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ORIGINAL ARTICLE

# Evaluation of anti-oxidative effects of propofol in experimental diabetes

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**Abstract** This study was designed to evaluate the serum oxidative status during general anaesthesia established with propofol in alloxan-induced diabetic mice. Sixty mice were randomly allocated into two equal groups. The mice in the diabetic group were injected intraperitoneally with alloxan. Diabetic and normal mice were further divided into five treatment groups of six mice per group. The control group consisted of no treatment whilst the experimental groups received one to four doses of propofol (100 mg/kg BW) at 60-min intervals. In each group, trunk blood samples were collected 30 min after the last injection for the measurement of serum glucose, malondialdehyde (MDA) concentrations, and superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities. Propofol reduced circulating MDA levels and increased serum GPX activity in both alloxan-treated and normal mice. Propofol had no effect on serum glucose concentration. Propofol's increasing effect on GPX activity was significantly greater in alloxan-treated mice (90.27% within 60 min, 131.58% within 120 min, and 77.04% within 180 min) compared to normal mice. Serum SOD activity was significantly higher during exposure to propofol (61% within 180 min) in diabetic mice but was not statistically altered during exposure to propofol over time in normal mice. The results of this study demonstrate ameliorative effects of propofol on oxidative status in an alloxan-induced model of diabetes in mice.

**Keywords** Oxidative stress · Alloxan · Propofol · Enzymes

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## Introduction

Diabetes is a systemic disease that involves the entire body. Therefore, diabetic patients are more predisposed to hospitalization than non-diabetics due to several disorders including cardiovascular and cerebrovascular diseases, infection, nephropathy, and lower-extremity amputations (Clement et al. 2004). It is estimated that diabetic patients have a 50% chance of requiring surgery during their lifetime (Hirsch et al. 1991). Patients with diabetes are susceptible to adverse outcomes that can prolong hospital stay and increase mortality (Krinsley 2003).

Increased oxidative stress through excessive production of reactive oxygen species (ROS) is a major cause of unfavourable evolution of diabetes (Baynes 1991; Baynes and Thorpe 1999; Ceriello 2000). Various mechanisms have been suggested to contribute to the formation of ROS in diabetic patients. Non-enzymatic glycosylation (glycation) followed by auto-oxidation of the glycation products is the main pathway of ROS production in diabetes. In addition, diabetes causes marked changes in component activity in the antioxidant defence system in different organs leading to increased intensity of oxidative stress (Ramakrishna and Jailkhani 2007). On the other hand, surgery by itself results in unfavourable alterations in normal body homeostasis. The involvement of ROS in surgical stress has been hypothesized (Tsuchiya et al.

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2008). Therefore, in addition to the normal risks of surgery, diabetics face additional threats when experiencing surgery. In this context, it seems obvious that improvement of oxidative status will be accompanied by fewer complications in diabetic patients.

Propofol (2,6-diisopropylphenol) is a widely used intravenous anaesthetic and potent sedative agent that is popular in critical care and operating room settings (Hutchens et al. 2006). Propofol has a phenolic chemical structure similar to α-tocopherol and presents antioxidant properties that have been reported both in vitro and in vivo (Ozturk et al. 2008).

This experimental study was conducted to investigate the effects of propofol anaesthesia (without surgery and other medications) on the oxidative stress in the blood of mice with alloxan-induced diabetes.

## Methods

The experiments were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources et al. 1996) and approved by the ethical committee. A total of 60 adult male NIH/Swiss mice each weighing 35–45 g were used. All animals underwent an acclimatisation period of 2 weeks prior to the experiment. Animals were housed six per cage in a room maintained at 23±1°C with an alternating 12-h light/dark cycle. Food and water were available ad libitum. Animals were used once only in all experiments.

The mice were randomly allocated into two equal groups, the alloxan-treated group and normal group. The mice in the alloxan-treated were fasted overnight with free access to water and injected intraperitoneally with alloxan (Sigma Chemical Co., St. Louis, USA) (180 mg/kg body weight) dissolved in a sterile normal saline solution. After 72 h, both diabetic and normal groups were divided into five treatment groups of six mice per group. The control group received no treatment whilst the other groups received one to four intraperitoneal doses of propofol (Pofol®, Jeil Pharmacy, Korea) (100 mg/kg BW) at 60-min intervals. Thus, for each time point, we injected 12 mice (half alloxan-treated and half normal). All rats recovered their righting reflex 25–30 min after the last propofol injection. Thirty minutes after the last injection, trunk blood was collected by decapitation. Samples were kept on ice, centrifuged at 4°C (4,000 g) for 15 min, and the serum was stored at −40°C pending further analysis.

Serum glucose was measured by a glucose oxidase/peroxidase method with 4-aminophenazone as the dye (Darman Kav. Co., Esfahan, Iran). Serum malondialdehyde concentrations (MDA), thiobarbituric acid reactive substances (TBARS), were determined calorimetrically by the Buege method (Buege and Aust 1978). In brief, 50 μl of serum was treated with 2 ml of TBA–TCA–HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl, and 15% TCA) and placed in a water bath for 15 min. After cooling, the flocculent precipitate was removed by centrifugation at 1,000 rpm for 10 min. The absorbance of the supernatant was measured against a reference blank at 535 nm. The concentration was calculated using an extinction coefficient of $1.56\times10^5$ $M^{-1}cm^{-1}$ and expressed as nanomoles per litre.

Total serum superoxide dismutase (SOD) activity was determined according to the method by Sun (Sun et al. 1988). This method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme activity causing 50% inhibition in the NBT reduction rate.

Plasma glutathione peroxidase (GPX) activity was measured using the Paglia and Valentine (1967) method. The test is based on the conversion of NADPH + $H^+$ into $NADP^+$. One unit of GPX was defined as 1 μmol ADPH + $H^+$/min/mg protein.

Serum total protein levels were measured using a Bio-Rad protein assay kit.

The data were expressed as the mean ± SEM. Two-way analysis of variance followed by Bonferroni post hoc test was used, and differences were considered significant when $p<0.05$. All statistical analyses were performed using the SigmaPlot11 (Systat Software Inc.).

## Result

Serum glucose concentration was significantly higher in the alloxan-treated mice than in normal mice, 10.61 and 4.8 mmol/L, respectively ($p<0.001$). There were no significant differences in serum glucose during exposure to propofol in both groups over time (Fig. 1). MDA excretion

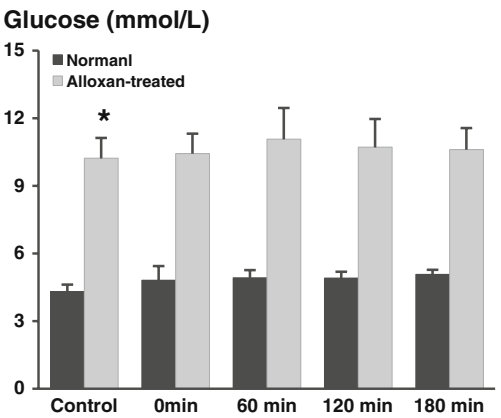


**Fig. 1** Effects of propofol on serum glucose concentration (mean ± SEM). * Indicates statistically significant difference ($p\leq0.001$) when compared to normal mice without propofol injection (control)

increased significantly in alloxan-treated animals compared to normal mice (5.21 and 2.41 nmol/L, respectively), whereas significantly smaller concentration of MDA were found during exposure to propofol in both alloxan-treated (−30.5% within 60 min, −37% within 120 min, and −39.71% within 180 min) and normal mice (−40.66% within 120 min and −45.23% within 180 min) (Fig. 2).

Serum activity of GPX was significantly reduced in alloxan-treated mice compared to the normal group (0.92 and 1.86 μm NADPH + $H^+$/min/mg protein, respectively). Measurements of GPX levels were significantly higher during exposure to propofol in alloxan-treated (96.49% within 60 min, 181.87% within 120 min, and 210.74% within 180 min) and normal mice (54.5% within 60 min, 78.49% within 120 min, and 110.75% within 180 min). Increasing effect of propofol administration on serum GPX activity was significantly greater in alloxan-treated mice (90.27% within 60 min, 131.58% within 120 min, and 77.04% within 180 min) compared to increasing rate of enzyme activity in normal mice (Fig. 3). Serum activity of SOD was significantly reduced in alloxan-treated mice compared to normal group (3.18 and 4.17, respectively). Measurements of SOD activity were significantly higher during exposure to propofol (61% within 180 min) in diabetic mice but were not statistically altered during exposure to propofol over time in normal mice (Fig. 4).

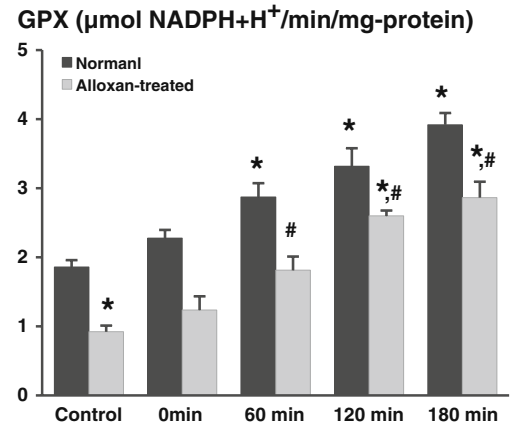


**Fig. 3** Effects of propofol on serum GPX activity (mean ± SEM). * Indicates statistically significant difference ($p \leq 0.01$) when compared to normal mice without propofol injection (control). # Indicates statistically significant difference ($p \leq 0.005$) when compared to alloxan-treated mice without propofol injection (control)

## Discussion

In the present study as expected, alloxan treatment induced a diabetic state characterized by hyperglycaemia and severe oxidative stress. Lipid peroxidation is a characteristic feature of diabetes mellitus. Several studies have reported elevated levels of lipid peroxides in the plasma of diabetic rats (Armstrong and al-Awadi 1991; Chis et al. 2009) and mice (Sharma and Garg 2009). Under physiological conditions, concentrations of lipid peroxides in tissues are low. Low levels of lipid peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled lipid peroxidation, thus leading to cellular infiltration and tissue damage (Metz et al. 1984). Measurement of TBARS is a commonly used assay that measures MDA as the degradation end product of lipid peroxidation (Walter et al. 2008). In the present study, the increased level of MDA in serum may be a reflection of the increased lipid peroxidation in different tissues of alloxan-treated mice. Alloxan breaks down DNA in pancreatic islets cells followed by hyper-

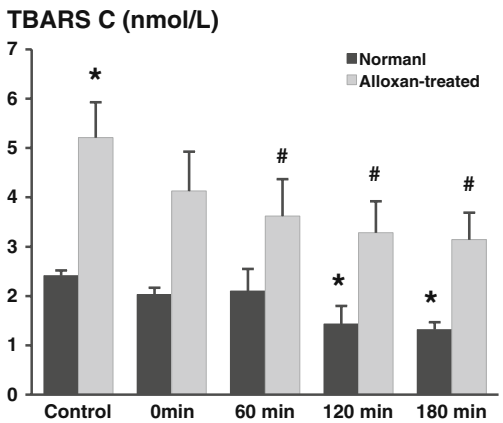


**Fig. 2** Effects of propofol on serum MDA (TBARS) concentration (mean ± SEM). * Indicates statistically significant difference ($p \leq 0.005$) when compared to normal mice without propofol injection (control). # Indicates statistically significant difference ($p \leq 0.005$) when compared to alloxan-treated mice without propofol injection (control)

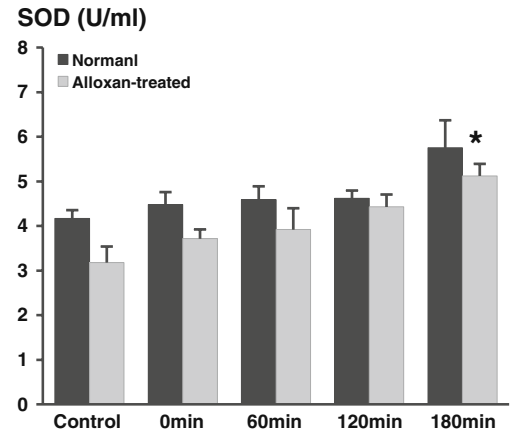


**Fig. 4** Effects of propofol on serum SOD activity (mean ± SEM). * Indicates statistically significant difference ($p < 0.05$) when compared to normal mice without propofol injection (control). # Indicates statistically significant difference ($p < 0.05$) when compared to alloxan-treated mice without propofol injection (control)

glycaemia and excessive production of ROS. Thus, the alloxan system was considered adequate for the study of a pathology in which free radicals might have a central role, such as diabetes (El-Missiry and El Gindy 2000). Our results showed that animals exposed to propofol have lower circulating measurements of MDA. This finding is consistent with several studies showing the inhibitory effect of propofol on lipid peroxidation (Kahraman et al. 1997; Runzer et al. 2002; Sanchez-Conde et al. 2008).

Oxidative stress in diabetes is associated with a reduction in the antioxidant system capacity, which can increase the harmful effects of toxic free radicals. Several enzymes are involved in this process. SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical, converting it into hydrogen peroxide and molecular oxygen (Heistad 2006). GPX is a selenium-containing enzyme which reduce $H_2O_2$ and organic hydroperoxides by employing glutathione (GSH) as an electron donor (Bae et al. 2009). Reduced activities of SOD and GPX in different tissues during diabetes have been reported, resulting in several deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Chis et al. 2009; Li 2007). We observed a significant increase in GPX activity in both normal and alloxan-treated groups within 60, 120, and 180 min of exposure to propofol. Increased SOD activity was observed in alloxan-treated mice within 180 min of propofol exposure, whereas systemic SOD activity was not altered in normal mice during exposure to propofol over the time. Allaouchiche et al. (2001) and more recently Abou-Elenain (2010) have found increased GPX levels during exposure to propofol in blood and bronchoalveolar lavage in pigs and humans respectively; whereas, the activity of SOD was not statistically altered. However, De La Cruz et al. found that propofol reduced MDA concentration, increased antioxidant GSH activity, and reduced levels of GPX in different tissues in rats (De La Cruz et al. 1998) and human platelets (De La Cruz et al. 1999). Another study reported increasing effects of propofol on SOD activity in rat intestinal mucosa (Liu et al. 2007). These discrepancies could be due to species differences, different propofol doses, and duration of exposure.

The antioxidant activity of propofol is due to its phenolic structure, similar to that of α-tocopherol. Propofol preferentially scavenges organoradical species since it is able to reduce significantly riboflavin radicals and the formation of MDA degradation products generated from lipid hydroperoxides of arachidonic acid (Green et al. 1994). In conclusion, our results suggest that propofol exposure has great ameliorative effects on oxidative status in alloxan-treated mice.

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