in Antalya, Turkey, 13-17 May 2009.
Correspondence: Orhan Veli Ozkan, Assistant Professor of General Surgery, Mustafa Kemal University, Faculty of Medicine, Department of General Surgery, 31100 Antakya, Hatay, Turkey.
e-mail: veliorhan@hotmail.com

proinflammatory and tissue injurious factors, leading to systemic inflammatory response syndrome, adult respiratory distress syndrome, and multi-organ disfunction syndrome. Altered mucosal and microvascular features subsequent to I/R injury such as swollen endothelial cells, capillary plugging, diminished intestinal blood supply and impaired mucosal barrier function result in transmigration of intestinal bacteria or endotoxins (Ozturk et al. 2002; Teke et al. 2007). The passage of indigenous bacteria from gastrointestinal system to sterile extraintestinal sites such as mesenteric lymph nodes, liver, lungs is called bacterial translocation, which is clinically related to burns, hemorrhagic shock, intestinal I/R and endotoxemia (Medeiros et al. 2006).

Although the definitive pathophysiology regarding the intestinal I/R injury still remains obscure, oxidative stress mediators such as reactive oxygen species (ROS), polymorphonuclear neutrophils (PMNLs), and nitric oxide (NO) are suggested to play a crucial role (Guneli et al. 2007). The role of ROS in I/R injury is supported by previous studies which have shown increased PMNLs around the I/R injured areas which release ROS (Yamaguchi et al. 1997) and reduced I/R injury secondary to the use of antioxidants such as melatonin, quercetin, ascorbic acid, L-carnitine, n-asetyl cystein, L-arginine and α-tocopherol (Ustundag et al. 2000; Mojzis et al. 2001; Giakoustidis et al. 2006; Chen et al. 2007; Dokmeci et al. 2007; Higa et al. 2007; Sayan et al. 2008).

Resveratrol (3,4`,5-trihydroxy-trans-stilbene) is a natural phytoalexin that is found in some dietary sources such as grapes, plums, peanuts, red wine and to a lesser extent white wine (de la Lastra and Villegas 2007; Ergun et al. 2007). It acts as a free radical scavenger and modulates a number of enzymes regulating cell life, namely cyclooxygenase (COX), iNOS, lipooxygenase and proteinkinase C (Feng et al. 2002). It is a strong antioxidant and anti-inflammatory agent (Martin et al. 2004; Elmali et al. 2007).

From a different point of view, female Wistar rats have been reported to be more resistant to lethal circulatory stress induced by trauma or intestinal ischemia (Homma et al. 2005; Deitch et al. 2008). Furthermore, Offner et al. (1999) identified male gender as an independent risk factor for the development of severe infection in surgical patients. Additionally, most reports concerning the effects of antioxidants on I/R injury were conducted exclusively with male animals. Resveratrol is an estrogen receptor agonist and causes a significant decrease in estrogen levels both in male and female rats due to a negative feedback effect (Soylemez et al. 2008). Since decreased estrogen levels impair normal cyclic activity in female rats, we thought that there was no need to follow oestrus cycle. Furthermore, resveratrol treatment was found to increase antioxidant levels in both genders (Gehm et al. 1997; Klinge et al. 2005; Yoshida et al. 2007; Soylemez et al. 2008). Based on the resistance of female gender against intestinal ischemia, and male gender being an independent risk factor itself, we performed our experiments in female rats. Therefore, our goal in the present study was to investigate the effects of resveratrol on lipid peroxidation, intestinal morphology and bacterial translocation in female rats subjected to early intestinal I/R injury.

Material and Methods

Animals

Female Wistar-albino rats weighing between 180 and 230 g were used in this study. Animals were kept under optimum conditions (21 ± 1°C, 40 to 70% humidity, 12/12 darkness-lightness cycle) at Kahramanmaras Sutcu Imam University's Laboratory Animal Unit and were allowed free access to food and water. This study was approved by the Local Ethics Committee of Kahramanmaras Sutcu Imam University. The operative procedure, use of anesthesia and animal care methods in the experiments were consistent with the guidelines in the National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH publication No.86-23, revised 1985, Bethesda, MD, USA).

Experimental Groups

Four groups of animals were used in this study: Sham (n = 8): These animals underwent laparotomy without I/R injury. Ischemia reperfusion (I/R, n = 8): These animals underwent laparotomy plus 60 min of ischemia followed by 60 min of reperfusion. I/R+vehicle (n = 8): Only 0.5% ethyl alcohol (0.3 ml/day, intraperitoneally) was admistered for 5 days before surgery and 15 min before ischemia as vehicle. I/R+Resveratrol (n = 8): These rats were treated with 15 mg/kg resveratrol in 0.3 ml 0.5% ethyl alcohol (intraperitoneally) once a day 5 days before surgery and 15 min before ischemia. Purified resveratrol was obtained from Sigma (St. Louis, MO, USA, 500mg) in transisomer form that is the most stable and pharmacologically active form. It is dissolved in 0.5% ethyl alcohol according to manufacturer protocol. In groups I/R, I/R+vehicle and I/R+Resveratrol, a laparotomy was performed under sterile conditions and the superior mesenteric artery (SMA) was occluded with a microvascular clamp as explained below.

Operative details

Following an overnight fasting, the rats were given anesthesia by an intramuscular injection of 50mg/kg ketamine (Ketalar; Parke Davis, Eczacibasi, Istanbul, Turkey) combined with xylazine, 10 mg/kg (Rompun; Bayer AG, Leverkusen, Germany). The rats were allowed to have spontaneous breathing during the surgery and their body temperature was kept at 37°C by a heating lamp. Prior to the midline laparotomy, the rats' skin was shaved and their abdominal walls were cleaned with 10% povidone iodine. After the manipulation of the small bowel and meticulous dissection of the SMA, an atraumatic microvascular clamp was used to occlude the SMA just after it branches out from the aorta. Occlusion of the superior mesenteric vein was avoided. Mesenteric ischemia was determined by the loss of mesenteric pulsations and the pale appearance of the intestines. The abdominal incision was closed by interrupted atraumatic 4-0 silk sutures after placing the bowel back into the abdomen. Following an ischemia period of 60 minutes, a relaparotomy was performed and the microvascular clamp was removed to provide a reperfusion period of 60 minutes. Restoration of the mesenteric pulsations and color confirmed the reperfusion. The bowel was then placed back into the abdomen once more for the reperfusion period. At the end of 60 minutes, the animals were sacrificed. An infusion of 10mL/kg of lactated Ringer's solution was given through the internal jugular venous cath-

eter during I/R to avoid dehyration of the rats. Ileal tissue samples were provided and stored at −80°C for future assays of malondialdehyde (MDA) levels, myeloperoxidase (MPO) and superoxide dismutase (SOD) activities and nitric oxide (NO) levels. An ileal segment of 2.5 cm was fixed by 10% formaldehyde for histopathological analysis and an additional 2.5 cm segment was taken for wet-to-dry weight ratios. Samples of mesenteric lymph nodes, liver and spleen were obtained for measurement of bacterial translocation.

Tissue Preparation for Biochemical Analysis

Preparation of Homogenate

As soon as the experimental rats were sacrificed, their intestinal tissues were quickly removed and blotted on filter paper. Tissue samples were homogenized with 10 volumes of ice-cold 0.25 M sucrose and centrifuged at 14,000 g to measure the levels of MDA, activities of MPO and SOD and NO levels.

Biochemical Analysis

Measurement of MDA levels

MDA levels provided a measurement of the gastrointestinal system lipid peroxidation level in tissue samples. MDA was measured according to a previously established procedure (Ohkawa et al. 1979). Briefly, the reaction mixture contained 0.1 mL of tissue sample, 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 20% acetic acid and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid. The pH was adjusted to 3.5, the volume was increased to 4.0 mL with distilled water and 5.0 mL of a mixture of n-butanol and pyridine (15 : 1, v/v) was added. The mixture was shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm. MDA levels were expressed as nmol/mg protein. All chemicals were obtained from the Sigma Chemical Corporation (St. Louis, MO, USA) with the exception of NAC®, which was from Bilim Husnu Arsan Drug Company (Istanbul, Turkey).

Measurement of MPO activity

MPO activity was determined by a modification of the O-dianisidine method (Fridovich, 1983; Worthington 1972). The assay mixture was placed in a cuvette of 1 cm path length and contained 0.3 mL of 0.1 M phosphate buffer (pH 6,0), 0.3 mL of 0.01 M H_20_2, 0.5 mL of 0.02 M O-dianisidine (freshly prepared) in deionized water and 10 μL of supernatant in a volume of 3.0 mL. After the supernatant was added, the change in absorbance at 460 nm was observed for 10 min. All measurements were carried out in duplicate. One unit of MPO was defined as that giving an increase in absorbance of 0.001 per min and the specific activity was expressed as U/mg protein.

Measurement of SOD activity

Superoxide dismutase (SOD) activity in the tissue was measured according to the method described by Fridovich (Fridovich 1983). This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with p-iodonitrotetrazolium violet (INT) to form a red formazon dye. The dye was measured at 505 nm. The assay medium consisted of the 0.01 mol phosphate buffer and 3-cyclohexilamino-1-propane sulfonic acid (CAPS) buffer solution (50 mmol CAPS, 0.94 mM ethylenediaminetetraacetic acid [EDTA] and saturated NaOH and the pH was adjusted to10.2. SOD activity was expressed as U/mg protein.

Measurement of Nitrate (NO_3^-) and Nitrite (NO_2^-)

The total amount of intestinal tissue NO metabolic products [nitrate (NO_3^-) + nitrite (NO_2^-)] was assayed by a modification of the cadmium (Cd)-reduction method (Cortas and Wakid 1990). After samples were deproteinated with Somogyi's reagent, nitrate was reduced by copper (Roviezzo et al. 2007)-coated Cd in a pH 9.7 glycine buffer. Cadmium granules (0.35-0.75 g) were rinsed three times with deionized distilled water, and swirled in a 5 nmol/L $CuSO_4$ solution in a glycine-NaOH buffer for 3 minutes. The Cu-coated granules were drained and used within 10 minutes. Freshly- activated Cd granules were put in 400 μL tissue supernatants, and vigoruosly shaken. The samples were centrifuged at 4000 rpm for 4 minutes, and then 200 μL of Griess's reagent was added (1 : 1 mixture of 1% sulfanilamide in 5% H_2PO_4 and 0.1% N-(L-naphtyl) ethylenediamine in distilled water) to 200 μL supernatants. The absorbances of the samples were analyzed spectrophotometrically using a microplate reader and quantified automatically against a KNO_3 standard curve, and the results were expressed as μmol NO metabolic products/mg protein in the intestinal tissue samples.

Protein measurement

Bovine serum albumin was used for standart protein curve analysis. Protein assays were conducted using the method of Lowry et al (Lowry et al. 1951).

Histopathological evaluation

Histopathological Analysis

Small intestinal tissue specimens for histological examination were fixed in 10% formalin, embedded in paraffin wax on the oriented edge, and cut into 5 μm thick sections. All tissue sections were stained with hematoxylin and eosin for histological examination. A six-level ranking system was used to evaluate histopathological damage in experimental groups (Park et al. 1990).

Grade 0: Normal

Grade 1: Mucosal cell desquamation not accompanied with necrosis

Grade 2: Mucosal villus necrosis with protection of crypts

Grade 3: Mucosal villus necrosis included crypts

Grade 4: Necrosis of the inner part of muscularis layer or complete mucosal necrosis accompanied with thinning of muscularis layer

Grade 5: Transmural necrosis.

Measurement of Bacterial Translocation

Bacteriological Assessment

All bacteriological procedures were performed under strict sterile conditions. The tissue was cut into small pieces using a sterile blade. All specimens (liver, spleen, and mesenteric samples) were placed into 1 ml of Mueller-Hinton broth (Merck, Germany). The samples were homogenized by sterile glass rods, and then 100 μL of the each samples was inoculated into blood agar (Sigma-Aldrich, Germany) and eosin-methylene-blue (EMB) agar (Merck, Germany) for colony counts.

The cultures were incubated for 48 h and observed for the presence of growth under either aerobic or anaerobic conditions.

Colonization was expressed as the number of colony-forming units per gram of tissue homogenate (CFU g^{-1}).

All isolates were identified using VITEK 2 (bioMerieux, France). Results were calculated as the number of colony-forming

units per gram of tissue homogenate (CFU g^{-1}).

Statistical Analysis

Data were analyzed by a statistical software (SPSS for Windows 11.5, SPSS Inc, Chicago, IL, USA). All the results were expressed as mean ± SEM. Differences among the groups (ANOVA), and multiple comparisons between the groups were analysed by a post-hoc test (Tukey's Honestly Significant Difference [HSD] test) while the Newman-Keuls test was used for the microbiological results. Kruskal-Wallis test and Mann-Whitney *U* test were performed for the histopathological analysis. $p < 0.05$ was considered to be statistically significant.

Results

Effects of Resveratrol on Antioxidant Activity in Intestinal Tissue Subjected to I/R in Rats

MDA and NO levels and enzymatic activity (SOD and MPO) in the ileal tissue are shown in Fig. 1A-D. Reperfusion of the SMA led to a substantial increase in MDA, NO and MPO levels in I/R and I/R+vehicle groups ($p < 0.001$). However, resveratrol treatment significantly inhibited their increased levels ($p < 0.001$).

The SOD activity in I/R and I/R+vehicle groups was significantly lower than that in sham group ($p < 0.001$). On the other hand, the SOD activity of resveratrol-treated group was significantly lower than sham group, but it was higher than I/R and I/R+vehicle groups ($p < 0.05$, Fig. 1C).

Effects of resveratrol on histopathological values

In the sham-operated groups, the results of the histopathological examinations of the small intestinal epithelium and villi were normal. In the resveratrol-treated group IV, grade 1-2 damage was detected (Fig. 2A-D). The median intestinal mucosal injury score in I/R and I/R+vehicle groups was significantly higher than in sham and resveratrol-treated groups ($p < 0.001$ and $p < 0.05$, respectively). Although the median histopathological injury score of resveratrol-treated animals was significantly lower than that of the other I/R groups, it was significantly higher than sham group ($p < 0.05$, Fig. 3).

Bacterial Translocation (BT)

The incidence of BT differed between the groups I/R and I/R+vehicle in MLN, spleen and liver ($p < 0.001$). Nevertheless, the treatment with resveratrol reduced BT in MLN, spleen and liver, compared to the other I/R groups ($p < 0.01$). Except in MLN, BT did not differ between the resvevatrol-treated and the sham group. BT in the resveratrol group was higher than the sham group in MLNs ($p < 0.01$) (Table 1).

Discussion

The intestine is known to be a tissue that is highly sensitive to ischemic injury. However, Parks et al. suggested that most of the tissue damage produced by the regional hypotension occurs during reperfusion (Parks and Granger 1986). Further tissue destruction secondary to reperfusion is attributed to the production of reactive oxygen species (ROS) following reoxygenation. Oxidative stress plays a crucial role in intestinal I/R injury. Activated neutrophils induce tissue damage through the production and release of

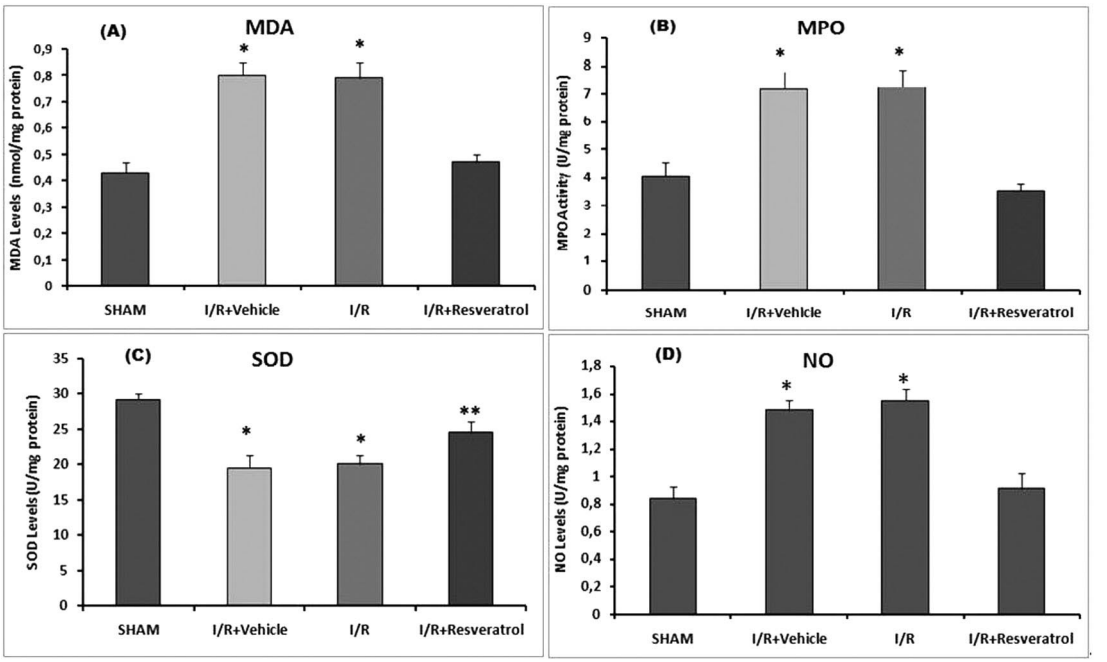

Fig. 1. Ileal tissue levels of malondialdehyde (MDA) and nitric oxide (NO) and enzymatic activity of superoxide dismutase (SOD) and myeloperoxidase (MPO). Mean ± SEM,
*: $p < 0.001$ compared with sham and I/R+Resveratrol groups, **: $p < 0.05$ compared with all the other groups, one-way ANOVA and Tukey's HSD tests were used.

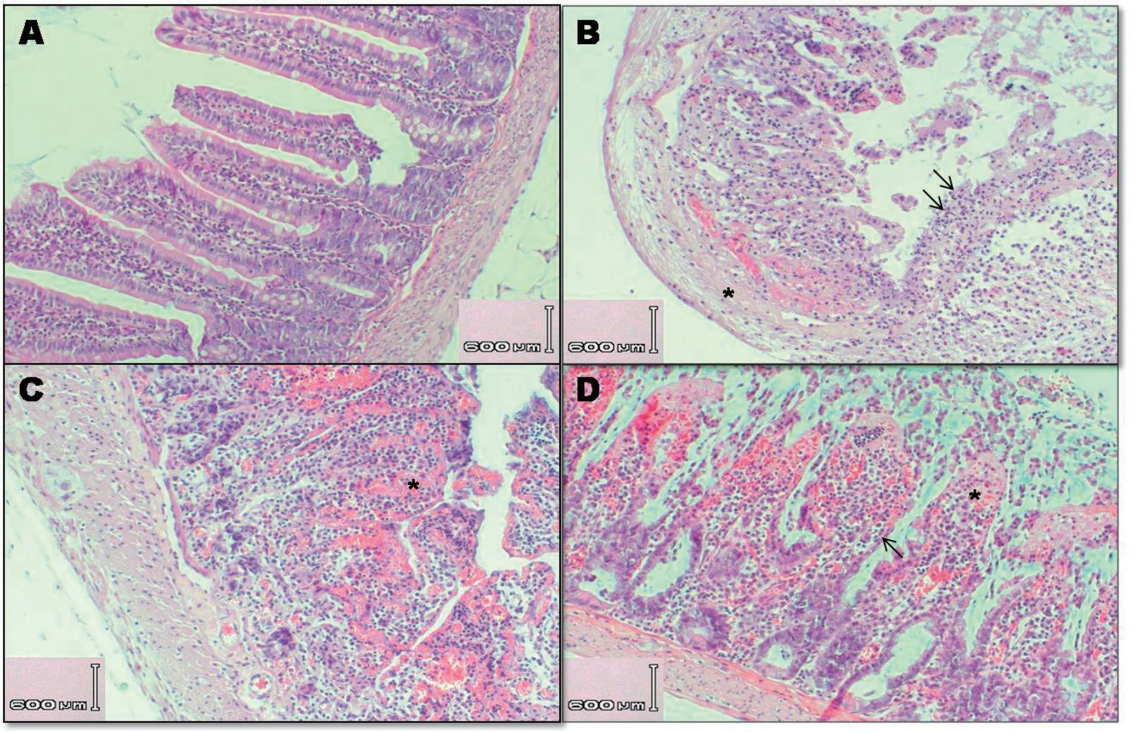

Fig. 2. Photomicrographs of ileal tissues stained by the hematoxylin and eosin
A: Sham: normal ileal tissue, grade 0
B: I/R: Complete mucosal necrosis (→) accompanied with thinning of muscularis layer (*), grade 4
C: I/R+vehicle: Mucosal villus necrosis (*) included crypts, grade 3
D: I/R+Resveratrol: Mucosal cell desquamation (→) with normal villi architecture (*), grade 1. Tissue sections were stained with hematoxylin and eosin (HE).

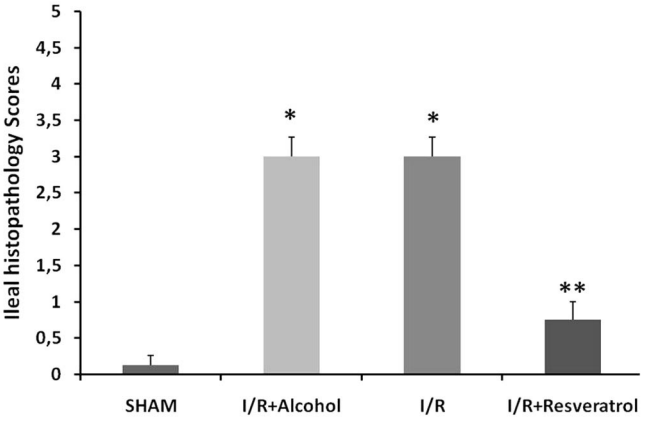

Fig. 3. Ileal histopathology scores
Shown are ileal histopathology scores (Mean ± SEM). *: $p < 0.001$ compared with Sham group, **: $p < 0.05$ compared with all the other groups. Mann-Whitney test was used.

ROS and cytotoxic proteins (proteases, MPO, lactoferrin) into the extracellular fluid which are part of the inflammatory cascades that trigger the radical-induced I/R injury (Guneli et al. 2007). Those cascades of cellular responses lead to inflammation, cellular death and organ failure. ROS intermediates and other oxygen radicals are shown to be associated with cellular injury and further necrosis due to peroxidation of cellular membrane lipids which in turn result in altered cellular function and structure. Excessive ROS accumulation may be associated with oxidative modification of cellular macromolecules such as lipids, proteins and nucleic acids, which has a deleterious potential (de la Lastra and Villegas 2007).

A growing body of research has concentrated on the efforts to prevent the synthesis or effects of ROS thus blocking their devastating consequences. Numerous experi-

Table 1. The average number of microorganisms as colony forming units (CFU) in the mesenteric lymph node (MLN), spleen and liver tissues.

	The average number of microorganisms (CFU)		
	MLN	Spleen	Liver
SHAM	0.0 ± 0.0	0.0 ± 0.0	149 ± 76
I/R	160 ± 87*	1053 ± 33*	1402 ± 425*
I/R+vehicle	147 ± 90*	953 ± 665*	1475 ± 498*
I/R+Resveratrol	37 ± 22[a]	0.0 ± 0.0	145 ± 5.2

*: $p < 0.001$ compared with Sham and I/R + Resveratrol groups, [a]: $p < 0.001$ compared with all the other groups one-way ANOVA and Newman-Keuls tests were used.

mental therapeutic measures involving ischemic preconditioning, anticomplement, anti-leucocyte and anti-inflammatory therapy with fluorocarbons, glutamine and glycine supplements, enteral feeding and antioxidants such as ascorbic acid, erythropoetin, pyrrolidine dithiocarbamate, resveratrol have been proposed as methods to avoid I/R injury (Lee et al. 2001; Arumugam et al. 2002a; 2002b; Mallick et al. 2004; Medeiros et al. 2006; Guneli et al. 2007; Higa et al. 2007; Teke et al. 2007). Recently, resveratrol has been investigated due to its biological attributes; namely antioxidant, anti-inflammatory, anti-aggregation, anti-atherogenic, estrogen-like growth-promoting effect, growth-inhibiting activity, immunomodulator, chemoprevention, anti-carcinogenic and anti-aging. Resveratrol is a natural phytoalexin which is an effective scavenger of hydroxyl and superoxide and exhibits a protective effect against lipid peroxidation in cell membranes and DNA damage caused by free radicals (Leonard et al. 2003). It presents a modulator role in regulating key enzymes including COX, iNOS, lipooxygenase, proteinkinase C (Ergun et al. 2007). It is a strong antioxidant and anti-inflammatory agent, inhibits lipid peroxidation and supresses overexpression of COX-2, iNOS expression and subsequent NO production in cultured cells (Tsai et al. 1999). Besides its antioxidant properties, it interferes with the release of inflammatory mediators from activated PMNLs and inhibits neutrophil infiltration, thus modulating the function of PMNLs.

Increased concentrations of MDA indicate the degree of lipid peroxidation verifying the oxidative damage in tissue thus it becomes a well-known marker of tissue injury (Karabulut et al. 2006). In the present study, a substantial increase in MDA levels in I/R and I/R+vehicle groups was noted. Resveratrol treatment was demonstrated to significantly inhibit their increased levels. This inhibition could be attributed to resveratrol's free radical scavenging activity as well as to its protective effect against lipid peroxidation in cellular membranes and DNA damage due to free radicals (Elmali et al. 2007).

MPO activity appears to a reliable index of polymorphonuclear leucocyte accumulation in the inflamed tissue and can be used to measure the extent of inflammation in intestinal tissue subjected to I/R injury. Intestinal MPO activity has been suggested to increase 3- fold after 60 minutes of intestinal ischemia and 8-9 fold after 60 minutes of reperfusion (Grisham et al. 1986). In our study, a significant increase in MPO activity in the I/R and I/R+vehicle groups was shown. Resveratrol treatment was demonstrated to significantly inhibit increased MPO activity. Diminished MPO activity in the resveratrol-treated group demonstrated that this anti-inflammatory agent prevented the activation and infiltration of PMNLs in the post-ischemic intestine.

In order to protect tissues against the devastating consequences of ROS, all cells present defense mechanisms that involve SOD, catalase, gluthatione reductase and glutathione peroxidase (de la Lastra and Villegas 2007). A growing body of data based on previous studies pointed out that antioxidant agents such as resveratrol might induce an increase in glutathione levels in a concentration dependent manner in human lymphocytes activated by H_2O_2 (Olas et al. 2004). Resveratrol also increased the amounts of several antioxidant enzymes involving gluthathione peroxidase, glutathione S-transferase and glutathione reductase (Yen et al. 2003). In our study, SOD activity in I/R and I/R+vehicle groups was significantly lower than in the sham group. The SOD activity of the resveratrol-treated group was significantly lower than the sham group but was higher than the I/R and I/R+vehicle groups. The higher SOD activity achieved in the resveratrol treated group in comparison with the I/R and I/R+vehicle groups seems to be in accordance with literature data (Sebai et al. 2008; Balakrishna et al. 2009).

NO plays a major role in maintaining mucosal integrity and is a significant mediator of vascular tone (Karabulut et al. 2006). Its protective or detrimental effect in intestinal I/R injury still remains undetermined. Excessive NO production has been attributed to inducible NO synthase which is not present under normal conditions but can be induced as a response to systemic infammation such as I/R injury. Endothelial nitric oxide synthase (eNOS) derived NO has been suggested to be protective at the onset of injury (Roviezzo et al. 2007). However, eNOS has been shown to be dysfunctional during oxidative stress. Superoxide anions may react with NO released by eNOS and then turn into the cytotoxic oxidant peroxynitrite (Munzel et al. 2005). The altered function of eNOS may cause intestinal I/R injury. In the present study, NO levels were increased in I/R and I/R+vehicle groups while resveratrol treatment significantly inhibited increased NO levels.

Different from our findings, Hassan-Khabbar et al in a recent publication (Hassan-Khabbar et al. 2008) showed that resveratrol in a low dose (0.2 mg/kg bw) has antioxidant properties and that in contrary a dose of 20 mg/kg shows a prooxidant effect and exacerbates the injury caused by ischemia/reperfusion in liver. But the route of administration in the above mentioned paper is intravenous.

Additionally, the different dosages, the application methods and reperfusion time chosen by Hassan-Khabbar et al could give rise to the current discrepancy. Furthermore, the dosage of resveratrol in our study is supported by several other studies (Karabulut et al. 2006; Elmali et al. 2007; Juan et al. 2009).

I/R injury is shown to resemble an acute inflammatory process. Neutrophil infiltration, segmental necrosis, shortening of villi, loss of intestinal villi, nuclear centralization and dimensional changes have been demonstrated as the histopathological findings of I/R injury (Teke et al. 2007). In our study, the median intestinal mucosal injury score in resveratrol treated group was significantly lower in comparison with I/R and I/R+vehicle groups thus demonstrating that resveratrol can minimize the tissue injury as an antioxidant, antiinflammatory and antileucocyte agent.

A normally functioning intestinal mucosa prevents the transfer of enteric bacteria and endotoxins into other organs and blood circulation. Gut barrier dysfunction is considered to be the major cause leading to infectious complications when patients suffer from impaired intestinal blood supply and insufficient nutrient support. Additionally, local production of ROS and cytokines which induce endothelial response and attract the circulating neutrophils into the region of local injury leads to a complex interaction between intestinal wall integrity and bacterial translocation. Overproduction of NO may impair the intestinal integrity thus resulting in the failure of the gut barrier. The reaction of NO with superoxide radicals in order to produce peroxynitrite (a potent oxidant) triggers apoptosis in enterocytes. Shedding of apoptotic enterocytes leads to space formation between cells through which BT can occur (Sorrells et al. 1996; Mishima et al. 1997; Ozturk et al. 2002). As we also demonstrated in the present study, resveratrol diminishes lipid peroxidation and thus prevents massive synthesis of oxidants and mucosal injury and blocks the cascade of events causing BT. Additionally, surgery is known to cause a transient immunosuppression leading to a susceptibility to infection. This could be the reason why we detect infection in the liver of sham group.

In conclusion, the antioxidant and anti-inflammatory properties of resveratrol may be beneficial in order to attenuate I/R injury. More rigorously designed future studies are required to precisely investigate the role of resveratrol regarding the prevention of intestinal I/R injury and to justify its potential use in clinical practice.

References

Arumugam, T.V., Shiels, I.A., Margolin, S.B., Taylor, S.M. & Brown, L. (2002a) Pirfenidone attenuates ischaemia-reperfusion injury in the rat small intestine. *Clin. Exp. Pharmacol. Physiol.,* **29**, 996-1000.

Arumugam, T.V., Shiels, I.A., Woodruff, T.M., Reid, R.C., Fairlie, D.P. & Taylor, S.M. (2002b) Protective effect of a new C5a receptor antagonist against ischemia-reperfusion injury in the rat small intestine. *J. Surg. Res.,* **103**, 260-267.

Balakrishna, S., Lomnicki, S., McAvey, K.M., Cole, R.B., Dellinger, B. & Cormier, S.A. (2009) Environmentally persistent free radicals amplify ultrafine particle mediated cellular oxidative stress and cytotoxicity. *Part Fibre Toxicol.,* **6**, 11.

Chen, C.F., Hsueh, C.W., Tang, T.S., Wang, D., Shen, C.Y. & Pei, J.S. (2007) Reperfusion liver injury-induced superoxide dismutase and catalase expressions and the protective effects of N-acetyl cysteine. *Transplant. Proc.,* **39**, 858-860.

Cortas, N.K. & Wakid, N.W. (1990) Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. *Clin. Chem.,* **36**, 1440-1443.

de la Lastra, C.A. & Villegas, I. (2007) Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications. *Biochem. Soc. Trans.,* **35**, 1156-1160.

Deitch, E.A., Feketeova, E., Lu, Q., Zaets, S., Berezina, T.L., Machiedo, G.W., Hauser, C.J., Livingston, D.H. & Xu, D.Z. (2008) Resistance of the female, as opposed to the male, intestine to I/R-mediated injury is associated with increased resistance to gut-induced distant organ injury. *Shock*, **29**, 78-83.

Dokmeci, D., Inan, M., Basaran, U.N., Yalcin, O., Aydogdu, N., Turan, F.N. & Uz, Y.H., (2007) Protective effect of L-carnitine on testicular ischaemia-reperfusion injury in rats. *Cell. Biochem. Funct.,* **25**, 611-618.

Elmali, N., Esenkaya, I., Karadag, N., Tas, F. & Elmali, N. (2007) Effects of resveratrol on skeletal muscle in ischemia-reperfusion injury. *Ulus. Travma Acil Cerrahi Derg.*,**13**, 274-280.

Ergun, O., Ergun, G., Oktem, G., Selvi, N., Dogan, H., Tuncyurek, M., Saydam, G. & Erdener, A. (2007) Enteral resveratrol supplementation attenuates intestinal epithelial inducible nitric oxide synthase activity and mucosal damage in experimental necrotizing enterocolitis. *J. Pediatr. Surg.*, **42**, 1687-1694.

Feng, Y.H., Zhou, W.L., Wu, Q.L., Li, X.Y., Zhao, W.M. & Zou, J.P. (2002) Low dose of resveratrol enhanced immune response of mice. *Acta Pharmacol. Sin.*, **23**, 893-897.

Fridovich, I. (1983) Superoxide radical: an endogenous toxicant. *Annu. Rev. Pharmacol. Toxicol.,* **23**, 239-257.

Gehm, B.D., McAndrews, J.M., Chien, P.Y. & Jameson, J.L. (1997) Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. *Proc. Natl. Acad. Sci. USA.*, **94**, 14138-14143.

Giakoustidis, D., Papageorgiou, G., Iliadis, S., Giakoustidis, A., Kostopoulou, E., Kontos, N., Botsoglou, E., Tsantilas, D., Papanikolaou, V. & Takoudas, D. (2006) The protective effect of alpha-tocopherol and GdCl3 against hepatic ischemia/ reperfusion injury. *Surg. Today,* **36**, 450-456.

Grisham, M.B., Hernandez, L.A. & Granger, D.N. (1986) Xanthine oxidase and neutrophil infiltration in intestinal ischemia. *Am. J. Physiol.,* **251**, G567-G574.

Guneli, E., Cavdar, Z., Islekel, H., Sarioglu, S., Erbayraktar, S., Kiray, M., Sokmen, S., Yilmaz, O. & Gokmen, N. (2007) Erythropoietin protects the intestine against ischemia/ reperfusion injury in rats. *Mol. Med.*, **13**, 509-517.

Hassan-Khabbar, S., Cottart, C.H., Wendum, D., Vibert, F., Clot, J.P., Savouret, J.F., Conti, M. & Nivet-Antoine, V. (2008) Postischemic treatment by trans-resveratrol in rat liver ischemia-reperfusion: a possible strategy in liver surgery. *Liver Transpl.,* **14**, 451-459.

Higa, O.H., Parra, E.R., Ab'Saber, A.M., Farhat, C., Higa, R. & Capelozzi, V.L. (2007) Protective effects of ascorbic acid pretreatment in a rat model of intestinal ischemia-reperfusion injury: a histomorphometric study. *Clinics,* **62**, 315-320.

Homma, H., Hoy, E., Xu, D.Z., Lu, Q., Feinman, R. & Deitch, E.A. (2005) The female intestine is more resistant than the male intestine to gut injury and inflammation when subjected to conditions associated with shock states. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **288**, G466-G472.

Juan, M.E., Maijó, M. & Planas, J.M. (2009) Quantification of trans-resveratrol and its metabolites in rat plasma and tissues by HPLC. *J. Pharm. Biomed. Anal.,* Apr 5. [Epub ahead of

print]
Karabulut, A.B., Kirimlioglu, V., Kirimlioglu, H., Yilmaz, S., Isik, B. & Isikgil, O. (2006) Protective effects of resveratrol on spleen and ileum in rats subjected to ischemia-reperfusion. *Transplant. Proc.*, **38**, 375-377.

Klinge, C.M., Blankenship, K.A., Risinger, K.E., Bhatnagar, S., Noisin, E.L., Sumanasekera, W.K., Zhao, L., Brey, D.M. & Keynton, R.S. (2005) Resveratrol and estradiol rapidly activate MAPK signaling through estrogen receptors alpha and beta in endothelial cells. *J. Biol. Chem.*, **280**, 7460-7468.

Lee, M.A., McCauley, R.D., Kong, S.E. & Hall, J.C. (2001) Pretreatment with glycine reduces the severity of warm intestinal ischemic-reperfusion injury in the rat. *Ann. Plast. Surg.,* **46**, 320-326.

Leonard, S.S., Xia, C., Jiang, B.H., Stinefelt, B., Klandorf, H., Harris, G.K. & Shi, X. (2003) Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. *Biochem. Biophys. Res. Commun.*, **309**, 1017-1026.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,***193**, 265-275

Mallick, I.H., Yang, W., Winslet, M.C. & Seifalian, A.M. (2004) Ischemia-reperfusion injury of the intestine and protective strategies against injury. *Dig. Dis. Sci.*, **49**, 1359-1377.

Martin, A.R., Villegas, I., La Casa, C. & de la Lastra, C.A. (2004) Resveratrol, a polyphenol found in grapes, suppresses oxidative damage and stimulates apoptosis during early colonic inflammation in rats. *Biochem. Pharmacol.*, **67**, 1399-1410.

Medeiros, A.C., Chacon, D.A., Sales, V.S., Egito, E.S., Brandao-Neto, J., Pinheiro, L.A. & Carvalho, M.R. (2006) Glucan and glutamine reduce bacterial translocation in rats subjected to intestinal ischemia-reperfusion. *J. Invest. Surg.,* **19**, 39-46.

Mishima, S., Xu, D., Lu, Q. & Deitch, E.A. (1997) Bacterial translocation is inhibited in inducible nitric oxide synthase knockout mice after endotoxin challenge but not in a model of bacterial overgrowth. *Arch. Surg.,* **132**, 1190-1195.

Mojzis, J., Hviscova, K., Germanova, D., Bukovicova, D. & Mirossay, L. (2001) Protective effect of quercetin on ischemia/reperfusion-induced gastric mucosal injury in rats. *Physiol. Res.*, **50**, 501-506.

Munzel, T., Daiber, A., Ullrich, V. & Mulsch, A. (2005) Vascular consequences of endothelial nitric oxide synthase uncoupling for the activity and expression of the soluble guanylyl cyclase and the cGMP-dependent protein kinase. *Arterioscler. Thromb. Vasc. Biol.*, **25**, 1551-1557.

Offner, P.J., Moore, E.E. & Biffl, W.L. (1999) Male gender is a risk factor for major infections after surgery. *Arch. Surg.,* **134**, 935-940.

Ohkawa, H., Ohishi, N. & Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, **95**, 351-358.

Olas, B., Wachowicz, B., Bald, E. & Glowacki, R. (2004) The protective effects of resveratrol against changes in blood platelet thiols induced by platinum compounds. *J. Physiol. Pharmacol.*, **55**, 467-476.

Ozturk, C., Avlan, D., Cinel, I., Cinel, L., Unlu, A., Camdeviren, H., Atik, U. & Oral, U. (2002) Selenium pretreatment prevents bacterial translocation in rat intestinal ischemia/reperfusion model. *Pharmacol. Res.*, **46**, 171-175.

Park, P.O., Haglund, U., Bulkley, G.B. & Falt, K. (1990) The sequence of development of intestinal tissue injury after strangulation ischemia and reperfusion. *Surgery*, **107**, 574-580.

Parks, D.A. & Granger, D.N. (1986) Contributions of ischemia and reperfusion to mucosal lesion formation. *Am. J. Physiol.*, **250**, G749-G753.

Roviezzo, F., Cuzzocrea, S., Di Lorenzo, A., Brancaleone, V., Mazzon, E., Di Paola, R., Bucci, M. & Cirino, G. (2007) Protective role of PI3-kinase-Akt-eNOS signalling pathway in intestinal injury associated with splanchnic artery occlusion shock. *Br. J. Pharmacol.*, **151**, 377-383.

Sayan, H., Ozacmak, V.H., Altaner, S., Aktas, R.G. & Arslan, S.O. (2008) Protective effects of L-arginine on rat terminal ileum subjected to ischemia/reperfusion. *J. Pediatr. Gastroenterol. Nutr.*, **46**, 29-35.

Sebai, H., Ben-Attia, M., Sani, M., Aouani, E. & Ghanem-Boughanmi, N. (2008) Protective effect of resveratrol on acute endotoxemia-induced nephrotoxicity in rat through nitric oxide independent mechanism. *Free Radic. Res.*, **42**, 913-920.

Sorrells, D.L., Friend, C., Koltuksuz, U., Courcoulas, A., Boyle, P., Garrett, M., Watkins, S., Rowe, M.I. & Ford, H.R. (1996) Inhibition of nitric oxide with aminoguanidine reduces bacterial translocation after endotoxin challenge in vivo. *Arch. Surg.*, **131**, 1155-1163.

Soylemez, S., Gurdal, H., Sepici, A. & Akar, F. (2008) The effect of long-term resveratrol treatment on relaxation to estrogen in aortae from male and female rats: role of nitric oxide and superoxide. *Vascul. Pharmacol.*, **49**, 97-105.

Teke, Z., Kabay, B., Aytekin, F.O., Yenisey, C., Demirkan, N.C., Sacar, M., Erdem, E. & Ozden, A. (2007) Pyrrolidine dithiocarbamate prevents 60 minutes of warm mesenteric ischemia/reperfusion injury in rats. *Am. J. Surg.*, **194**, 255-262.

Tsai, S.H., Lin-Shiau, S.Y. & Lin, J.K. (1999) Suppression of nitric oxide synthase and the down-regulation of the activation of NFkappaB in macrophages by resveratrol. *Br. J. Pharmacol.* **126**, 673-680.

Ustundag, B., Kazez, A., Demirbag, M., Canatan, H., Halifeoglu, I. & Ozercan, I. H. (2000) Protective effect of melatonin on antioxidative system in experimental ischemia-reperfusion of rat small intestine. *Cell. Physiol. Biochem.*, **10**, 229-236.

Worthington, E.M. (1972) Freehold (NJ):, Worthington Biochemical Corporation, 100-101 p.

Yamaguchi, Y., Akizuki, E., Ichiguchi, O., Matsumura, F., Goto, M., Miyanari, N., Mori, K., Yamada, S. & Ogawa, M. (1997) Neutrophil elastase inhibitor reduces neutrophil chemoattractant production after ischemia-reperfusion in rat liver. *Gastroenterol.,* **112**, 551-560.

Yen, G.C., Duh, P.D. & Lin, C.W. (2003) Effects of resveratrol and 4-hexylresorcinol on hydrogen peroxide-induced oxidative DNA damage in human lymphocytes. *Free Radic. Res.*, **37**, 509-514.

Yoshida, Y., Shioi, T. & Izumi, T. (2007) Resveratrol ameliorates experimental autoimmune myocardilis. *Circ. J.,* **71**, 397-404.