

## Mega Doses of Resveratrol Enhance Oxidative and Nitrosative Stress and Accelerate Inflammations in Glycerol-Rat Model

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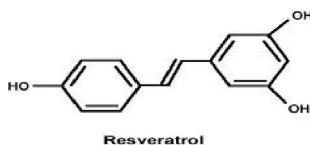
**Abstract:** Resveratrol is a naturally occurring polyphenol that possesses some antioxidant and anti-inflammatory properties. It can cause a significant effect on the inflammatory process seen in glycerol-induced renal injury. Renal injury associated with an increase of oxidative stress has been reported in the clinical and experimental use of some therapeutic agents (such as glycerol). The present study was designed to investigate the effect of supplementing different doses of resveratrol on overcoming acute renal failure induced by glycerol injection in rats, and the possible mechanism by which resveratrol exerts its protective effect in ameliorating glycerol-nephrotoxicity. Five groups of rats were used: a normal control group treated with normal saline solution (10 ml/kg) and four groups injected intramuscularly with 1:1 (v/v) solution of glycerol and saline (10 ml/kg), then three groups of them were dosed orally once daily with 200, 1000, 3000 mg/kg, b.wt/day resveratrol in DMSO for 28 days by gavage. Results of the present study revealed that, glycerol injection deteriorated renal function as evidenced by significant increase in hematological measurements, serum urea, creatinine,  $\text{Na}^+$  and  $\text{K}^+$  levels. In addition, glycerol treated rats exhibited a significant increase in serum and renal malondialdehyde (MDA) and nitrite / nitrate ( $\text{NO}_x$ ) levels as well as renal myeloperoxidase (MPO), with a significant decrease in sodium, potassium adenosine triphosphate activity ( $\text{Na}^+$ ,  $\text{K}^+$ -ATPase) in renal tissues. Concurrent administration of resveratrol in doses 200, 1000 mg/kg, bwt/day with glycerol significantly reduced the increased MDA and  $\text{NO}_x$  and MPO in renal tissue, restored the altered antioxidant enzyme activities and hematological parameters, and normalized activity of ( $\text{Na}^+$ ,  $\text{K}^+$ -ATPase) in renal tissues. Administration of resveratrol at a dose of 3000 mg/kg, bwt/day, increase nephrotoxicity and inflammation as assessed by different hematological and enzymatic parameters. These findings imply the potential usefulness of resveratrol in low and medium doses as a protective agent to guard against renal injury induced by glycerol injection. Generally, resveratrol can have a significant effect on the inflammatory process seen in glycerol-induced renal injury.

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### 1. Introduction

Resveratrol (3,5,4= trans-trihydroxystilbene), a natural phytoalexin present in grapes, peanuts, mulberries and red wine, has various pharmacological effects, including anti-inflammatory properties, modulation of lipid metabolism and prevention of cancer. Its anti-inflammatory effect is related to inhibiting oxidation, leukocyte priming, and expression of inflammatory mediators (**Savouret and Quesne, 2002**). It is one of the ingredients in the traditional Asian medicine Ko-jo-kon for treatment of fungal, inflammatory, hypertensive, allergic, and lipid diseases (**Docherty et al., 2003**).



**Figure (1): Structure of Resveratrol**

Resveratrol has been found to prevent and cure

cardiovascular diseases and improve microcirculatory disorders by protecting the vascular endothelium, as well as inhibiting platelet aggregation and inhibiting cellular events associated with tumor initiation (**Aggarwal et al., 2004**). In addition, resveratrol has been recently proposed as a potential antioxidant that could obviously inhibit free radical generation in red cell membrane, heart, liver, brain, kidney which reflected beneficial effects to prevent functional injury and improve nerve function and promote restoration after trauma (**Rahman et al., 2006**). For these reasons, resveratrol is an attractive pharmaceutical candidate.

Glycerol is a chemical compound with three hydrophilic alcohol hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. Glycerol is a central component of the structure of many lipids and is a precursor for the synthesis of triacylglycerols, phospholipids, glucose, protein, pyruvate and proteins (**Cetin et al., 2007**). Kidneys are dynamic organs and represent the major control system maintaining the body

haemostasis; they are affected by many chemicals and drugs. Kidney is highly susceptible to toxicants for two reasons. First, a high volume of blood flow through it, second, it filters large amounts of toxins which can be concentrated in the kidney tubules. Nephrotoxicity is the toxicity to the kidney. It can result in systemic toxicity causing decreased ability to excrete body wastes, inability to maintain body fluids, and electrolyte balance (Ajith *et al.*, 2007).

Renal injury associated with an increase of oxidative stress has been reported in the clinical and experimental use of some therapeutic agents (such as glycerol) and several anti-inflammatory drugs (Hickey *et al.*, 2001). Glycerol-induced acute renal failure (ARF) is characterized by myoglobinuria, tubular necrosis and enhanced renal vasoconstriction. Although the mechanism by which glycerol induces ARF is not clear, increased free radical generation has always been consistently demonstrated (Dubey *et al.*, 2000).

With this background, the present study was designed to investigate the possible protective effect of resveratrol on nephrotoxicity induced by glycerol and the possible mechanism by which resveratrol exerts its protective effect in ameliorating glycerol-nephrotoxicity. Also to investigate the effect of supplementing different doses of resveratrol in overcoming acute renal failure induced by glycerol injection in adult albino rats.

## 2. Material and Methods

### Chemicals:

Resveratrol was purchased from (Sigma–Aldrich Chemical Co. St.Louis, Mo,USA). Glycerol was of analytical pure grade and purchased from El-Gomhuria Co. Cairo, Egypt.

### Animals:

40 Male (Sprague Dawley) rats (150-160) g were obtained from Animal House of National Research Center, Cairo, Egypt. Rats were kept under controlled conditions, fed standard chow diet and provided with water *ad libitum*. The rats were weighted and randomly allocated into 5 groups (8 animals each). All animals were deprived of water overnight, at 16 hrs later, on the day of the experiment, the first group (G1) was injected with normal saline solution (10 ml/kg) in the hind limb musculature, such that each limb received one half of the required dose. Groups (2-5) injected with 1:1 (v/v) solution of glycerol and saline (10 ml/kg) according to Shimizu *et al.* (1998). After this, the animals fed free access to water and food. Different treatment concentrations of resveratrol were prepared by dissolving the powder in 10% Dimethylsulfoxide

DMSO according to (Juan *et al.*, 2001). On the second day of the experiment, group 3, group 4 and group 5 were given orally once daily resveratrol 200, 1000, 3000 mg/kg. b. wt/day respectively, for 28 days by gavage (10 ml/kg b. wt/day) respectively. At the end of the study, rats were deprived of food overnight, anesthetized with diethyl ether and blood were collected by cardiac puncture (1 ml into EDTA for hematology). About 2-4 ml blood was centrifuged at 4000 rpm for 15 minutes, and the serum was immediately removed from the cells. Kidneys were isolated, washed with cold saline and blotted dry with filter paper, and kept for the biochemical analysis.

### Biochemical analysis:

In blood: the following hematological variables were determined Erythrocyte count, hemoglobin, hematocrit, total differential leukocyte count and platelet count. In serum: the estimation of urea, creatinine, Na<sup>+</sup> and K<sup>+</sup> levels were made using standard diagnostic kits (RANDOX kits). Serum MDA and Nitrite/nitrate NO<sub>x</sub> level were described by Uchiyama and Mihara (1978) and Miranda *et al.*, 2001 respectively.

### Renal tissues analysis:

For determination of the biochemical parameters in the renal tissues, the isolated kidney was quickly weighted and homogenized in 4 volumes of ice cold deionized water to yield 20% w/v homogenate using ice cold Teflon homogenizer. A portion of the homogenate was mixed with a cold 2.3 % KCl solution (1:1), centrifuged at 4°C for 15 minutes. The supernatant was used for determination of MDA level by Uchiyama and Mihara (1978) and Nitrite/nitrate (NO<sub>x</sub>) (Miranda *et al.*, 2001). For assessment of sodium, potassium adenosine triphosphate (Na<sup>+</sup>, K<sup>+</sup> - ATPase) activity in microsomal fraction, a portion of kidney homogenate was mixed with equal volume of sucrose–imidazole buffer pH 7.2 (50 mmol imidazole, 2 mmol EDTA and 0.6 mol sucrose), centrifuged at 4° C for 15 minutes, the resultant supernatant was separated and ultracentrifuged at 4000 r.p.m at 4° C for 60 minutes. Then discarded and the sediment was resuspended in one third of the original volume of the buffer. The microsomal fraction was incubated with deoxycholate for demasking of all the latent Na<sup>+</sup>, K<sup>+</sup> - ATPase activity in the preparation as described by Jorgensen and Skuo (1971). The activity of Na<sup>+</sup>, K<sup>+</sup> - ATPase was assayed according to Rasch (1986). Enzyme activity was expressed as μmol pi/h/mg protein. Myeloperoxidase enzyme activity (MPO), an indicator of polymorphonuclear leukocyte (PMNs) accumulation, was measured in tissues in a

procedure similar to that documented by **Krawisz *et al.* (1984)**. Tissue samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0) and centrifuged for 10 min; pellets were suspended in 50 mM PB containing 0.5 % hexa-decyltrimethyl-ammonium bromide (HETAB). After three freeze and thaw cycles with sonication between cycles, the samples were centrifuged for 10 min. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mM PB, O-dianisidine, and 20mM H<sub>2</sub>O<sub>2</sub> solution. One unit of enzyme activity was defined as the amount of MPO that cause a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/g wet tissue.

#### Statistical analysis:

Data analyses were performed using SPSS software version 14.0 for windows. All data were

expressed as mean  $\pm$  SD. Analysis of variance was used to test for differences between the groups.

#### Results

Table (1) represents the results of the hematological tests carried out at the end of the study, it was noticed from the table that glycerol injection caused a significant reduction ( $p < 0.05$ ) in the erythrocytes, hemoglobin and hematocrit counts when compared to control group G1. Resveratrol administration of doses 200 and 1000 mg/kg, b. wt/day normalizes the result, on the other hand G5 administered with high resveratrol dose (3000 mg/kg, b. wt/day) show some clinical signs of toxicity including reducing hemoglobin, hematocrit and red cell count. There was a significant increase in white blood cells count in G2 (glycerol injected) and G5 (high resveratrol dose) thus indicating inflammations, while supplementation of resveratrol at low and medium doses normalizes the results.

**Table (1): Effect of experimental treatments on hematological variables of control and treated rats**

Groups Parameters	G1 control	G2	G3	G4	G5
<b>Hemogram</b>					
<b>Erythrocytes</b> 10 <sup>12</sup> /L	6.9 $\pm$ 0.1	4.5 $\pm$ 0.2	6.54 $\pm$ 0.5	6.9 $\pm$ 0.2	3.8 $\pm$ 0.5
<b>Hemoglobin</b> g/L	130.3 $\pm$ 2.0	80.3 $\pm$ 2.5	120.5 $\pm$ 3.5	125.4 $\pm$ 4.1	60.5 $\pm$ 1.6
<b>Hematocrit</b>	0.39 $\pm$ 0.004	0.34 $\pm$ 0.003	0.32 $\pm$ 0.004	0.35 $\pm$ 0.006	0.28 $\pm$ 0.004
<b>White blood cells</b>					
<b>Leucocytes</b> 10 <sup>9</sup> /L	6.77 $\pm$ 0.41	10.68 $\pm$ 0.45	8.54 $\pm$ 0.6	8.83 $\pm$ 0.2	12.63 $\pm$ 0.6
<b>Neutrophils %</b>	17.93 $\pm$ 3.2	23.2 $\pm$ 3.1	20.64 $\pm$ 2.5	19.63 $\pm$ 1.6	25.63 $\pm$ 3.5
<b>Lymphocytes</b> %	78.08 $\pm$ 2.66	89.02 $\pm$ 2.8	82.95 $\pm$ 1.5	86.63 $\pm$ 2.5	112 $\pm$ 6.5
<b>Monocytes%</b>	0.36 $\pm$ 0.8	0.53 $\pm$ 0.6	0.47 $\pm$ 0.8	0.49 $\pm$ 0.1	0.74 $\pm$ 8.1
<b>Eosinophils%</b>	1.77 $\pm$ 0.28	2.98 $\pm$ 0.19	2.1 $\pm$ 0.43	2.5 $\pm$ 0.42	3.2 $\pm$ 0.29
<b>Basophiles%</b>	1.8 $\pm$ 0.99	3.0 $\pm$ 0.99	2.4 $\pm$ 0.13	2.6 $\pm$ 0.17	3.8 $\pm$ 0.91
<b>Platelets</b> 10 <sup>9</sup> /L	657 $\pm$ 26	620 $\pm$ 23	635 $\pm$ 22	632 $\pm$ 24	608 $\pm$ 18

Values are expressed as means  $\pm$  S.D

**Table (2): Effect of experimental treatments on serum kidney functions of control and treated rats.**

Groups Parameters	G1 control	G2	G3	G4	G5
<b>Urea mg/dl</b>	34.76 $\pm$ 1.04	77.83 $\pm$ 6.9	47.96 $\pm$ 2.1	49.83 $\pm$ 3.2	89.65 $\pm$ 7.9
<b>Creatinine mg/dl</b>	1.047 $\pm$ 0.04	1.7 $\pm$ 0.13	1.34 $\pm$ 0.15	1.26 $\pm$ 0.16	1.9 $\pm$ 0.06
<b>Na<sup>+</sup> mmol/ L</b>	143.08 $\pm$ 6.46	155.73 $\pm$ 7.6	151.86 $\pm$ 5.7	149.4 $\pm$ 6.2	160.83 $\pm$ 5.4
<b>K<sup>+</sup> mmol/ L</b>	4.94 $\pm$ 0.1	5.5 $\pm$ 0.6	5.1 $\pm$ 0.5	5.3 $\pm$ 0.5	5.8 $\pm$ 0.4

Values rare expressed as meanS  $\pm$  S.D

As shown in table (2), glycerol injection (G2) induced a significant increase in urea, creatinine, Na<sup>+</sup> and K<sup>+</sup> levels compared with the normal group G1,

thus indicating renal injury. Concurrent administration of resveratrol either in dose 200 or 1000 mg/kg, b. wt/day (G3 and G4, respectively)

represented a significant reduction in urea, creatinine,  $\text{Na}^+$  and  $\text{K}^+$  levels. The reduction of these levels is not dose-dependent. Administration of resveratrol at

a level of 3000 mg/kg, b. wt/day induced a highly significant increase in the levels when compared to control group (G1) and glycerol injected group (G2).

**Table (3): Effect of experimental treatments on serum MDA and NOx levels and renal MDA, NOx and Na,K –ATPase activity of control and treated rats.**

Groups Parameters	G1 control	G2	G3	G4	G5
Renal MPO	3.72± 0.4	12.84±0.92	7.84±0.54	6.4±0.32	14.83±0.95
Serum MDA nmol/ml	3.05±0.11	4.86±0.86	3.68±0.73	3.49±0.62	5.74±0.73
Renal MDA nmol/g tissue	74.8±2.19	140.76±9.6	90.84±7.1	94.82±6.9	149.6±10.4
Serum NOx nmol/ml	27.29±1.9	45.35±4.1	32.72±1.8	35.82±2.1	50.66±3.9
Renal NOx nmol/g tissue	129.6±9.5	250.62±13.6	159.63±11.7	151.82±12.53	279.3±12.7
Na,K –ATPase Mmolpi/h/mg protein	30.87±0.8	16.30±1.4	28.59±2.3	27.5±2.6	14.6±2.4

Values are expressed as means ± S.D

Data represented in table (3) showed that glycerol injected rats exhibited a significant increase in both serum and renal MDA and NOx levels accompanied by significant decrease in renal  $\text{Na}^+$ ,  $\text{K}^+$  -ATPase activity as compared to the normal control group G1. Resveratrol treatment either in dose 200 or 1000 mg/kg, b. wt/day reduced the elevated MDA and NOx levels both in serum and renal tissue and restored renal  $\text{Na}^+$ ,  $\text{K}^+$  -ATPase activity to approach the normal control value. By contrast, rats administered with 3000 mg/kg, b. wt/day (G5) showed significant increase in both serum and renal MDA and NOx, with a noticeable reduction in renal  $\text{Na}^+$ ,  $\text{K}^+$  -ATPase activity as compared to the normal control group G1 and glycerol injected group G2. A pronounced increase was observed in the concentration of MPO activity indicating inflammation in G2 and G5 when compared to other groups.

#### 4. Discussion

In this study, we observed that induction of renal failure resulted in a significant renal damage, as evidenced by increased lipid peroxidation and the impairment of renal function. Furthermore these functional and structural alterations were associated with enhanced oxidative and nitrosative stress in glycerol injected rats. To date, few studies have evaluated the toxicity of resveratrol in animals.

Experimentally, glycerol injection was used to induce renal damage. Exogenous glycerol taken up from dietary fats is released during digestion into the blood stream and can enter the glycolysis or

gluconeogenesis pathways depending on the physiological conditions. Animals are deprived of drinking water before the i.m. injection of glycerol because hypovolaemia facilitates tubular precipitation of myoglobin casts and helps in accelerating renal damage. Glycerol, when injected intramuscularly in large doses to dehydrated rats, produces acute renal failure (ARF) similar to that observed in patients with crush syndrome. The use of 3000 mg resveratrol was suitable to induce nephrotoxicity in rat as stated early by Bertilli *et al.* (1996) who reported that after oral administration of resveratrol in rats, the agent accumulated mainly in heart muscle and presented a strong affinity for liver and kidneys.

Juan *et al.* (2002) administered a single dose of 2000 mg resveratrol per kilogram body weight did not cause any detectable, toxicologically significant changes in the rats. Other published experiments in rats tend to use dose levels less than 2000 mg resveratrol per kilogram body weight and for durations that are shorter than 4 weeks. Here, the study report the toxicological effects in rats of 200, 1000, and 3000 mg resveratrol per kilogram body weight (kg b. wt) administered orally for 4 weeks. These data identify the kidney as a target organ for toxicity caused by the highest dose of resveratrol and provide data that will be useful in supporting the safety evaluation of resveratrol for clinical use.

Moreover, the administration of resveratrol intraperitoneally has been previously reported by Soares *et al.*, 2002. Results showed that, glycerol injection and high dose of resveratrol caused a

significant reduction in the erythrocytes, hemoglobin and hematocrit counts when compared to control group G1. This may be due to iron release from heme pigment myoglobin causing alterations in renal function/structure (**Singh et al., 2003**). Resveratrol administration of doses 200 and 1000 mg/kg, b. wt /day normalizes the result. Anemia occurred in the rats treated with 3000 mg/kg b. wt/ day dose may have been related to the renal injury, as reduced erythropoietin synthesis in the kidneys would have occurred. The resulted anemia may have been a direct effect of resveratrol on red blood cells. White blood cell counts were significantly increased in animals in the 3000 mg/kg/ b. wt day dose level. The elevations may have been associated with the renal pelvic inflammation. Low and medium doses of resveratrol 200 and 1000 mg/kg, b. wt, can exert beneficial effects on renal injury, shock and multiple organ dysfunction syndromes via inhibiting oxidation, leukocyte priming and expression of inflammatory mediators as well as by protecting microcirculation (**Dong, 2003**). **Shigematsu et al.(2003)** indicated that in pathological conditions such as inflammation and shock, resveratrol can suppress leukocyte adhesion to endothelium and subsequently reduce infiltration of leukocytes into inflammatory sites as well as affect the kinetic behaviors of calcium channels to dilate vessels and improve microcirculatory disorders.

Data showed that glycerol-injected rats, presented increases in serum creatinine and urea as well as excretion of sodium and potassium. Oral administration of 3000 mg/kg/ b. wt day to rats for 28 days resulted in nephrotoxicity observed as elevated serum urea, creatinine,  $\text{Na}^+$  and  $\text{K}^+$  levels.

Kidney is vulnerable to damage because of larger perfusion and the increased concentration of excreted compounds that occur in renal tubular cells (**Mohamed et al., 2003**). Urea is the major nitrogen-containing metabolic product of protein metabolism. Serum levels of urea and creatinine were used as indicators of renal function. Elevated blood urea is known to be correlated with an increased protein catabolism in mammals and/or the conversion of ammonia to urea, as a result of increased synthesis of arginase enzyme involved in urea production (**Hooper et al., 1998**). In the present study, treatment of rats with resveratrol at doses 200 and 1000 mg/kg, b.wt /day renders rats less susceptible to kidney damage induced by injection with glycerol. This protection was evidenced in the serum as the elevated levels of both urea and creatinine were markedly lowered below those elicited by the nephrotoxicant.

Glycerol injected rats exhibited a significant increase in both serum and renal MDA and NOx

levels. These results suggest that i.m. administration of glycerol induces the production of free radicals with consequent changes in energy homeostasis, renal cell injury and acute renal insufficiency (**Nath, 2006**). Glycerol, acting as a scavenger molecule, produces secondary scavenger-derived free radicals capable of damaging DNA. In addition, considering that the kidney is responsible for the metabolism of 20% of all glycerol, the glyceraldehydes produced may auto-oxidize in the presence of oxygen, yielding superoxide radical with accumulation of hydrogen peroxide (**Chander et al., 2003**).

Resveratrol treatment either in dose 200 or 1000 mg/kg, b. wt/day reduced the elevated MDA and NOx levels both in serum and renal tissue. By contrast, rats administered with 3000 mg/kg, b. wt/day (G5) showed significant increase in both serum and renal MDA and NOx. A study by **Chan and Chang (2006)** found that low doses of resveratrol act as a scavenger for ROS radicals or enhancer for ethanol derived free radicals generating, while administration of high doses of resveratrol induced generation of ROS and enhanced intracellular oxidation stress and nephrotoxicity. However, no previous work has examined the dosage effects of resveratrol on cell death. Mechanistically, A study by **Banerjee et al.( 2002)** showed that higher concentrations of resveratrol induced the generation of ROS and synergistically enhanced intracellular oxidative stress in toxicant rats, promoting apoptosis or necrosis.

In the present study, failure caused an increase in the renal MDA level, an indicator of lipid peroxidation, and depleted the antioxidant pool. Moreover, oxidative injury of the tissues was accompanied by neutrophil infiltration, as evidenced by high tissue MPO levels. The protection against oxidative injury accomplished by resveratrol was also accompanied by the amelioration of renal dysfunction (**Giovannini et al., 2001**). Furthermore, **Chanvitayapongs et al. (1997)** showed that resveratrol not only possessed antioxidant properties but could also reduce the cell death induced by oxidized lipoproteins. In an *in vitro* study **Lu et al.(2002)** examining the antioxidant activities of resveratrol and its analogues, the results exhibited that they had various potencies in inhibiting lipid peroxidation in rat brain, kidney and liver homogenates and rat erythrocyte hemolysis. This indicating that resveratrol thereby reduces the inflammatory process and minimizing renal damage. In rodents, resveratrol preserves anti-oxidant enzyme activities such as superoxide dismutase, glutathione peroxidase and catalase (**Meng et al., 2005**). It has also been shown *in vitro* systems to scavenge superoxide and block oxidation of low-density

lipoproteins( LDL) by metals and the RNS, peroxynitrite (ONOO) generated in biological systems by the reaction of NO and superoxide (**Hung et al., 2002**).

In addition, resveratrol can increase the antioxidant capacity of the kidneys and plasma by modulating the endogenous antioxidant system (**Rodrigo and Bosco, 2006**). Previous studies by Bertelli *et al.* (1996), Susana and Gustavibarj (1999) showed that resveratrol maintains antioxidant as assessed by tissue levels of nitric oxide .

Resveratrol treatment either in dose 200 or 1000 mg/kg, b. wt/day reduced the elevated NOx levels in serum and renal tissue, while rats administered with 3000 mg/kg, b. wt/day (G5) showed significant increase NOx level. Peroxynitrite is a potent oxidant formed by the non enzymatic reaction between the superoxide anion and nitric oxide (NO) and is involved in several pathological conditions, such as inflammation , as well as in renal and cardiovascular diseases (**Chander et al., 2005**).

Protein tyrosine nitration is considered a consequence of peroxynitrite formation. Higher nitrotyrosine was observed in the renal cortex of glycerol- treated rats indicate the increase of the nitration of tyrosines. NO production can lead to peroxynitrite anion formation that may contribute to the renal injury provoked by glycerol. Resveratrol, as a potent scavenger of peroxy radicals, can also decrease the amount of peroxynitrite in the blood and kidneys (**Olas et al., 2006**) Generally , resveratrol acts as a renoprotective agent against nephrotoxicity induced by glycerol possibly through modulation of NOx (**Naderali et al., 2000**).

Glycerol injection causes significant decrease in renal Na<sup>+</sup>, K<sup>+</sup>-ATPase, while resveratrol treatment either in dose 200 or 1000 mg/kg, b. wt/day restored renal Na<sup>+</sup>,K<sup>+</sup>-ATPase activity to approach the normal control value. Rats administered with 3000 mg/kg, b. wt/day (G5) showed significant reduction in renal Na<sup>+</sup>, K<sup>+</sup>-ATPase activity. It is known that Na<sup>+</sup>, K<sup>+</sup>-ATPase is a membrane bound sulfhydryl containing enzyme, whose activity is essential for maintenance of cellular homeostasis. In the current study, Na<sup>+</sup>, K<sup>+</sup>-ATPase activity was significantly reduced in the kidney on glycerol injected rats. Reduction in the renal Na<sup>+</sup>, K<sup>+</sup>-ATPase activity can be related to the enhanced oxidative and nitrosative stress observed in the present study. It is known that the activity of that enzyme is modulated by the physical and chemical properties of the membrane. Consequently, it is likely that glycerol-enhanced lipid peroxidation may impair the optimum interaction between the membrane phospholipid and Na<sup>+</sup>, K<sup>+</sup>-ATPase (**Kuhnle et al., 2002**). It was noticed that

administration of resveratrol in doses 200, 1000 mg/kg b. wt/ day succeeded to restore renal Na<sup>+</sup>, K<sup>+</sup>-ATPase activity. That observation could be related to its potent antioxidant properties as well as its ability to restore NOx levels in the present study. A study by **Yang and Pio, 2003** stated that resveratrol markedly improved the Na<sup>+</sup>, K<sup>+</sup>-ATPase activities in injured rats. Another study by **Mizutani et al., 2001** showed that resveratrol at the dose of 200 mg/kg could effectively increase Na<sup>+</sup>, K<sup>+</sup>-ATPase activity .

A pronounced increase was observed in the concentration of MPO activity indicating inflammation in G2 and G5 when compared to other groups. This may due to oxidative injury of the tissues which accompanied by neutrophil infiltration, as evidenced by high tissue MPO levels. Myeloperoxidase (MPO) is an enzyme that is found predominantly in the azurophilic granules of polymorphonuclear leukocytes (PMNL). Tissue MPO activity is frequently utilized to estimate tissue PMNL accumulation and correlates significantly with the number of PMNL determined histochemically in inflamed tissues (**Chander and Chopra, 2006**). Neutrophils are known to release MPO as a response to various stimulatory substances (**Reiter et al., 2001**). In the present study, the elevated tissue MPO activity indicates the contribution of neutrophil infiltration in glycerol-induced renal injury. Administration of resveratrol at doses 200 or 1000 mg/kg, b. wt/day reduces the MPO activity as observed in G3and G4. These results suggest that the mechanism of the protective effect of resveratrol involves the inhibition of inflammatory cell infiltration. As a result, administration of 200 and 1000 mg resveratrol/ kg b. wt/day did not result in nephrotoxic findings.

In conclusion, this study demonstrates that resveratrol, in doses 200 to 1000 mg / kg b. wt/day reduces glycerol-induced renal injury, and that the protective effect of resveratrol can be attributed, at least in part, to its ability to balance oxidative and nitrosative stress, to inhibit neutrophil infiltration and to regulate the inflammatory mediators, suggesting a future role in the treatment of renal failures. High doses of resveratrol increase renal toxicity as assessed enzymatic and renal tests.

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