International Journal of Scientific and Technological Research
ISSN 2422-8702 (Online)
Vol 4, No.10, 2018 

# Metformin Attenuated Hyperoxia Induced Oxidative Damages on Different Tissues in Young Rats

Erdal Ince
Department of Medical Science Biology, Cerrahpasa medical faculty,
Istanbul University – Cerrahpasa, Istanbul, Turkey
E-mail: ince@istanbul.edu.tr

**Abstract**
To the best of our knowledge, this is the first study investigating the protective effect of metformin against hyperoxia-induced oxidative and inflammatory damage to the liver, thymus, spleen, kidney and lung tissues of young rats. 40 Wistar albino separated to four equal groups such as: normoxia control group (NC), normoxia plus metformin group (NM, 25 mg/kg of metformin), hyperoxia (≥85% $O_2$) group (HC), hyperoxia plus metformin group (HM, ≥85% $O_2$; 25 mg/kg of metformin). To evaluate the antioxidant effect of metformin on rat tissues, we performed lipid peroxidation, protein oxidation , Na K ATPase activity and antioxidant capacity [ enzymatic: glutathione peroxidase (GP), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) activity; non-enzymatic: glutathione (GSH) level] measurements. We also measured serum interleukin (IL)-1β, IL-6, IL-10 and tumor necrosis factor alfa (TNFα) cytokine levels and nuclear factor kappa B (NF-κB) activation to evaluate the anti-inflammatory effects of metformin. A statistically significant decrease was observed in the HM group in terms of tissue lipid peroxidation, protein oxidation, and DNA fragmentation measurements compared to the HC group, while enzymatic and non-enzymatic antioxidant capacity and Na K ATPase activity increased significantly. In the HM group, plasma IL-1β, IL-6, and TNF-α levels and NF-κB activation. decreased and IL-10 increased significantly compared to the HC group. In conclusion, metformin has a protective role against hyperoxia-induced oxidative and inflammatory damage in the liver, thymus, spleen, kidney and lung tissues of young rats.

**Key words:** hyperoxia, oxidative stress, inflammation, metformin

**Abbreviations:**
ROS Reactive oxygen species
LP Lipid peroxidation
PO Protein oxidation
GSH Reduced glutathione
GP Glutathione peroxidase
GR Glutathione reductase
SOD Superoxide dismutase
CAT Catalase
MDA Malondialdehyde

## 1-Intoduction

Human life and most physiological processes depend on a continuous supply of oxygen to sustain cell function (Leach *et al.*, 1998). Oxygen has double edged properties, being essential for life; it can also aggravate the damage within the cell by oxidative events. When cells use oxygen to generate energy and metabolic processes, free radicals are created by aerobic and anaerobic process (Pham-Huy *et al.*, 2008). Production of various types of reactive nitrogen and oxygen species can directly harm biomolecules and can lead to cell death, especially under prolonged oxygen supplementation (Potteti *et al.*, 2013). Cellular defenses against increased oxidative stress such as antioxidant enzymes and non-protein thiols become depleted by the excess production of ROS instigating cellular injury and death ( Davies, 2000). Accumulating evidence has demonstrated that exposure to high concentrations of oxygen may lead to damaging effects of hyperoxia in various tissues and organs. are associated with the production of reactive oxygen species (ROS), including superoxide, hydrogen peroxide and the hydroxyl radical

(Asikainen and White, 2004). Although ROS are by-products of normal oxygen metabolism, their increased generation in hyperoxia, especially when the cell's antioxidant defenses are limited, augments damage to proteins, lipids and DNA, leading to possible cell injury or altered cell proliferation (Yee *et al.*, 2006). Resistance to hyperoxia, above the normal physiological levels of O2 (0.21), can be used as an indicator of the longevity potential of an organism. In accordance with these findings, there are positive correlation between mammalian longevity and reduction of oxidative stress (Per *et al.*, 2018). Under normobaric circumstances, the side effects of oxygen are initially restricted to the lungs. However, when hyperoxia manifests for prolonged periods or under hyperbaric conditions, other organs are concurrently at risk as more oxygen is dissolved in plasma (Turrens, 2003). The effects of hyperoxia on various organs (e.g., lungs, brain, or retina) have been investigated sufficiently in recent years (Hinkelbein *et.al.*, 2010; Spelten *et al.*,2014). İn addition, ROS are capable of promoting inflammation, resulting in the release of inflammatory mediators and secondary tissue damage (Auten *et.al.*, 2001). The end result of excess ROS generation lead to the release of inflammatory mediators, which can also make nuclear factor kappa beta (NF-κB) activated, thus generating a large number of inflammatory mediators, to have an inflammatory cascade amplification effect, aggravating tissue damage (Mensack and Murtaugh, 1999). The ROS leukocyte interaction further exacerbates injury. As stated before, in the lung, hyperoxia causes a massive production of reactive oxygen species (ROS), which in turn initiate inflammatory response, destruction of the alveolar-capillary barrier, and impaired gas exchange (Mantell *et al.*, 1999). The excessive production of ROS under hyperoxic conditions leads to modifications of macromolecules and pulmonary cell death (Husari *et al.*,2006). Many studies have shown that the brain has sensitive to oxidative stress because of their low endogenous antioxidant, relative to other tisuues (Vidal-Jorge *et.al.*, 2017). In addition, hyperoxia induced neurodegeneration involves NF-κB activation, microglial activation and increased cyclooxygenase type 2 (COX2) immunoreactivity, all of which are indicative of an enhanced neuro-inflammatory response. ROS producing enzymes including nitric oxide synthase, COX2, and NADPH oxidase are all induced by NF-κB activation suggesting that hyperoxia induced ROS in brain may be related to NF-κB activation of inflammatory enzymes (Hinkelbein *et al.*, 2018). Lymphoid tissues (thymus, spleen) are responsible for regulating immune responses and function. Also, the lymphoid tissue cells are sensitive to changes in the antioxidant status because they carry out important functions by generating a high number of oxygen free radicals (Ozerkan *et al.*, 2014). Moreover, the cells of the immune system have a high percentage of polyunsaturated fatty acids in their plasma membranes, and therefore, it is not surprising that these cells usually contain a higher concentration of antioxidants compared with other cells (Hatam and Kayden, 1979). Interestingly, currently there is no available information about liver, lymphoid tissue toxicity in hyperoxia condition. Many therapeutic approaches have been utilised for prevent oxidative stress/inflammatory including the use of oral hypoglycaemic agents. in hyperoxia in animal models and in humans.

Metformin is an oral biguanide antihyperglycemic drug which can enhance insulin sensitivity (El Messaoudi *et al.*, 2011). Metformin has been shown to have antioxidant properties and can decrease lipid peroxidation in various tissues (Bhamra *et al.*, 2008). Recent studies established that the combination of metformin and non-steroidal anti-inflammatory drugs (NSAID) treatment is strong anti-inflammatory effect and improves metabolic syndrome. İn addition, it has been shown that metformin has potent anti-inflammatory properties in type 2 of diabetes. ( Arai *et al.*, 2010). These pleiotropic effects of metformin are probably mediated via activation of AMP-activated protein kinase (AMPK). AMPK agonists have anti-inflammatory properties in vitro, as shown in multiple cell types exposed to lipopolysaccharides (LPS), including macrophages and airway epithelial cells and in vivo, as demonstrated in obese mice with allergic eosinic inflammation and in mice with inflammatory bowel disease (Chen *et al.*, 2015). Because inflammation is an important contributor to the pathogenesis of hyperoxia induced oxidative tissue damage, treatment with the potent anti-inflammatory AMP-activated protein kinase (AMPK) agonist metformin may result in the identification of a novel therapy for disease such as chronic obstructive pulmonary disease. However, no data are available about the antioxidant and antiinflammatory action of metformin on hyperoxia in liver lymphoid tissues.

Thus, the main aim of this experimental study was to investigate the potential protective effect of metformin on the liver, thymus, spleen, kidney and lung tissues of young rats against maternal ethanol-induced oxidative and inflammatory damage

## 2-Materials and Methods

### *2.1.Experimental procedure*

All experimental protocols were approved by the Cerrahpasa Medical Faculty Animal Care and Use Committee and we followed the National Institutes of Health (Washington, DC) Guide for the Care and Use of Laboratory Animals..Ten adult male and 30 adult female Wistar rats (aged 6–8 weeks; Cerrahpasa

Medical Faculty Experimental Research Centre, Istanbul/Turkey) weighing 180–200 g were used for this experimental study. They were placed in a secluded, temperature- and humidity-controlled room (22°C ± 3°C and 60% ± 5%, respectively) in which a 12-h/12-h light-dark cycle was maintained.
Wistar albino rats separated to four equal groups such as: normoxia control group (NC), normoxia plus metformin group (NM, 25 mg/kg of metformin), hyperoxia (≥85% $O_2$) group (HC), hyperoxia plus metformin group (HM, ≥85% $O_2$; 25 mg/kg of metformin). Each
group consisted of 10 rats in the beginning of the study. Normoxia and gelsolin groups were
kept at room air. Hyperoxia and hyperoxia plus gelsolin groups were put in plexiglass chambers which could allow maintaining oxygen levels≥ 85% together with their mothers and oxygen level was monitored in the chambers.
During the study, all groups were given equal volumes of intraperitoneal (i.p.) injections. The metformin preparation was diluted with in water. The NC group was given serum physiological at ambient air. The normoxia plus metformin groups were given /kg bw/day metformin at ambient air. The Hyperoxia group was given≥85% O2 as well as i.p. serum physiological. The hyperoxia plus metformin groups were given≥85% O2 and daily injected subcutaneously either with 100 µl 0.9% NaCl or metformin 25mg/kg bw/ day. Experimental first day was accepted beginning of the application to animal. At the end of the 7 days of treatment, the rats were fasted overnight. The next morning, under light ether anesthesia (inhaled 4000 ppm/h), the heart was punctured using an injector, and the blood was collected into biochemical test tubes. Blood test tubes were centrifuged at 2000*g* for 10 min at room temperature, and the serum was separated. After removing the blood samples, the rats were sacrificed under general anesthesia by an intraperitoneal injection of ketamine hydrochloride (60 mg/kg, Ketalar) and xylazine hydrochloride (10 mg/kg) and lymphoid (thymus, spleen, and lymph nodes) and nonlymphoid (liver) tissues were surgically removed.

### *2.2. Biochemical analyses*

Tissues used for biochemical studies were frozen in liquid nitrogen and kept at –80°C until further use. Tissue samples were excised and weighed, and a part of it was minced and homogenized well in liquid nitrogen. Then, the homogenate was sonicated for 60 s at 220 V (Sonic Dismembrator, Model 300 (Fisher, Germany) in each cold assay buffer. Metformin (25 or 100 mg/kg; D150959; Sigma-Aldrich, St. Louis, MO) All chemicals used for the experiments were of analytical grade and purchased from Merck (Darmstrat Germany) and Sigma-Aldrich (MO, USA)

#### *2.2.1. Assessment of biochemical structure*

*Determine of malondialdehyde (MDA) level*

The thiobarbituric acid (TBA) test was used for determining MDA level in cell. (Ohkawa et al., 1979). Formed MDA and TBA complex given colored structure and these stuctures were measured spectrophotometrically at 532 nm to define MDA levels. The level was determined
as nmol/mg protein.

*Protein Oxidation measurement*

To demonstrate oxidative protein damage, the amount of tissue PC was measured by the dinitrophenylhydrazine (DNPH) derivatization method as described by Levine et al. (1994) Briefly, proteins dissolved in 6% SDS were mixed with an equal volume of DNPH solution and incubated at room temperature. Derived proteins were precipitated with TCA and the pellets were dissolved in 6M guanidine. Reading was done at 370 nm absorbance and the amount of PC was expressed as nmol carbonyl/g of tissue.

*$Na^+$ $K^+$ ATPase activity measurement*

It is based on the measurement of inorganic phosphate that is formed from 3 mmol/l disodyum adosine triphosphate added to the medium during the incubation period (Reading and Isbir, 1980). The medium incubated in a $37C^0$ water bath for 5 min with a mixture of 100 nmol/l $Na_2Cl$, 5mmol KCl, 6 mmol $MgCl_2$, 0.1mmol EDTA, 30 mmol Tris- HCl (pH 7.4). Following the preincubation period, $Na_2ATP$, at final concentration of 3 mmol/l, was added to each tube and incubated at $37C^0$ for 30 min.after the incubation, the tubes were placed in an ice bath and the reaction was stopped. Subsequently, the level of inorganic phosphate was determined in a spectrophotometer at anexcitation wavelength of 690 nm. The specific activity of the enzyme is expressed as nanomoles Pi per miligram of protein per hour.

*Reduced glutathione (GSH) measurement:*
GSH measurements were made using the procedure of Beutler et al. (1963). The formation of 5-thio-2-nitrobenzoate was monitored spectrophotometrically at 412 nm. Briefly, 2 ml of 0.3 mol/l phosphate solution (Na2HPO4.2H2O) was added over 0.5 ml of the supernatant after 10 min centrifugation at 2000 g, after which 0.2 ml of eluted separator dissolved in 1% sodium citrate (dithiobisnitrobenzoate) was added. Readings were measured at 412 nm absorbance and the amount of GSH was expressed as μmol/g of tissue.

*Glutathione peroxidase (GP) activity measurement:*
GP activity was measured according to the method of Paglia and Valentin (1967). In the presence of a reaction solution containing sodium phosphate buffer, NADPH2, sodium azide, EDTA, reduced GSH and GR, the oxidation rate of NADPH2 at 340 nm absorbance was followed. The enzyme activity was calculated as μmol NADPH/min/g.

*Glutathione Reductase (GR) activity measurement:*
GR catalyzes the reduction of glutathione disulfide (GSSG) to reduced GSH. As GP-GR is an important system in ethanol metabolism, GR was selected from other detoxification enzymes. The GR activity measurement was made according to the commodity specified by Beutler. The GSH oxidation of NADPH was calculated by spectrophotometric monitoring at 340 nm. The enzyme activity was calculated as gmol NADPH min/g. (Beutler, 1985)

*Superoxide dismutase (SOD) enzyme activity measurement:*
SOD enzyme values were determined by the method modified by Winterbourn et al.(1975).The principle of this method is based on the reduction of nitro blue tetrazolium (NBT) by the xanthine-xanthine oxidase system, a superoxide producer. The superoxide radicals formed result in colored NBT-diformasan by reducing NBT in the environment. SOD activity was expressed as unit/gram (U/g) of tissue protein.

*Catalase (CAT) enzyme activity measurement:*
CAT activity was measured by the method defined by Aebi (1985). CAT catalyzes the conversion reaction of $H_2O_2$ to H2O. This conversion can be followed by a decrease in absorbance at 240nm. A total of 0.2 ml $H_2O_2$ solution (30 mM) + phosphate buffer was added to each sample of 0.4 ml tissue homogenate during the experiment. Enzyme activity was calculated as unit/gram (U/g) of tissue unit.

*Protein measurement:*
Tissue protein concentrations in the supernatant were determined by the Bradford method (1976) using bovine serum albumin as the standard.

*Determination of rat plasma cytokine and measurement of NF-κB activity:*
Blood was collected directly from the heart in a heparinized tube and was centrifuged at 1,000 x g for 5 min to collect plasma which was then stored at -20°C. TNF-α, IL-1β, IL-6 as PICs and IL-10 rat serum amounts as anti-inflammatory cytokines (AIC) were determined by commercially available ELISA kits (BioSource Europe S.A. Nivelles, Belgium). Measurements were made according to the manufacturer's protocol. Serum NF-κB activity was determined according to the procedure provided by the manufacturer (Cayman Chemicals, Michigan, USA).

*Statistical analysis:*
Results were reported as mean ± SD. All data were analyzed statistically using the ANOVA analysis followed by the Tukey's post-hoc test. Statistical significance was set at $p < 0.05$.

**Results**

*Effect of metformin on hiperoxia induced liver, thymus, spleen, kidney and lung tissue damage*
As seen in Figures 1A, B and C, MDA, PC and $Na^+/K^+$-ATPase activities values which we used as oxidative stress markers in tissues of young rats increased significantly in HC group compared to the NC and NM groups ($P<0.05$ - $P<0.001$). Thus, metformin significantly reduced lipid peroxidation, protein oxidation and rised $Na^+/K^+$-ATPase activities in tissues ($P<0.05$ - $P<0.001$). As showed in Table 1, plasma 8-hydroxy-2' -deoxyguanosine (8-OHdG), ALT, AST and LDH content of HC model group were

higher than those of control group. Metformin significantly reduced 8-OHdG ,ALT, AST and LDH content in HM rats, compared with HC model group.

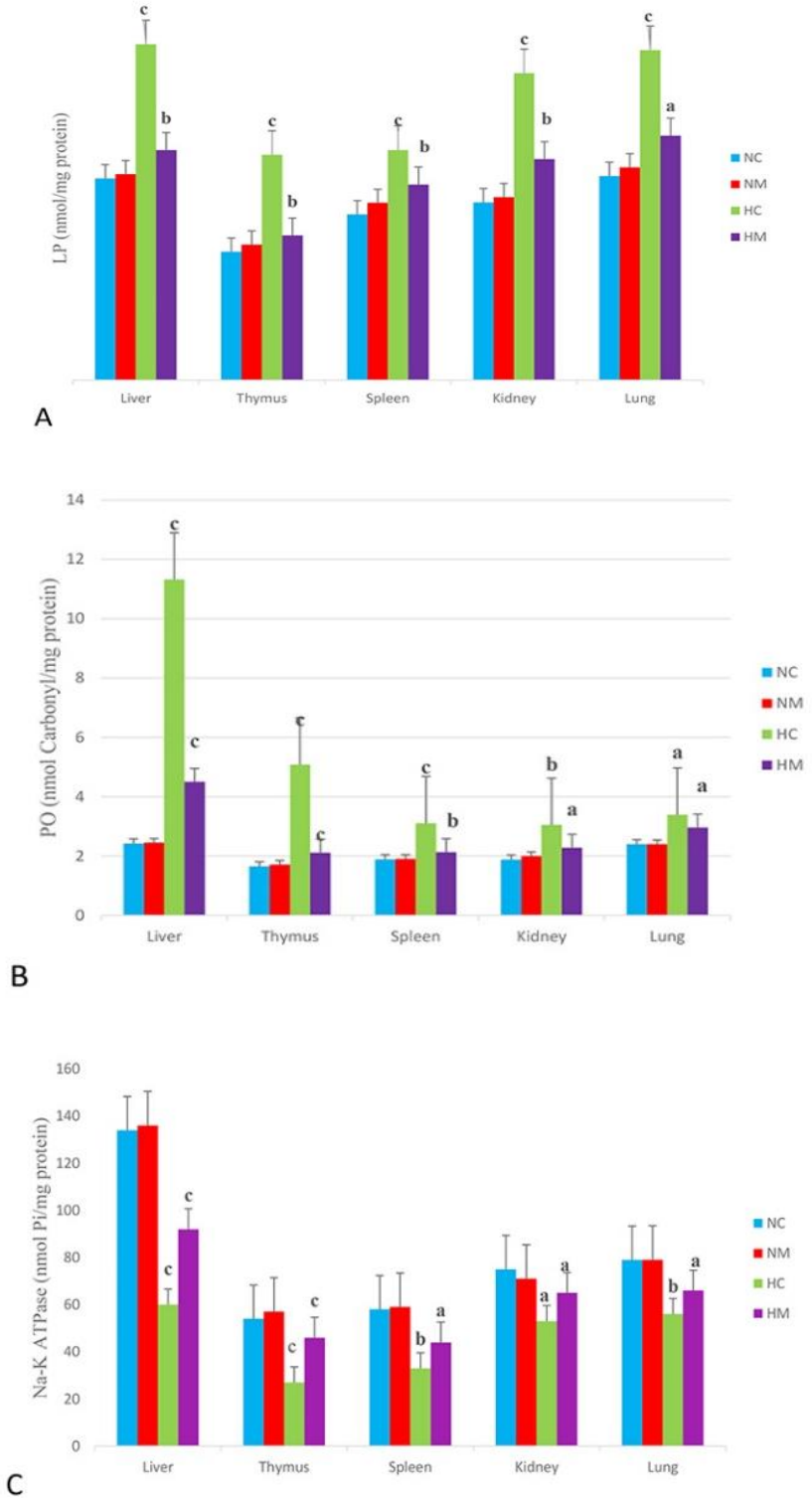


Figure 1. Effect of hyperoxia and/or metformin on lipid peroxidation levels (A), protein oxidation levels (B) and Na+/K+-ATPase activities (C) in liver, thymus, spleen, kidney and lung tissues of young rats. Normobaric control (NC), normobaric metformin (NM), hyperoxia (HC) and metformin treated hyperoxia (HM) groups. For each group (n= 10). a $p < 0.05$, b $p < 0.01$, c $p < 0.001$ vs, control group. a $p < 0.05$, b $p < 0.01$, c $p < 0.001$ vs, hyperoxia group.

 

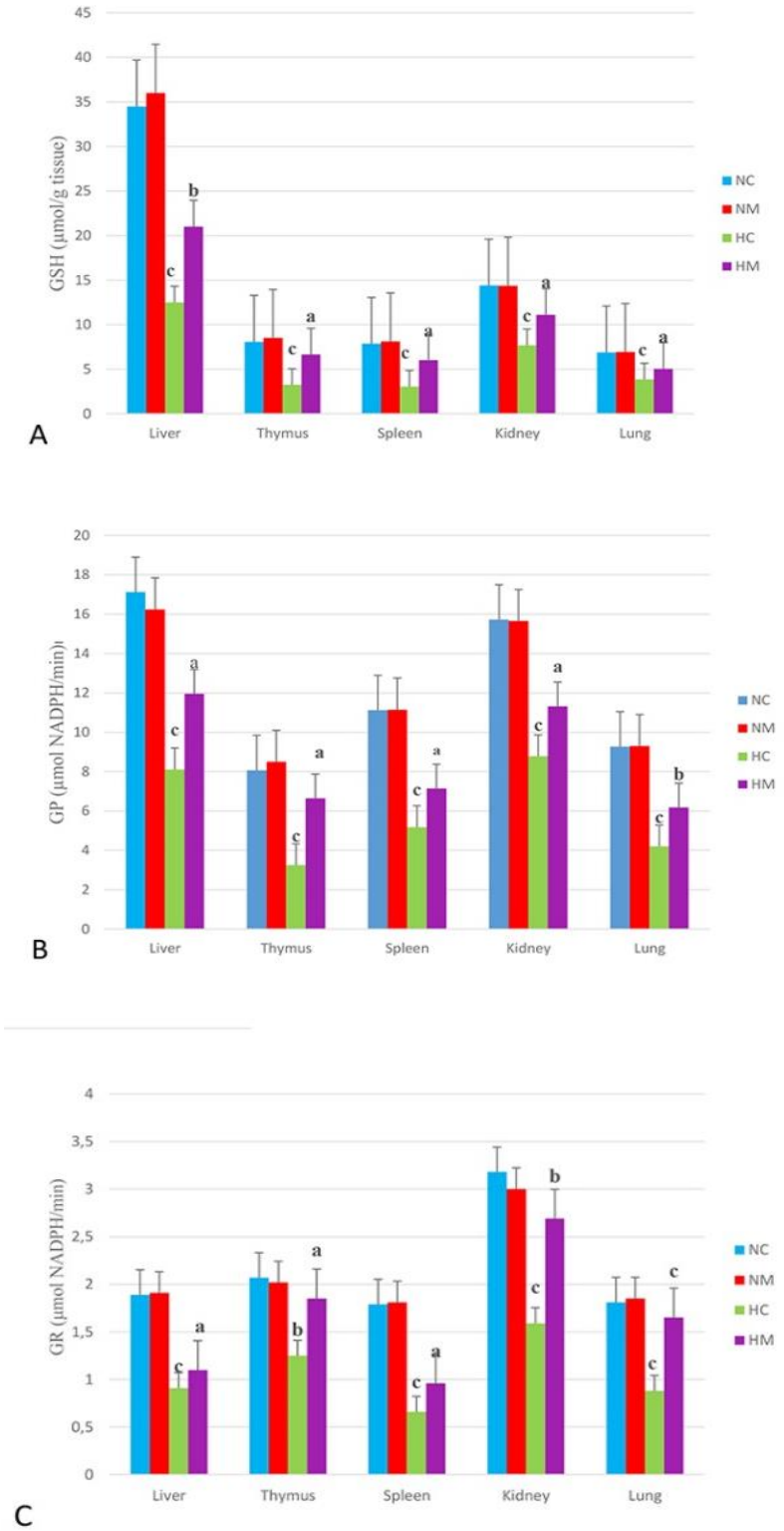


**Figure 2.** Effect of hyperoxia and/or metformin on reduced glutathione (GSH, **A**) levels, glutathione peroxidase (GP, **B**) and glutathione reductase (GR, **C**) activities in liver, thymus, spleen, kidney and lung tissues of young rats. Normobaric control (NC), normobaric metformin (NM), hyperoxia (HC) and metformin treated hyperoxia (HM) groups. For each group (n= 10). $^{a}$ $p < 0.05$, $^{b}$ $p < 0.01$, $^{c}$ $p < 0.001$ *vs*, control group. $^{a}$ $p < 0.05$, $^{b}$ $p < 0.01$, $^{c}$ $p < 0.001$ *vs*, hyperoxia group.

 

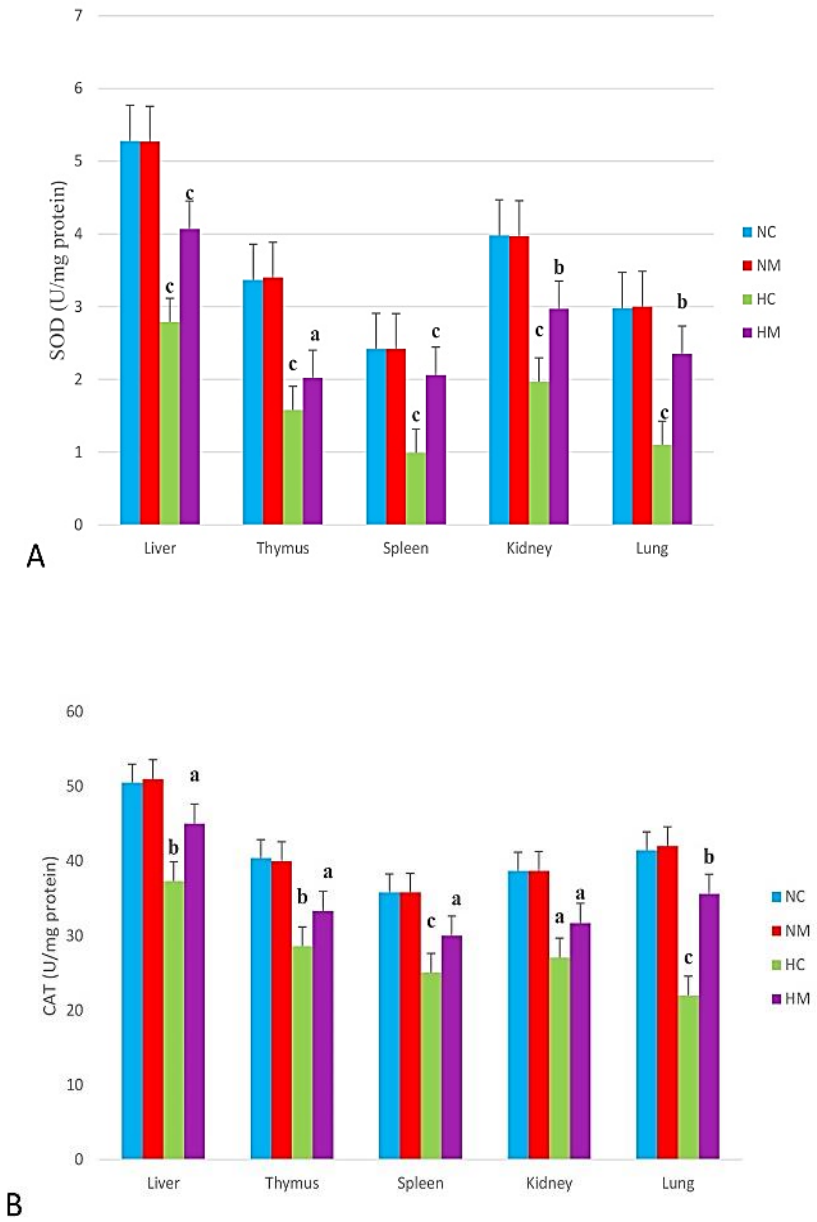


**Figure 3.** Effect of hyperoxia and/or metformin on super oxide dismutase (SOD, **A**) and catalase (CAT, **B**) activities in liver, thymus, spleen, kidney and lung tissues of young rats. Normobaric control (NC), normobaric metformin (NM), hyperoxia (HC) and metformin treated hyperoxia (HM) groups. For each group (n= 10). $^{a}$ $p < 0.05$, $^{b}$ $p < 0.01$, $^{c}$ $p < 0.001$ *vs*, control group. $^{a}$ $p < 0.05$, $^{b}$ $p < 0.01$, $^{c}$ $p < 0.001$ *vs*, hyperoxia group.

 

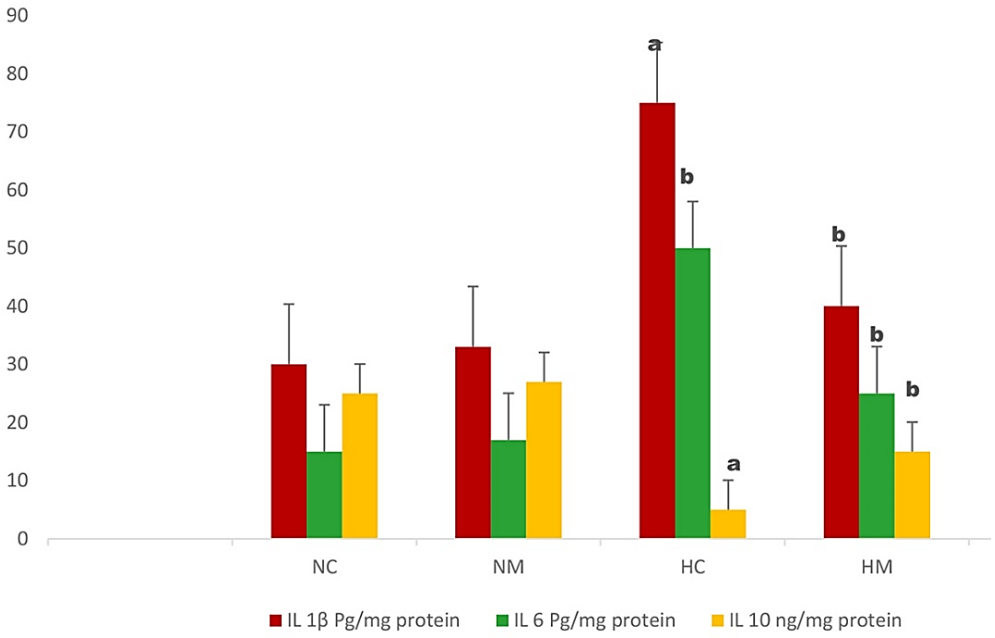


**Figure 4.** Effect of hyperoxia and/or metformin on IL-1β, IL-6, IL-10 levels. Normobaric control (NC), normobaric metformin (NM), hyperoxia (HC) and metformin treated hyperoxia (HM) groups. For each group (n= 10). $^{a}$ $p < 0.05$, $^{b}$ $p < 0.01$, $^{c}$ $p < 0.001$ *vs*, control group. $^{a}$ $p < 0.05$, $^{b}$ $p < 0.01$, $^{c}$ $p < 0.001$ *vs*, hyperoxia group.

**Table 1.** Plasma nuclear factor kappa b (NF κB) activity, 8 hydroxy-2-deoxyguanosine (8-OHdG) levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activity of normobaric control (NC), normobaric metformin (NM), hyperoxia (HC) and metformin treated hyperoxia (HM) groups. For each group (n= 10).
$^{a}$ $p < 0.05$, $^{b}$ $p < 0.01$, $^{c}$ $p < 0.001$ *vs*, control group. $^{a}$ $p < 0.05$, $^{b}$ $p < 0.01$, $^{c}$ $p < 0.001$ *vs*, hyperoxia group.

| | **NC** | **NM** | **HC** | **HM** |
|---|---|---|---|---|
| NF κB Activity | 100 ± 12 | 100 ± 5 | 224 ± 24$^{c}$ | 159 ± 37$^{b}$ |
| 8-OHdG (ng/ml) | 0.35 ± 0.03 | 0.34 ± 0.03 | 5.84 ± 0.4$^{c}$ | 3.7 ± 0.4$^{c}$ |
| Serum ALT (U/L) | 28.8 ± 2.3 | 30.5 ± 2.8 | 57.1 ± 7.2$^{c}$ | 34.4 ± 1.5$^{b}$ |
| Serum AST (U/L) | 54,6 ± 3.8 | 67.2 ± 4.8 | 98.6 ± 9.9$^{c}$ | 47.1 ± 4.8$^{c}$ |
| LDH (U/L) | 1095 ± 119 | 1396 ± 208 | 4709 ± 345$^{c}$ | 2541 ± 304$^{a}$ |

*The effect of metformin on antioxidant capacity:*
Non-enzymatic GSH levels in liver, thymus, spleen, kidney and lung tissues were significantly decreased in group HC compared to groups NC and NM (Figure 2A, P<0.05 - P<0.001). Thus, metformin increased GSH reduction in tissues (P<0.05 - P<0.001).
Antioxidant enzyme (GP, GR, GST, SOD and CAT) activities showed a significant decrease in HC compared to groups NC and NM (Figure 2B, C and Figure 3A, B P<0.05 - P<0.001). whereas enzyme activities showed a significant increase in treated hyperoxia with metformin group versus HC group (P<0.05 - P<0.001).

*Effect of metformin on inflammatory responses:*
Serum IL-1β, IL-6, and TNF-α values increased significantly in group HC compared to groups NC and NM, while serum IL-10 values showed a significant decrease (Figures 4, P<0.05 - P<0.001). In other words, metformin showed a significant decrease in serum IL-1β, IL-6, and TNF-α values, whereas serum IL-10 values increased significantly (P<0.05 - P<0.001). Serum NF-κB activity increased significantly in group HC compared to groups NC and NM, whereas metformin administration significantly decreased NF-κB activation (Table 1, P<0.05 - P<0.001).

**Discussion**
This is the first study focusing on the protective effect of metformin against hyperoxia-induced oxidative and inflammatory damage in liver, thymus, spleen, kidney and lung of young rats. As implied in many studies, a major indicator of the cellular and molecular mechanisms of hyperoxia-induced tissue injury is the formation of ROS (superoxides, hydrogen peroxide, hydroxy-radicals) [Mostafavi *et al.*, 2008]. Oxidative stress terms a condition in which the production of oxidants exceeds the organism's anti-oxidative capacity, a disequilibrium inevitably followed by damage of the molecular cell compounds. A linear increase of ROS concentration with oxygen tension in mitochondria has been observed (Turrens, 2003). Superoxide radical ($\bullet O_2^-$) is generated by the mitochondrial electron transport chain by the transfer of an electron to molecular $O_2$. Additionally, oxygen tends to build superoxides with particular metals, destroying double bonds in fatty acids. In the present study, MDA, a marker of LP, increased in the liver, thymus, spleen, kidney and lung tissues of young rats, indicating the presence of hyperoxia-induced oxidative damage. In accordance with the present findings, previous experimental animal model studies have shown that hyperoxia alone failed to increase LP and decreased DNA damage in liver and brain tissues. (Endesfelder *et al.*, 2014). The pulmonary cellular response to hyperoxic exposure and increased ROS is well described. Anatomically, the pulmonary epithelial surface is vulnerable to a destructive inflammatory response. This inflammation damages the alveolar capillary barrier leading to impaired gas exchange and pulmonary edema. Reactive O2 species induces pulmonary cell secretion of chemoattractants, and cytokines stimulate macrophage and monocyte mobilization and accumulation into the lungs, leading to additional ROS (Romashko *et al*., 2003). Mid levels of O2 such as 40% is given for medical intervention because of beneficial for patient outcome but higher levels of O2 – may induce harmful side effects (Lee and Galbraith, 1992) such as the formation of fibrous tissue in the liver, intrapulmonary arteriovenous shunting and severely changing lung function can ocur and the irreversible brain damage in infants (Ahotupa *et al.*, 1992). Presently,≥85% O2 is accepted excessive oxygen by authority (Lee and Galbraith, 1992) and not known to be toxic therefore this level was used in this work to see cytotoxic effects on rat tissues. The results of the present study demonstrated that hyperoxia caused oxidative tissue damage in the liver, thymus, spleen, kidney and lung tissues of the young rats, as assessed by increased MDA and PO levels; concomitant reduction in GSH levels and $Na^+/K^+$-ATPase, GP, GR, GST, SOD and CAT activities and serum IL-10. Furthermore, the hyperoxial-induced ROS evaluation in serum IL-1β, IL-6, and TNF-α values and NF-κB activation were increased.
Oxidative stress may ocur in imbalance between pro-oxidants (intrinsic or extrinsic) and antioxidants condition (Winterbourn and Hampton, 2008). If this status negatively changing, free radicals and macromolecular damage occur in the cell such as enzyme and transporter activity, protein conformation and interactions, DNA binding, trafficking, and degradation (Jones, 2006). Oxidative damage to proteins can cause an increase in carbonyl groups due to amino acids sensitive to oxidation such as histidine, proline, arginine, and lysine. Protein (carbonyl) oxidation and LP levels were widely used as biomarkers of tissue damage or oxidative stress. The present study showed PC increases in liver, lymphoid tissue homogenates of young rats exposed to hyperoxia. It also showed for the first time that metformin decreased MDA and PC amounts in liver and lymphoid tissues. $Na^+/K^+$-ATPase is an enzyme located in the cell membrane responsible for the active transport of various ions, thus maintaining the ionic and osmotic balance of the cells. Membrane-bound enzymes, such as $Na^+/K^+$-ATPase, require phospholipids

for the maintenance of their activity and are susceptible to structural changes due to LP (Prabbu and Balasubramanian, 2003). Therefore, the assessment of $Na^+/K^+$-ATPase activity can be used as an index for oxidant-induced tissue injury and LP (Tuna *et al*., 2001). This can explain why liver and other tissue (thymus, spleen, kidney and lung) injuries were accompanied by a simultaneous increase in MDA and PO levels and a decrease in $Na^+/K^+$-ATPase activity in the present study. Metformin is a potent antidiabetic drug that is commonly used in type 2 diabetic patients to lower glucose levels in the circulation. Compared with other treatment modalities for type 2 diabetes, patients treated with metformin were protected against mortality in cardiac disease and had less cancer, suggesting that metformin has various biological functions other than its blood glucose-lowering effect in diabetic patients (Evans *et al.*, 2005). Many of the pleiotropic effects of metformin are related to reduced inflammation, cancer, and cardiovascular disease and improved vascular function. Our results of diminished MDA PC and $Na^+/K^+$-ATPase activity directly reflect the free radical scavenging capacity of metformin in liver, thymus, spleen, kidney and lung tissues. Balancing of hyperoxia-induced oxidative stress, protection against ROS and other free radicals depends on endogenous antioxidant capacity. SOD reduces the formation of superoxide radicals and lipid peroxides and even accelerates their clearance, thereby reducing oxidative damage in the liver, lymphoid tissue, kidney and lung cells (Dink *et al.*, 2015). GSH functions in the inactivation of lipid peroxide radicals and ROS-stimulated reactive toxic intermediates, in the protection of protein sulfhydryl groups, and acts as a cofactor for GP and GR (Meister, 1983). One of the most important targets of the reaction catalyzed by the GR enzyme is maintenance of the reduced glutathione/oxidized glutathione (GSH/GSSG) ratio in the cellular environment. GP and CAT protect SOD from inactivation by $H_2O_2$ and protects SOD, GP, and CAT from superoxide anions. The GP, GR, SOD, CAT, and GSH values reflect the body's ability to clean oxygen radicals (Vidyasagar *et al.*, 2004). In the present study, we found a significant decrease in GP, GR, GST, SOD, and CAT enzyme activities and GSH levels in the liver and lymphoid tissues, kidney and lung tissues of young rats in the group that received hyperoxia. Increased oxidation of GSH can lead to a decrease in GR activity and an increase in GSSG and may lead to a decrease in GP enzyme activity, which acts particularly in eliminating hydroperoxides, including lipid hydroperoxides. In other words, metformin increased the antioxidant capacity of the liver, thymus, spleen, kidney and lung tissues of young rat exposed to hyperoxia. These results indicate that metformin has a scavenging effect on hydroxyl, superoxide and peroxyl radicals. Evidence is accumulating that these beneficial effects of metformin on inflammation and the cardiovasculature are mediated via activation of AMPK-dependent signaling ( Park *et al.* 2012) and subsequent inhibition of NF-κB signaling ( Salminen *et al.*, 2011). Inflammation is an important player in the pathogenesis of hyperoxia induced oxidative stress, because it may contribute to severe tissue damage and fibrosis, and because treatment with anti-inflammatory agents provides protection against hyperoxia-induced neonatal lung disease or experimental bronchopulmonary dysplasia.( Yi *et al.*, 2004). Recent findings suggest that increased NF-kB activity as a result of hyperoxia -induced oxidative stress plays an important role in the formation of oxidative cell damage, particularly in lung and brain tissue ( Salminen *et al.*, 2011). In this experimental study, a significant increase in serum NF-kB activity in HC group compared to the NC and NM groups strongly emphasizes the role of NF-kB in hyperoxia -induced oxidative stress. In the present study, metformin was related to a significant decrease in serum NF-kB activity. We believe that metformin plays a protective role against oxidative damage by increasing the antioxidant levels with ROS scavenging.

In addition, proinflammatory cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-1β, may have procoagulant effects. In addition, metformin decreases the activity of the potent fibrinolytic inhibitor PAI-1, thereby promoting fibrin degradation and reducing fibrin deposition (Landin *et al.*, 1994). Previous studies have shown that increased hyperoxia-induced TNF-α causes liver and brain damage, and IL-6, and IL-10 protect these tissues against inflammatory responses. In some studies, IL-10 has been shown to inhibit increased NF-kB activation after stimulation, activity of nuclear factor, and binding of DNA activity from cytoplasm to nucleus. In our study, serum NF-kB activation and IL-10 levels decreased in parallel with the increase in TNFα, IL-1β, and IL- 6 levels in young rats exposed to hyperoxia-induced oxygen toxicity. These findings clearly show that proinflammatory responses play a role in the hyperoxia-stimulated oxidative damage mechanism in the liver, thymus, spleen, kidney and lung tissues of young rats. The inhibitory effect of metformin on proinflammatory production, including TNFα, IL-1β and IL-6. In this study, while metformin decreased serum NF-kB activation and TNF-α, IL-1β, and IL-6 cytokine levels, on the other hand, it increased IL-10 cytokine levels. As a result, it can be concluded that hyperoxia and supplemental oxygene cause biochemical alterations in experimental animals' tissue. Physiological side effects can occur such as induced radical formation and oxidant injury.

In conclusion, on the basis of results of this work we can say that metformin has a protective effect against the oxidative damage caused by hyperoxia in the liver and lymphoid tissues of young rats. Results

from this study indicate that metformin might be also clinically beneficial for the hyperoxia induced toxicity in normal cell.

**Funding**
This study was supported by Istanbul University Scientific Research Projects Committee (BEKADEP, Project number: T-92/160695).

**References:**

Leach R.M. **&** Treacher D.F., (1998). Oxygen transport-2. Tissue hypoxia. *BMJ*, 317, 1370- 1373.

Pham-Huy L.A.,He H.**&** Pham-Huy C. (2008). Free Radicals, Antioxidants in Disease and Health. *Int J Biomed Sci*,4 (2), 89-96.

Potteti, H.R., Reddy, N.M., Hei, T.K. et al., (2013). The NRF2 activation and antioxidative response are not impaired overall during hyperoxia- induced lung epithelial cell death. *Oxid. Med. Cell. Longev*.,798–401.

Davies K.J. (2000). Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. *IUBMB Life*, 50,279–289.

Asikainen T.M., **&** White C.W. (2004). Pulmonary antioxidant defenses in the preterm newborn with respiratory distress and bronchopulmonary dysplasia in evolution: implications for antioxidant therapy. *Antioxid. Redox Signal.*,1,155–167.

Yee M.,Vitiello P.F., Roper J.M. et al. (2006). Type II epithelial cells are critical target for hyperoxia-mediated impairment of postnatal lung development. Am. J. Physiol., 291(5), L1101–L1111.

Per S., Kose M., Ozdemir A.**&** Pandir D. (2018). Hepatoprotective effects of capping protein gelsolin against hyperoxiainduced hepatotoxicity, oxidative stress and DNA damage in neonatal rats. *Env. Toxicol. & Pharmcol*., 58,189-195.

Turrens J.F. (2003). Mitochondrial formation of reactive oxygen species. *J. Physiol*.,552,335–44.

Hinkelbein J., Feldmann R.E. **&** Jr. Kalenka A. (2010). Time-dependent alterations of cerebral proteins following short-term normobaric hyperoxia. *Mol. Cell. Biochem.*,339,9–21.

Spelten O., Wetsch W.A., Wrettos G., Kalenka A.**&** Hinkelbein J. (2014). Response of rat lung tissue to short-term hyperoxia: A proteomic approach. *Mol. Cell. Biochem*., 383,231–242.

Auten R.L., Jr, Mason S.N., Tanaka D.T., Welty-Wolf K. **&**Whorton M.H. (2001). Anti-neutrophil chemokine preserves alveolar development in hyperoxia-exposed newborn rats. *Am. J. Physiol. Lung Cell Mol. Physiol.*,281(2),L336–44.

Mensack S.M. **&** Murtaugh R. (1999). Oxygen toxicity, *Comp. Cont. Edu. Prac. Veterinar*., 1(4), 341–351.

Mantell L.L., Horowitz S., Davis J.M. **&** Kazzaz J.A. Hyperoxia-induced cell death in the lung—The correlation of apoptosis, necrosis, and inflammation. Ann. N. Y. Acad. Sci.,887,171–180.

Husari A.W., Dbaibo G.S., Bitar H., Khayat A, Panjarian S.,**&** et al. (2006). Apoptosis and the activity of ceramide, Bax and Bcl-2 in the lungs of neonatal rats exposed to limited and prolonged hyperoxia. *Respir. Res.*,7,100. doi: 10.1186/1465-9921-7-100.

Vidal-Jorge M., Sanchez-Guerrero A., Mur-Bonet G., Castro L. **&** et al. (2017). Does Normobaric Hyperoxia Cause Oxidative Stress in the Injured Brain? A Microdialysis Study Using 8-Iso-Prostaglandin F2α as a Biomarker. J. Neurotravma, 4(19),2731-2742.

Hinkelbein J., Braunecker S., Danz M., Böhm L.**&** Hohn A. (2018). Time Dependent Pathway Activation of Signalling Cascades in Rat Organs after Short-TermHyperoxia. *Int J Mol Sci*.,19(7), E1960. doi: 10.3390/ijms19071960).

Ozerkan D., Ozsoy N. **&** Cebesoy S. (2014). Response of thymus lymphocytes to streptozotocin-induced diabetes and exogenous vitamin C administration in rats. *Microscopy (Oxf)*, 63(6), 409-17.

Hatam L.**&** Kayden H.J. (1979). A high performance liquid chromatographic method for the determination of tocopherol in plasma and cellular elements of the blood. *J. Lipid Res.* 20, 639-645.

El Messaoudi S., Rongen G.A., De-Boer R.A. **&** Riksen N.P. (2011). The cardioprotective effects of metformin. *Curr. Opin. Lipidol*., 22,445-453.

Bhamra G.S., Hausenloy D.J. **&** Davidson S.M. (2008). Metformin protects the ischemic heart by the Akt-mediated inhibition of mitochondrial permeability transition pore opening. *Basic Res. Cardiol*., 103,274-284.

Arai M., Uchiba M., Komura H., Mizuochi Y., Harada N. **&** Okajima K. (2010). Metformin, an antidiabetic agent, suppresses the production of tumor necrosis factor and tissue factor by inhibiting early growth response factor-1 expression in human monocytes in vitro. *J. Pharmacol. Exp. Ther.*, 334,206–213.

Chen X., Walther F.J., Sengers R.M.A., Laghmani E.H., Salam A., **&** et al. (2015). Metformin attenuates hyperoxia-induced lung injury in neonatal rats by reducing the inflammatory response. *Am. J. Physiol. Lung Cell Mol. Physiol*., 309(3), L262–L270.

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

Levine R.L., Williams J.A., Stadtman E.R. **&** Shacter E. (1994). Carbonyl assays for determination of oxidatively modified proteins. *Methods in Enzymology*, 233,346-357.

Reading H.W.**&** İsbir T. (1980). The role of cation activated ATPase in transmitter release from the art iris. QJ Exp. Physiol. 65, 105.

Beutler E., Duron O.**&** Kelly B. (1963). An improved method for the determination of blood glutathione. *J. Lab. Clin. Med.* 61, 822–826

Paglia D.E., Valentine W.N. (1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase.. J. Lab. Clin. Med..**70**, 158-169

Beutler E. (1985). Red cell metabolism. In a manual of biochemical methods. pp.72-131, 3th edition Grune&Stratton, New York, NY.

Winterbourn C.C., Hawkins R.E., Brain M. **&** Carrel W. (1975). The estimation of red cell superoxide dismutase activity. J. Lab. Clin. Med.. 55, 337-341.

Aebi H. (1985): Catalase. Methods Enzymol. 125-126.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding Anal Biochem 1976;72:248-254.

Mostafavi S., Ray D., Warde-Farley D., Grouios C. **&** Morris Q. (2008). GeneMANIA: A real-time multiple association network integration algorithm for predicting gene function. *Genome Biol.*, 9 doi: 10.1186/gb-2008-9-s1-s4.

Endesfelder, S., Zaak, I. **&** Weichelt, U. (2014). Caffeine protects neuronal cells against injury causedby hyperoxia in the immature brain. *Free Radic. Biol. Med.*, 67, 221–234.

Romashko J., Horowitz S. **&** Franek W.R. MAPK pathways mediate hyperoxia-induced oncotic cell death in lung epithelial cells. *Free Radical Biology and Medicine*, 35(8), 978–99.

Lee, W.M. **&** Galbraith, R.M. (1992). The extracellular actin-scavenger system and actin

toxicity. *N. Engl. J. Med*., 326, 1335–1341.

Ahotupa, M., Mäntylä, E., Peltola, V. **&** et al. (1992). Pro-oxidant effects of normobaric

hyperoxia in rat tissues. *Acta Phys. Scan*., 145, 151–157.

Winterbourn, C.C. **&** Hampton, M.B. (2008). Thiol chemistry and specificity in redox signaling. *Free Radic. Biol. Med.*, 45, 549–561.

Jones, D.P. (2006). Redefining oxidativestress. *Antioxid. Redox Signal.*, 8, 1865–1879.

Prabbu R. **&** Balasubramanian K.A. (2003): Effect of oxidants on small intestinal brush border membranes and colonic apical membranes- a comparative study. *Comp. Biochem. Physiol. Toxicol. Pharmacol.* 134, 329-339

Tuna M., Polat S., Erman T., Gocer A.I., Tuna N., Tamer L., Kaya M. **&** Cetinalp E. (2001). Effect of anti rat interleukin-6 antibody after spinal cord injury in the rat: inducible nitric oxide synthase expression sodium and potassium activated, magnesium-dependent adenosin 5 phosphatase and superoxide dismutase activation, and ultra sutructural changes. *J. Neurosurg.* 95, 64-73.

Evans J.M., Donnelly L.A., Emslie-Smith A.M., Alessi D.R. **&** Morris A.D. (2005). Metformin and reduced risk of cancer in diabetic patients. *BMJ* 330: 1304–1305.

Ding R.B., Tian K., Cao Y.W., Bao J.L., Wang M., He C., Hu Y., Su H., Wan J.B. (2015). Protective effect of panax notoginseng saponins on acute ethanol-induced liver injury is associated with ameliorating hepatic lipid accumulation and reducing ethanol-mediated oxidative stress. *J. Agric. Food Chem*.,63,2413–2422.

Meister A. (1983). Glutathione, ascorbate and cellular protection. *Cancer Res.*,54, 1969-1974.

Vidyasagar J., Karunakar N., Riddy M.S., Rajnayarana R. (2004). Oxidative stres and antioxidant status in acute organophosphorus insecticide poising. *Indian J. Pharmacol.*, 36, 76- 79.

Park C.S., Bang B.R., Kwon H.S., Moon K.A., Kim T.B., Lee K.Y., Moon H.B. **&** Cho YS. (2012). Metformin reduces airway inflammation and remodeling via activation of AMP-activated protein kinase. Biochem. Pharmacol., 84, 1660–1670.

Salminen A., Hyttinen J.M. **&** Kaarniranta K. (2011). AMP-activated protein kinase inhibits NF-κB signaling and inflammation: impact on healthspan and lifespan. *J. Mol. Med. (Berl)*, 89, 667–676.

Yi M., Jankov R.P., Belcastro R., Humes D., Copland I., Shek S. **&** et al. (2004). Opposing effects of 60% oxygen and neutrophil influx on alveologenesis in the neonatal rat. *Am. J. Respir. Crit. Care Med.*, 170,1188–1196.

Landin K., Tengborn L. **&** Smith U. (1994). Effects of metformin and metoprolol CR on hormones and fibrinolytic variables during a hyperinsulinemic, euglycemic clamp in man. *Thromb. Haemost.*, 71,783–787.