

## RESEARCH ARTICLE

# Preventive Effects of Resveratrol against Azoxymethane Induced Damage in Rat Liver

Simay Gürocak<sup>1\*</sup>, Ercan Karabulut<sup>2</sup>, Neşe Karadağ<sup>3</sup>, Dincer Ozgor<sup>4</sup>, Neslihan Ozkeles<sup>1</sup>, Aysun Bay Karabulut<sup>5</sup>

### Abstract

**Background:** In recent years, due to modern lifestyles and exposure to chemical carcinogens, cancer cases are steadily increasing. From this standpoint, azoxymethane (AOM), a chemical carcinogen which causes de novo liver damage, and resveratrol, which is an antioxidant found in foods and protects against oxidative stress damage, are of interest. We here aimed to evaluate whether resveratrol could protect the liver tissues from the effects of AOM. **Materials and Methods:** The study was conducted in 4 groups, each consisting of seven rats, the first receiving only AOM (2 times per week, 5 mg/kg), group 2 AOM and resveratrol (2 times a week, 20 mg/kg), group 3 assessed only as a control and group 4 administered only resveratrol. At the end of the seventh week, the rats were sacrificed. Rat liver MDA, NO, GSH levels were analyzed biochemically, as well as the tissues being evaluated histopathologically. **Results:** MDA and NO increased in AOM group as signs of increased oxidative stress. The group concomitantly administered resveratrol was been found to be significantly decreased in MDA and NO levels and increased in GSH activity. However, there were no significant findings on histopathological evaluation. **Conclusions:** In the light of these results, resveratrol appears to exert protective effect on oxidative stress in the liver tissue due to deleterious effects of chemical carcinogens.

**Keywords:** Oxidative stress - azoxymethane - resveratrol - liver damage

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### Introduction

Liver diseases due to viral, chemical substances and other factors are a life-threatening condition resulting in a 50-90% mortality rate in the absence of liver transplantation (Deng et al., 2012). Loss of liver function has detrimental effects on multiple organs, both due to the release of toxic factors from the injured liver and to the loss of key hepatic detoxifying pathways. Azoxymethane (AOM) is a potent carcinogen for the liver (Chastre et al., 2012). In experimental animal studies also could be used to make hepatotoxicity (Khurana et al., 2010). But especially AOM has also been used in the investigation of the preventive effect of many vegetables and fruits on colon cancer (Robertis et al., 2011). Also resveratrol is present in foods such as peanuts, grapes and wine (Giovinazzo et al., 2012).

Resveratrol (3,4,5- trihydroxy-trans-stilbene) is a natural polyphenol (Giovinazzo et al., 2012). It has a potent antioxidant (Khan et al., 2013) and anti-inflammatory (Pallarès et al., 2012) properties, which can play a crucial role against carcinogenesis (Bishayee et al., 2010).

Many experimental studies are conducted to support a key role of reactive oxygen species (ROS) in the numerous mechanisms of seemingly unrelated hepatopathies (Zigdon et al., 2013). This species are powerful oxidizing agents. They are capable of damaging DNA. Increasing amounts of reactive species may promote the development of malignancy.

The aim of the present study was to investigate the protective effects of resveratrol on oxidative stress in the azoxymethane (AOM) induced hepatotoxic effect. The effects of resveratrol on AOM-induced hepatotoxicity were evaluated in relation to liver pathology, hepatic glutathione, MDA and NO levels in rats with toxic liver injury.

### Materials and Methods

Six months old female rats (200 gr) (sprague downey) were used in the study. Rats were obtained from Inonu University, Animal Laboratory, Malatya Turkey. The study was approved by the Inonu University Ethics Committee. Rats were housed with free access to standard laboratory chow and water under constant conditions of temperature,

<sup>1</sup>Department of Radiation Oncology, <sup>3</sup>Department of Patology, <sup>4</sup>Department of General Surgery, <sup>5</sup>Department of Biochemistry, Inonu University School of Medicine, Malatya, <sup>2</sup>Department of Pharmacology, Çukurova University School of Medicine, Adana, Turkey  
\*For correspondence: [simay.gurocak@inonu.edu.tr](mailto:simay.gurocak@inonu.edu.tr)

humidity and light cycling. Rats were allocated randomly into 4 groups, each consisting of seven (n:7) animals groups. Group I was administered with AOM (Twice a week for 7 weeks, 5 mg/kg subcutaneous azoximethan).

Group II was administered AOM and resveratrol (Twice a week for 7 weeks, 5 mg/kg subcutaneous azoximethan, 20 mg/kg of resveratrol). Group III served as control. Group IV was administered resveratrol (Twice a week for 7 weeks, 20 mg/kg of resveratrol)

Intraperitoneal injection of the AOM (Sigma- Aldrich, St- Louis, CO) dissolved in 100 µl saline. Control animals received an equivalent volume of saline.

All of the tissues were homogenized in ice-cold 140 mM KCl at 16,000 rpm for 2 min using a homogenizer (Ultra-Turrax T 25 basic homogenizer, IKA, Germany). The MDA levels were measured at this homogenate stage. The homogenate was then centrifuged at 5000× g for 60 min to remove debris. All preparation procedures were performed at 4°C.

Livers were fixed overnight by immersion in 10% buffered formalin. Parafin- embedded specimens were prepared and 6 µm sections and stained with hematoxylin-eosin coloration. Hepatic histopathological changes were assessed under the light microscope by pathologist uninformed about the groups. For assessment of mitotic figures, the presence of pleomorphism, necrosis, anointment, apoptosis.

#### Determination of total hepatic glutathione content

In the determination of glutathione, the method developed by Fairbanks and Klee, which is based upon the reaction of sulfhydryl groups with Elman marker, was used (Fairbanks et al., 1986). Sample absorbances were multiplied by the factor obtained from standard graphic and GSH activity was calculated as µmol /L. Sample absorbances were multiplied by dilution factors and the factor obtained from standard graphics and the amount of MDA was calculated as nmol /L.

#### Determination of total MDA content

MDA in liver homogenate was determined by the method of Uchiyama and Mihara (Uchiyama et al., 1978). 15 of a 1% phosphoric acid solution were added to supernatant of the tissue; pipetted into a tube together with 0,6 % thiobarbituric acid solution. The mixture was heated in boiling water for 45 min. After cooling, the colour was extracted into 4 ml of n-butanol. The absorbance was measured in a spectrophotometer (Ultraspec Plus, Pharmacia LKB Biochrom, UK) at wavelengths of 535 and 525 nm. The difference in reading was used to calculate the thiobarbituric acid-reactive substances of lipid peroxidation. A standard curve was prepared from a standard solution of 1,1,3,3-tetramethoxypropane, results are given as nmol per g.

#### Determination of total NO content

NO amount was determined by Cortas and Wakid method, which is based on the spectrophometric measurement of the coloured complex produced by the interaction of NO formed by NOS activity in the environment with Griess reactive (Cortas et al., 1990).

**Table 1. Values of Biochemical Markers of Oxidative Stress**

	Groups			
	AOM	ARES	C	RES
GSH*	1.31±0.37 <sup>b,c</sup>	2.03±0.48 <sup>d</sup>	3.21±0.22	2.837±0.397
MDA*	87.05±7.79 <sup>a,b,c</sup>	55.14±7.06 <sup>d,e</sup>	20.51±1.45	23.483±3.490
NO*	14.87±1.57 <sup>b,c</sup>	8.76±0.98 <sup>e</sup>	2.13±0.38 <sup>f</sup>	6.790±0.890

\*P Value=0.008. <sup>a</sup>AOM between ARES groups difference were significant (p<0.008); <sup>b</sup>AOM between K groups difference were significant (p<0.008); <sup>c</sup>AOM between RES groups difference were significant (p<0.008); <sup>d</sup>ARES between K groups difference were significant (p<0.008); <sup>e</sup>ARES between RES groups difference were significant (p<0.008); <sup>f</sup>K between RES groups difference were significant (p<0.008)

#### Statistical analysis

The statistical package for social sciences (SPSS) version 15,0 was used for statistical analysis. Compliance with the normal distribution of the data was performed by Shapiro wilk test. In terms of the variables MDA and GSH was used to compare groups by Kruskal Wallis test. Identified significant differences in multiple comparisons test was used for Bonferoni corrected mann- whitney U test.

## Results

The results for MDA, GSH, and NO levels in the four groups are listed in Table 1. AOM group and the between C and RES groups difference were significant on the GSH levels. GSH levels were significantly different between the C and ARES group. GSH level was decreased in AOM group compared to C group, increased in the ARES group but not statistically significant. The GSH in resveratrol-treated rats were significantly higher than in the AOM-only group (P<0.008).

MDA was evaluated. AOM group and the between RES, C and ARES groups difference were significant. MDA was significantly difference between C and RES. MDA was significantly higher than those of in the AOM-only group (P<0.008).

NO was evaluated. AOM group and the between C and RES groups difference were significant. ARES between RES groups difference were significant, too. C between RES group difference were significant (P<0.008).

## Discussion

Liver damage induced of chemical cancerogen agents can be threatening of life. Azoxymethane (AOM) is an effective chemical cancerogen used for hepatic chemical damage in animal models (Khurana et al., 2010). Also it has been used in animal studies evaluating efficacy of preventative treatment for azoxymethane-induced carcinogenesis (Liao et al., 2013). Due to the results of our work, rat liver is negatively effected by AOM. Parellel to our study, Thrane et al. (2012) reported a model in which, AOM is associated with liver injury leading up to Fulminant Hepatic Failure (FMF) (Thrane et al., 2012). The other toxins with the most widely used experimental studies are acetaminophen, carbontetrachloride and galactosamine. They are not quotable, produce incoherent toxicity, fail to generate a clinically significant lesion and require supportive therapy. Carbontetrachloride have

the inconsistent results between experiments and across species (Cengiz et al., 2013, Wang et al., 2013). Lee et al. (2012) reported that resveratrol treatment partially prevented carbon tetrachloride-induced acute liver damage (Lee et al., 2012). Also in our study resveratrol reduced the oxidative stress created by the AOM.

In vitro effect of resveratrol against oxidative injury of human coronary artery endothelial cells. Resveratrol is used especially for the hydroxyl radical-induced lipid peroxidation with the aim of preventing in living cells (Bishayee et al., 2010). Between in vitro and in live cells are proved that resveratrol not only make the direct scavenging effect in vitro but also may the disclose indirect antioxidant effect by an increase in the expression of intracellular antioxidant enzymes (Sayın et al., 2011). However this effect of resveratrol is not clear, may be connected to antioxidant and free-radical scavenging effect.

Resveratrol was shown to protect hepatic antioxidant systems against ischemia reperfusion-induced oxidative stress, protect from oxidative DNA damage caused by hydrogen peroxide and peroxynitrite (Sayın et al., 2011; Hamburger et al., 2013)

Malondialdehyde (MDA), that is a lipid peroxidation end-product, connect to the cross and cause to modification on enzyme activity and ion permeability (Lamichhane et al., 2012). Lipid peroxidation and oxidative stress are evaluated by MDA level. In the our study, the levels of MDA were significantly higher than in the AOM group. In the resveratrol- treated rats, levels of MDA were significantly lower than in the AOM group.

Glutathione (GSH) is an antioxidant, protecting damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. The ratio of reduced glutathione to oxidized glutathione within cells is used as a measure of cellular toxicity. GSH plays main role in the protection against oxidative stress, as a cofactor of glutathione peroxidases attend in the elimination of lipid hydroperoxides and hydrogen peroxide. Glutathione also stimulated the transcription of many genes involved in the cell cycle, apoptosis and differentiation.

In the current study, levels of GSH in the AOM group were significantly lower resveratrol- treated group. These results are concordant with previous studies. Increased GSH levels in the resveratrol treated group may be related to its free radical cleaning and antioxidant effect.

Nitric Oxide (NO) is a free radical generated from L- arginine by nitric oxide synthase in living organisms. Chronic expression of NO is related with various carcinomas and inflammatory conditions including Type-1 diabetes, arthritis and ulcerative colitis. In parallel; The levels of NO in resveratrol treated rats were significantly lower than those of the AOM group. In our study NO levels significantly increased in resveratrol group.

Increased formation of ROS may initiate the development of malignancy, and the 'normal' rates of ROS may account for the increased risk of cancer development in the aged.

Consequently resveratrol may be useful for protective effect of oxidative stress by induction as a chemical

carcinogen in the liver. In this study, we observed that resveratrol decreased the oxidative damage of chemical carcinogen AOM. Nevertheless, more investigations are required to conclude the anticarcinogen and protected effect of resvaratrol in clinical and experimental models.

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