Chapter 12
The Protective Effects of Natural Polyphenolic Complexes of Grape Wine on Organisms Exposed to Oxidative and Nitrosative Stress Under Diabetes Mellitus

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Abstract Peripheral nerve damage is a significant complication of diabetes mellitus. The ingestion of polyphenols available naturally in a variety of plant products may provide impressive protection against such damage. The natural polyphenol complex of grape wine has a significant anti-diabetic effect. It can protect against dehydration at the level of the whole organism and deter free radical-induced damage to the sciatic nerve, the spinal cord, kidney and retina. The levels of nitrosylated and PARylated proteins can be restored to near control levels by the extract. The biochemical mechanisms of action of the natural polyphenol complex of grape wine require further research, but may be considered as a valuable therapeutic approach for the treatment of diabetic complications.

12.1 Introduction

Diabetes Melitus (DM) has reached epidemic proportions with 6–8 % of the population of the developed countries suffering from the disease. The statistical research conducted by the World Health Organization (WHO) predicts an increase in the number of diabetics in 2025 to up to 380 million people. In Ukraine, more than one million patients with DM are registered. The number of children with diabetes under the age of 5 from 1985 till 2009 has increased by seven times. Today, in Ukraine, more than 8,000 children are affected by DM. The growth in the incidence of diabetes, the

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early disability of the working population causing a significant economic damage to the country, reduced quality of life and a shortening of its duration in patients with diabetes have attracted the attention of scientists to this problem.

Hyperglycemia is the main established pathogenetic factor for the development of DM, which occurs against the background of insulin deficiency, and is the prime determinant of the diagnosis of type 1 DM. Under hyperglycemia, the violation of the electron transport chain leads to the overproduction of superoxide anion, which, interacting with other reactive oxygen species (ROS), activates free radical oxidation leading to oxidative stress (OS) [1]. Oxidative stress, in its turn, causes the disruption of cellular homeostasis, accumulation of molecules with altered structure, damage to the structure of lipids, proteins and DNA. In response to DNA damage, the nuclear enzyme poly(ADP-ribose) polymerase (PARP-1) is activated. Poly(ADP-ribosyl)ation of numerous nuclear proteins takes place causing significant energy depletion of cells. Under certain conditions, it can cause cell death [22]. It has been proven that PARP-1 can poly(ADP-ribosyl)ate glyceraldehyde 3-phosphate dehydrogenase (the enzyme of glycolysis) that leads to the inhibition of glycolytic glucose utilization at the level of glyceraldehyde 3-phosphate formation with subsequent accumulation of the intermediate products of glycolysis. This activates a number of signaling and metabolic pathways, including polyol and hexosamine pathways, activation of PKC and accumulation of methylglyoxal, uncharacteristic for normal physiological conditions. Such changes in metabolism (e.g. accumulation of fructose, sorbitol, methylglyoxal, advanced glycation end products, etc.) is a trigger for the development of diabetic angiopathy, such as damage of retinae (retinopatia), kidneys (nephropatia), the peripheral nervous system (neuropatia), etc.

Activation of alternative pathways of glucose utilization leads to an increase in nonenzymic glycosylation under diabetic conditions. Under the interaction of AGEs with specific receptors (RAGES), the cascade of mechanisms is activated, causing increased gene expression that encodes a number of proinflammatory cytokines (TNF-α, IL 1,6), vasoconstrictors – endothelin 1, adhesion molecules (ICAM-1, VCAM-1) and growth factors. They disturb the function of blood vessels and contribute to premature development of atherosclerosis and inflammatory processes [19], induce the super-activity of the local renal renin-angiotensin system, help to increase glomerular blood pressure and filtration rate, etc. The latter injuries cause the development of microalbuminuria and microproteinuria. Accordingly, we can assume that the effect of OS and AGEs primarily leads to the development of diabetic nephropathy (DN).

Diabetic nephropathy occurs as a result of a complex variety of pathological processes which are formed primarily in the capillaries and small vessels of the kidneys. Like all microangiopathies, this disease is realized through the emergence and progression of endothelial dysfunction.

As well known, kidney glomerulus consists of endotheliocytes that cover the capillaries from the inside and podocytes that cover the capillaries from the outside, providing a filter barrier and mesangial cells. These structures are the elements of smooth muscle tissue that are located around the capillaries and involved in the regulation of blood flow velocity. Experimental DM (EDM) is characterized by podocyte apoptosis

that is accompanied by a sharp decrease in their number. All of these changes can lead to a thickening of the filtering barrier with the involvement of proteins, (collagen IV, in particular) with further progression of fibrogenesis until the complete loss of the physiological function of the glomerulus. This is the reason why inflammatory processes in the glomerulus are accompanied by an increase in body size and weight [15].

DM retinopatia at early stages is characterized by a partial increase in permeability of retinal vessels, the loss of vessel pericytes followed by a thickening of the vessel walls, their degeneration, poor blood circulation in the eye and the development of hypoxia [8]. A decrease in perfusion of capillaries and the resultant hypoxia leads to neovascularization, excessive development of abnormal endothelium and the accumulation of collagen. Angiogenesis is accompanied by the modification of endothelium and basement membrane degradation. All of this eventually leads to retinal detachment [4].

Accumulation of fructose, sorbitol, methylglyoxal, and the advanced glication end products is a trigger for the development of DN. Initial metabolic changes occurring in the nerve fibers cause disruption of their functions, and eventually lead to changes in their structure. In particular, the activation of the polyol pathway in nervous tissue with the characteristic accumulation of sorbitol and fructose, which consistently causes a decrease in activity of Na^+, K^+-ATPase and the level of mioinozytol, leads to the retention of Na^+ and water, swelling of the myelin sheath, its further demyelination and reduction of motor and the sensory nerve conduction velocity of the peripheral nervous system.

Simultaneously, in parallel with it, there is a total destruction of the organism by ROS that, in addition to DNA damage, causes damage of axon membrane structures of peripheral nerve fibers and, as a result, damage of the nerve cell structures and functions. In addition to direct damaging effects, the accumulation of ROS affects the energy metabolism in neurocites and development of endoneurial hypoxia. Such a comprehensive total damage causes demyelination and degeneration of the nerve fibers and reduces their functional activity. Under these conditions, the antioxidant system of the organism plays an important role, particularly its enzymatic component, the main task of which is the neutralization of free radicals. Under DM it has reduced activity. Strengthening of the antioxidant system by exogenous antioxidants could provide a protective effect to all body systems.

Research into natural antioxidants – polyphenol complexes of grape wine, including proanthocyanidins, derived flavan-3-ols and several other derivatives of flavonoids, which are effective in preventing cardiovascular diseases, have become promising recently [16]. It is known that polyphenols of grape wine are able to interact with plasma proteins and cellular elements of blood, prevent premature oxidation of their molecular complexes, which occurs under oxidative and nitrosative stress. Significant bactericidal and antiviral effect of these substances has been shown [5]. The protective effects of the polyphenols complexes of grape wine on some systems and organs under oxidative stress and during the metabolic syndrome have been detected [17, 18].

The protective properties of natural grape polyphenol complexes under streptozotocin-induced DM and their effect on the enzymic antioxidant system during the development of angiopathies have been sparsely investigated. The goal of our work,

therefore, has been to investigate the protective antioxidant effect of polyphenol complexes of grape wine on the enzymatic antioxidant system, its ability to prevent the formation of nitrotyrosine modified proteins and activation of PARP-1 in different tissues of rats with streptozotocin-induced DM.

12.2 Materials and Methods

All animal care and procedures were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes Directive of 24 November 1986 (86/609/ECC) and were approved by Bioethics Committee of the Ivan Franko National University of Lviv Protocol for Animal Studies, Lviv, Ukraine. Male Wistar rats, of 190–210 g body weight, were fed a standard rat chow and had access to water *ad libitum*.

The specimen of natural polyphenols complexes of grape wine was received by evaporation of red wine in rotary evaporators LABOROTA 4000 (Heidolph, Germany). The red wine was made by the classical technology from Cabernet Sauvignon (clone C337/S04C3) grapes and contained phenolic compounds (2,309 mg/L), proanthocyanidines (936 mg/L) and pigment polymers (444 mg/L).

The rats were separated into four groups of seven animals each: Group 1 – normal untreated control, Group 2 – polyphenol treated, Group 3 – streptozotocin (STZ) treated, and Group 4 – polyphenol and STZ treated. The STZ treatment was a single i.p. injection of 50 mg STZ/kg body weight. The specimen treatment was an oral dose (300 ml/70 kg body weight/day) that constituted 23.5 mg/kg body weight/day administered daily for 2 weeks prior to the STZ injection and daily for 4 weeks after the STZ injection. Group 2 received a specimen of natural polyphenol complexes of grape wine for 6 weeks. Blood samples for glucose measurements were taken from the tail vein 72 h after the STZ injection and the day prior to the study termination. All of the rats with blood glucose of 14 mmol/L or more were considered diabetic.

The animals were sedated by CO_2 and immediately sacrificed by cervical dislocation. One sciatic nerve, an eye and a part of the spinal cord and dorsal root ganglia (lumbar and sacral nerve roots) from each rat were fixed in 10 % neutral-buffered formalin for further assessment of poly(ADP-ribose) by conventional immunohistochemistry. The second sciatic nerve, eye bowl and another part of the spinal cord and dorsal root ganglia from each rat were immediately frozen in liquid nitrogen for subsequent Western blot analyses of the protein 3-nitrotyrosine content.

To assess nitrosylated proteins by Western blot analysis, tissue samples were transferred to an extraction buffer (1:10 wt/vol), containing 50 mM Tris–HCl (pH 7.2), 150 mM NaCl, 0.1 % sodium dodecyl sulfate (SDS), 1 % NP-40, 5 mM EDTA, 1 mM EGTA, 1 % sodium deoxycholate, and the protease/phosphatase inhibitors: leupeptin (10 μg/ml), aprotinin (20 μg/ml), benzamidine (10 mM), phenylmethylsulfonyl fluoride (1 mM), sodium orthovanadate (1 mM), and homogenized on ice. The homogenate was sonicated (3×5 s) and centrifuged at 14,000 g for 20 min. All the

afore-mentioned steps were performed at 4 °C. The lysates (20 μg of total protein per lane) were mixed with the equal volume of sample-loading buffer, containing 62.5 mM Tris–HCl (pH 6.8), 2 % SDS, 5 % β-mercaptoethanol, 10 % glycerol and 0.025 % bromophenol blue, heated at 95 °C for 5 min, separated on 10 % SDS-PAGE and transferred onto a nitrocellulose membrane.

Free binding sites were blocked in 2 % (w/v) bovine serum albumin (BSA) in phosphate buffer saline (PBS) containing 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.7 mM KH_2PO_4, pH 7.3, and 0.05 % Tween 20, for 1 h, after which nitrotyrosine antibodies were applied for 2 h. The horseradish peroxidase-conjugated secondary antibody was then applied for 1 h. After extensive washing, protein bands detected by the antibodies were visualized with the ECL Detection Reagents (Amercham, USA). The total content of all nitrosylated proteins was quantified by densitometry (Gel Pro Analyzer 3.1, Media Cybernetics USA). Membranes were then stripped in the 25 mM glycine-HCl, pH 2.5 buffer containing 1 % SDS, and reprobed with β-actin antibody to confirm equal protein loading.

All sections were processed by a single investigator and evaluated blindly. Poly(ADP-ribose) immunoreactivities in the sciatic nerve, spinal cord, dorsal root ganglia neurons (DRG) and retina were assessed by immunohistochemical techniques (light and fluorescent microscopy). Tissues were fixed in 10 % neutral-buffered formalin and 7 μm sections were prepared from paraffin embedded tissues. Paraffin sections were deparaffinized in xylene, hydrated in decreasing concentrations of ethanol and washed in water. Optimal staining was achieved with antigen retrieval solution.

For immunofluorescent histochemistry, non-specific binding was blocked with the 10 % of normal goat serum and 1 % BSA in PBS buffer in the humidity chamber for 1 h. Firstly, mouse monoclonal antipoly(ADP-ribose) antibody was diluted 1:100 in 1 % BSA in PBS, and applied overnight at 4 °C in the humidity chamber. Secondly, Alexa Fluor 488 goat anti-mouse antibody was diluted 1:200 in PBS and applied for 2 h at room temperature. Sections were mounted in Prolong Gold Antifade Reagent.

For light microscopy, endogenous peroxidase was quenched with 0.3 % H_2O_2 for 20 min. Non-specific binding was blocked with the 10 % of normal goat serum and 1 % BSA in PBS buffer in the humidity chamber for 1 h. Then, avidin/biotin blocking kit (Vector Laboratory Inc., USA) was used to block endogenous biotin and avidin. Mouse monoclonal anti-poly(ADP-ribose) antibody (diluted 1:100 in 1 % BSA in PBS) was applied overnight at 4 °C in the humidity chamber. The detection was performed using the secondary biotin-conjugated goat anti-mouse antibody (diluted 1:200 in PBS and applied for 2 h at room temperature) and Vectastain Elite ABC kit (Vector Laboratory Inc., USA). The positive signals were visualised with the 3,3′-diaminobenzidine (DAB substrate kit, Vector Laboratory Inc., USA). The sections were counterstained with hematoxylin, dehydrated and mounted in the micromount mounting medium (Surgipath Medical Ind., Inc, USA).

Negative controls included elimination of the primary antibody. At least ten fields of each section were examined to select one representative image. Representative images were microphotographed and the intensity signal was quantified with ImageJ 1.32 software (National Institutes of Health, USA). Low power observations of

Table 12.1 Body weight and blood glucose concentration in control and diabetic rats with or without polyphenol complexes consumption (mean±s.e.m, n=5–7)

Indicators / Rodent group	Blood glucose (mmol/l)		Body weight (g)	
	Initial§	Final	Initial§	Final
C	5.7±0.5	6.2±0.5	206.0±16.1	279.0±19.6
C+Pol	5.9±0.2	6.3±0.2	202.0±17.3	276.0±20.0
D	18.5±0.9**	26.8±2.2**	200.0±6.8	209.0±30.0**
D+Pol	20.0±0.3**	26.9±1.9**	198.0±6.4	234.5±10.5*,#

Notes: *C* control, *C+Pol* control+specimen of polyphenols, *D* diabetic, *D+Pol* diabetic+specimen of polyphenols. *P<0.05 and **P<0.01 vs. control, #P<0.05 vs. control and diabetic rats without polyphenol specimen consumption. § – third day after induction of diabetes

sciatic nerve, spinal cord, DRG and retina sections stained for poly(ADP-ribose) were made using a Nikon Optiphot 2 imaging microscope. Color images were captured with a DCM310 microscope CMOS camera. Low power images were generated with a 40× acroplan objective using the automatic capturing feature of the ScopePhoto software.

Tissues homogenization was carried out using hand homogenizers in the presence of 0.1 M phosphate buffer (1:10 wt/vol) pH 7.0 on ice. Homogenized samples of spinal cord were centrifuged for 30 min at 14,000 g at 4 °C. After the removal of a thin lipid layer, it was recentrifuged for 15 min at 10,000 g at 4 °C. Samples of sciatic nerves and DRG were centrifuged for 20 min at 10,000 g at 4 °C.

The activity of superoxide dismutase (SOD) was determined by Chevari method, catalase by the Corolyk method, glutathione peroxidase by the Moin method, glutathione reductase by the Goldberg method. The MDA level was analyzed with 2-thiobarbituric acid by the Timyrbulatov method. The concentration of protein was determined by the Lowry method.

Differences among experimental groups were determined by ANOVA and the significance of between-group differences was assessed by Student–Newman–Keul's multiple range test. Significance was defined at P≤0.05.

12.3 Results and Discussion

At the end of the experiment, the final body weight of control rats and rats consuming the specimen of natural polyphenol complexes of grapes increased by 36 % compared with the body weight of animals before the experiment (Table 12.1). In contrast to the control groups, the body weight of rats with diabetes slightly decreased. At the same time, the body weight of rats with diabetes that consumed the specimen increased by 18 %. In groups of rats with STZ-induced diabetes, this phenomenon can be explained by the "overproduction" of urea. The latter was excreted from the body by the osmotic diuresis together with corresponding amount of water, K^+ and Na^+ ions [13]. This process leads to dehydration, which further increases due to the release of free fatty acids from adipocytes followed by their conversion to ketone
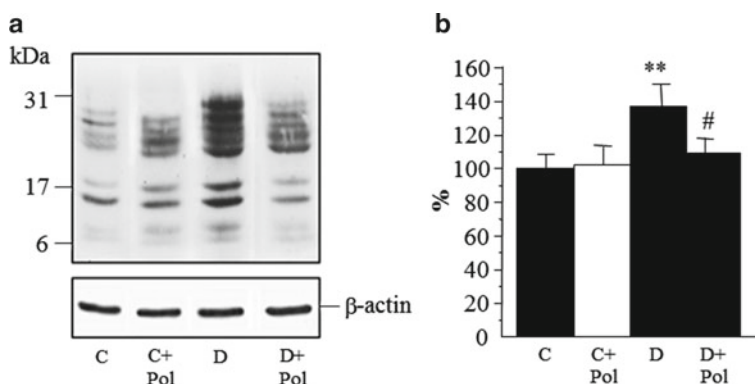
Fig. 12.1 Representative Western blot analyses of nitrotyrosine-modified proteins in the retinae (**a**) of control and diabetic rats with and without polyphenol consumption. Total nitrotyrosine content (**b**). Total nitrotyrosine protein content in control rats is taken as 100 %. Equal protein loading was confirmed with β-actin antibody. *C* control groups, *D* diabetic groups, *Pol* polyphenols complexes of grape wine. The data are expressed as mean±s.e.m., n=5–7. **p<0.01 vs. controls; #p<0.05 vs. diabetic rats without polyphenols consumption

bodies (acetoacetate and β-hydroxybutyrate) leading to ketoacidosis. The ketone bodies "provoke" an increase in osmotic diuresis and loss of electrolytes [14]. Such changes of the level of body water affect the general metabolism and body weight. The indices of body weight in groups of rats with STZ-induced diabetes that consumed the specimen increased significantly to control values.

The results presented in Table 12.1, comparing indices at the beginning and the end of the experiment, show an increase in hyperglycemia in the groups affected by DM and the absence of any glycemic effect of the polyphenolic complexes.

Other researchers have shown that relatively high doses of resveratrol, which are basically a part of the polyphenol complex of grape wine, significantly reduced glucose levels to normal in rats with STZ-induced diabetes [23]. The results indicate that a decrease in the level of blood glucose of diabetic animals depends upon the variety of grapes and the amount of the dose.

Reactive nitrogen compounds, including peroxynitrite play a crucial role in the pathogenesis of diabetes and its complications [9, 22]. It has been recently revealed that nitrotyrosine (NT), a marker of oxidative-nitrosative stress, is accumulated in vascular endothelium, myocardium [22], retina [7], blood flow [2], kidneys, and the peripheral nervous system of diabetic rodents as well as in obese fatty Zucker rats, Zucker diabetic fatty rats and leptin knockout (ob/ob) mice [3, 20]. These data indicate that peroxynitrite-induced injuries are present in the early and late stages of Type 1 and Type 2 DM and during the prediabetic stages. Peroxynitrite is a powerful oxidant in the pathogenesis of diabetic complications [10, 20], including endothelial [22], peripheral and autonomic neuropathy [10], and retinopathy [12].

Western blot analysis showed an increase in the content of NT in the retina of rats with DM by 38 % compared with control (Fig. 12.1). Nitrosative stress was partially normalized by the consumption of polyphenol complexes of grape wine, and the
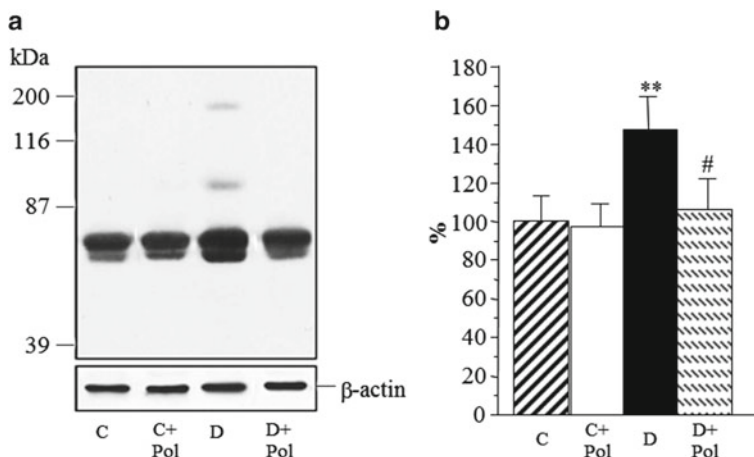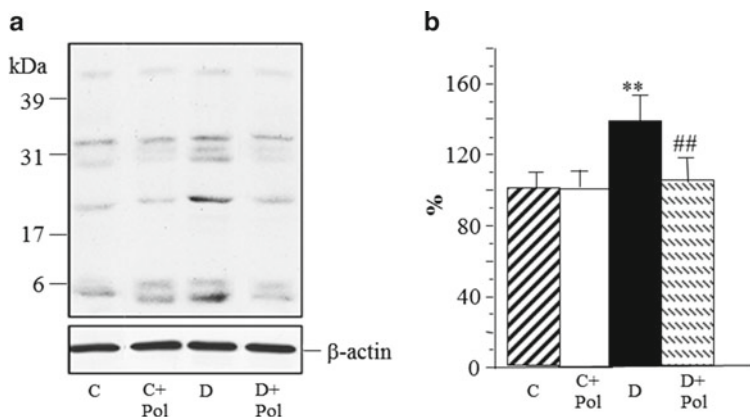
Fig. 12.2 Representative Western blot analyses of nitrotyrosine-modified proteins in the sciatic nerve (**a**) of control and diabetic rats with and without polyphenols consumption. Total nitrotyrosine context (**b**). Total nitrotyrosine protein content in control rats is taken as 100 %. Equal protein loading was confirmed with β-actin antibody. *C* control groups, *D* diabetic groups, *Pol* polyphenols complexes of grape wine. The data are expressed as mean ± s.e.m., n = 5–7. **p < 0.01 vs. controls; ##p < 0.01 vs. diabetic rats without polyphenols consumption
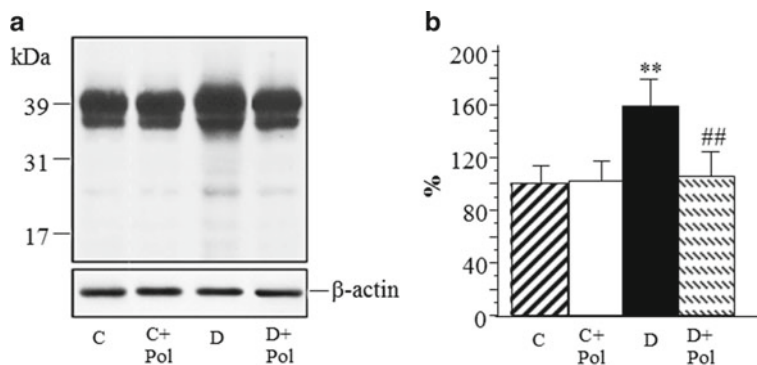
Fig. 12.3 Representative Western blot analyses of nitrotyrosine-modified proteins in the DRG (**a**) of control and diabetic rats with and without polyphenols consumption. Total nitrotyrosine context (**b**). Total nitrotyrosine protein content in control rats is taken as 100 %. Equal protein loading was confirmed with β-actin antibody. *C* control groups, *D* diabetic groups, *Pol* polyphenols complexes of grape wine. The data are expressed as mean ± s.e.m., n = 5–7. **p < 0.01 vs controls, ##p < 0.01 vs. diabetic rats without polyphenols consumption

recovery of the level of nitrotyrosine was almost to control levels (P < 0.05). The similar increase in the content of nitrotirosine-modified proteins was observed by 48 % in the sciatic nerve (Fig. 12.2), by 60 % in the DRG (Fig. 12.3) and by 40 % in the spinal cord (Fig. 12.4) of the rats with diabetes. After consumption of the specimen of natural polyphenol complexes by rats with DM, we observed a partial

Fig. 12.4 Representative Western blot analyses of nitrotyrosine-modified proteins in the spinal cord (**a**) of control and diabetic rats with and without polyphenols consumption. Total nitrotyrosine context (**b**). Total nitrotyrosine protein content in control rats is taken as 100 %. Equal protein loading was confirmed with β-actin antibody. *C* control, *D* diabetic groups, *Pol* polyphenols complexes of grape wine. The data are expressed as mean ± s.e.m., n = 5–7. **p < 0.01 vs. controls, ##p < 0.01 vs. diabetic rats without polyphenols consumption

(sciatic nerve) and full (DRG, spinal cord) return of the contents of NT to the control level.

It may be assumed that polyphenol complexes of grape wine partially or completely prevent an increase in the content of nitrotyrosine-modified proteins and this can recover or stabilize the function of retinae and the peripheral nervous system of the body affected by diabetes. The damage caused by peroxynitrite can lead to motor and sensory neuropathies through various mechanisms, including activation of PARP-1 [20]. Thus, in response to DNA damage by reactive oxygen species, the reparatory complex, which includes PARP enzyme poly(ADP-ribosyl)ation over 300 core proteins and some cytoplasmic proteins, is activated. Therefore, the activation of PARP-1 and oxidative/nitrosative stress can be mutually reinforcing, and the determination of their levels is necessary in order to evaluate the degree of tissue damage under conditions of diabetes. The content of poly(ADP-ribosyl)ated proteins recorded in microphotographs of retinae increased by 47 % in rats with diabetes, and this increase was normalized by the consumption of polyphenols (Fig. 12.5).

The intensity of luminescence in immunofluorescent microphotographs, which identified the level of poly(ADP-ribosyl)ated proteins in cells of the sciatic nerve, increased by 51 % in rats with diabetes [11]. This increase was normalized by polyphenol consumption (Fig. 12.6). At the same time, the level of poly(ADP-ribosyl)ated proteins in spinal cord and DRG in control and diabetic rats did not differ significantly (Fig. 12.7).

As was noted above, the abnormal changes in neuropathy occur in and extend from the distal parts of the nerves. Thus, in the diabetic neuropathy, the loss of sensory sensitivity is first to occur. After that, demyelination of nerve conduction with
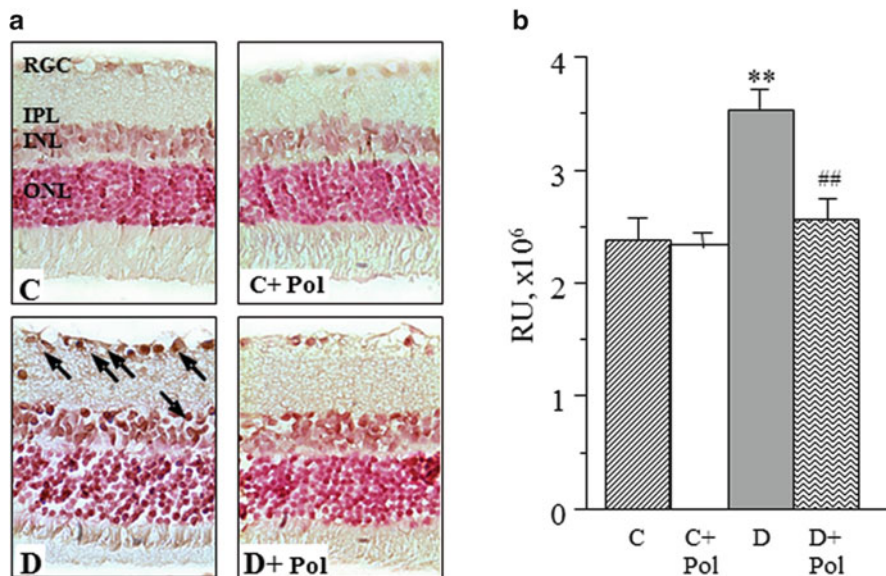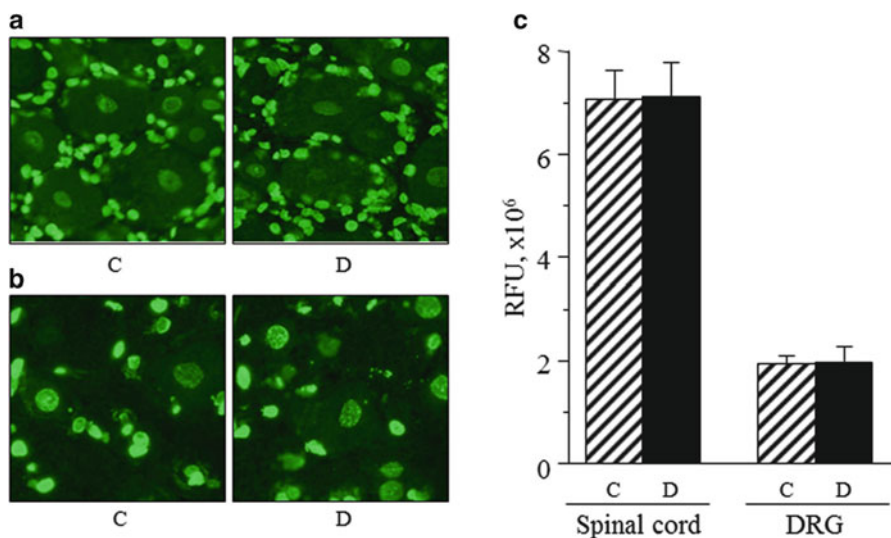
Fig. 12.5 Representative microphotographs of immunofluorescent staining of poly (ADP-ribose) in retine of control and diabetic rats with and without polyphenol consumption. Microphotographs (40×) of immunohistochemical staining of poly(ADP-ribose) in retinae of control and diabetic rats with and without polyphenol consumption (**a**). Total poly(ADP-ribose) content (relative units per image) in retinae (**b**). *Arrows* show examples of poly(ADP-ribosyl)ated proteins of retinae cells. *RGC* retinal ganglion cells, *IPL* inner plexiform layer, *ONL* outer nuclear layer, *INL* inner nuclear layer. Means ± s.e.m., n = 10–15 per group. *C* control rats, *D* diabetic rats, *Pol* polyphenol complexes of grape wine. **p < 0.01 vs. controls, ##p < 0.01 vs. diabetic rats without polyphenol consumption

loss of the sensory and motor nerve velocity takes place. Degeneration both of a single axon and the nerve fiber occurs in the direction from the periphery to the center and is characterized by the term "death back" [6]. It should be noted that our data indicate that PARP-1 is activated in the sciatic nerves, but the activation is absent in the spinal cord and DRG of rats with DM (Fig. 12.7). This confirmed and extended the interpretation of previous studies, which have shown that structurally different inhibitors of PARP-1 restored the conductivity of both motor and sensory nerves, offset the phenomenon of neurovascular dysfunction, restored the energy of the peripheral nervous system and removed signs of sensory neuropathy in rats with 4 weeks of diabetes [20]. Therefore, the activation of PARP-1 in the sciatic nerve and its absence in the spinal cord and DRG indicate that this enzyme occupies a central role in the development of an early neuropathy in the peripheral nervous system during DM [21]. The ability of polyphenols to inhibit PARP-1 supports their potential to prevent the early development of angiopathy during diabetes.

The activity of the enzymatic antioxidant system during DM can change in different ways in different tissues, but always decreases. Consistent with this idea, a
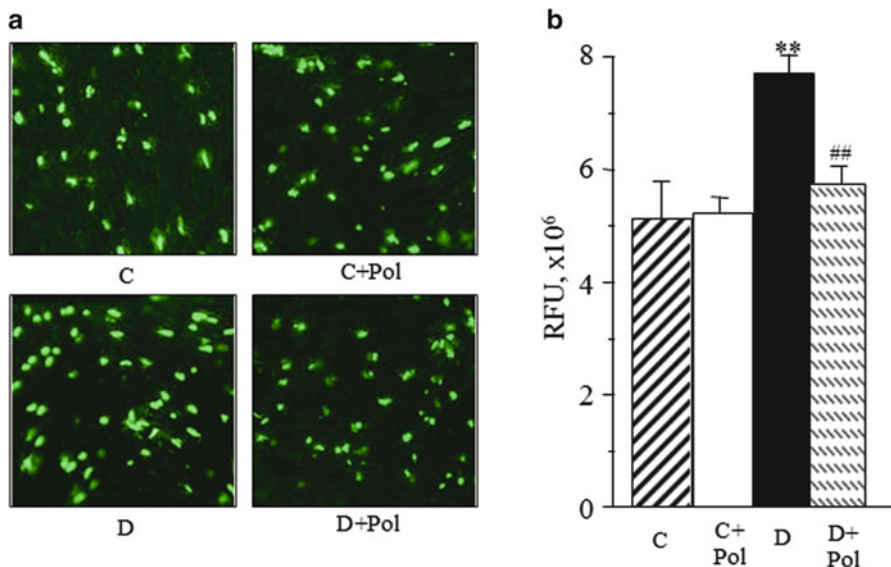
Fig. 12.6 Representative microphotographs (100×) of immunofluorescent staining of poly(ADP-ribose) in sciatic nerves (**a**). Intensity of poly(ADP-ribose) fluorescence (relative fluorescence units per image) in sciatic nerves (**b**). *C* control groups, *D* diabetic groups, *Pol* polyphenol complexes of grape wine. Data are expressed as mean ± s.e.m., n = 5–7. **p < 0.01 vs. controls, ##p < 0.01 vs. diabetic rats without polyphenols consumption


Fig. 12.7 Representative microphotographs (100×) of immunofluorescent staining of poly(ADP-ribose) in spinal cord (**a**) and in DRG (**b**). Intensity of poly(ADP-ribose) fluorescence (relative fluorescence units per image) in DRG and spinal cord (**c**). *C* control groups, *D* diabetic groups, *Pol* polyphenol complexes of grape wine. Data are expressed as mean ± s.e.m., n = 5–7
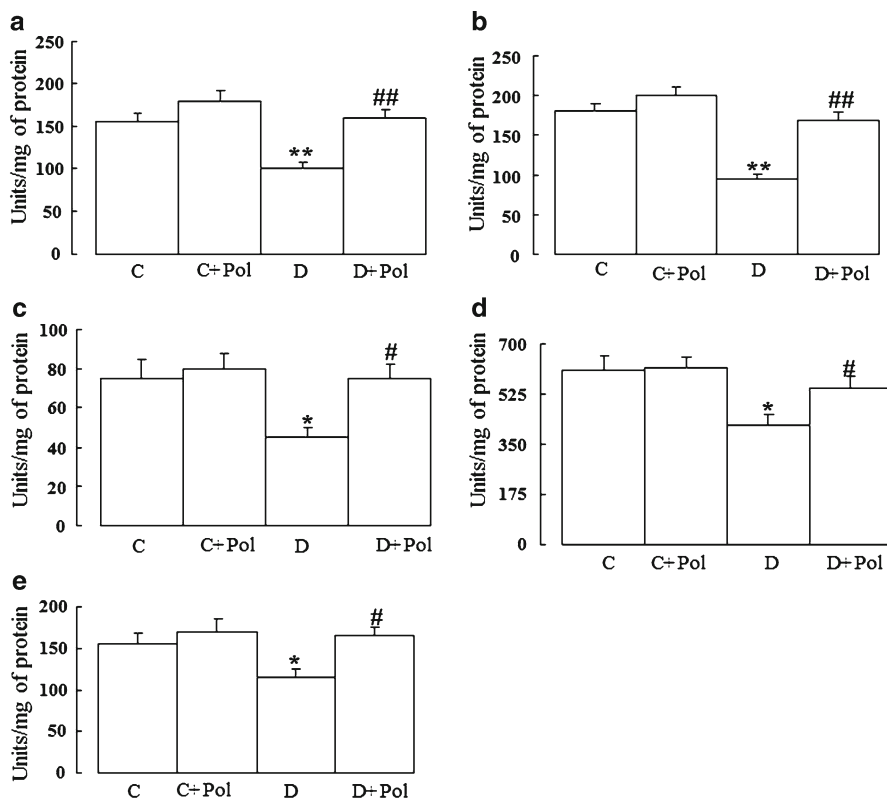
Fig. 12.8 The activity of superoxide dismutase in the kidney (**a**), retina (**b**), sciatic nerve (**c**), spinal cord (**d**) and DRG (**e**). *C* control, *C + Pol* control + polyphenol complexes of grape wine, *D* diabetic, *D + Pol* diabetic + polyphenols complexes of grape wine. Mean ± s.e.m., n = 5–7. *P < 0.05 and **P < 0.01 vs. controls; #P < 0.05 and ##P < 0.01 vs. untreated diabetic group

significant decrease in the activity of superoxide dismutase was shown in the sciatic nerve, dorsal root ganglia and spinal cord, kidney and retina (by 40, 26, 32, 31 and 47 %, respectively) (Fig. 12.8).

A decrease in the activity of SOD is directly associated with the accumulation of superoxide anions, which is typical for most tissues under DM conditions. Violation of the mechanism of utilization of these anions leads to the formation of another oxidant – peroxynitrite ($ONOO^-$), which can interact with proteins, nitrite them on tyrosine residues and thereby alter their biological properties [24]. Besides peroxynitrite, the formation of products of protonation of nitric oxide (e.g., NO^+, NO^-, NO^{2-}, NO^{3-}) has been reported. These moeties are especially detrimental for enzymes, because they can modify amino acid residues that can ultimately decrease SOD activity. The direct interaction of NO with Cu^{2+} in the active center of SOD causes an inhibition of its activity. It is complemented by the non-enzymatic glycosylation of amino acid residues also affecting SOD activity.
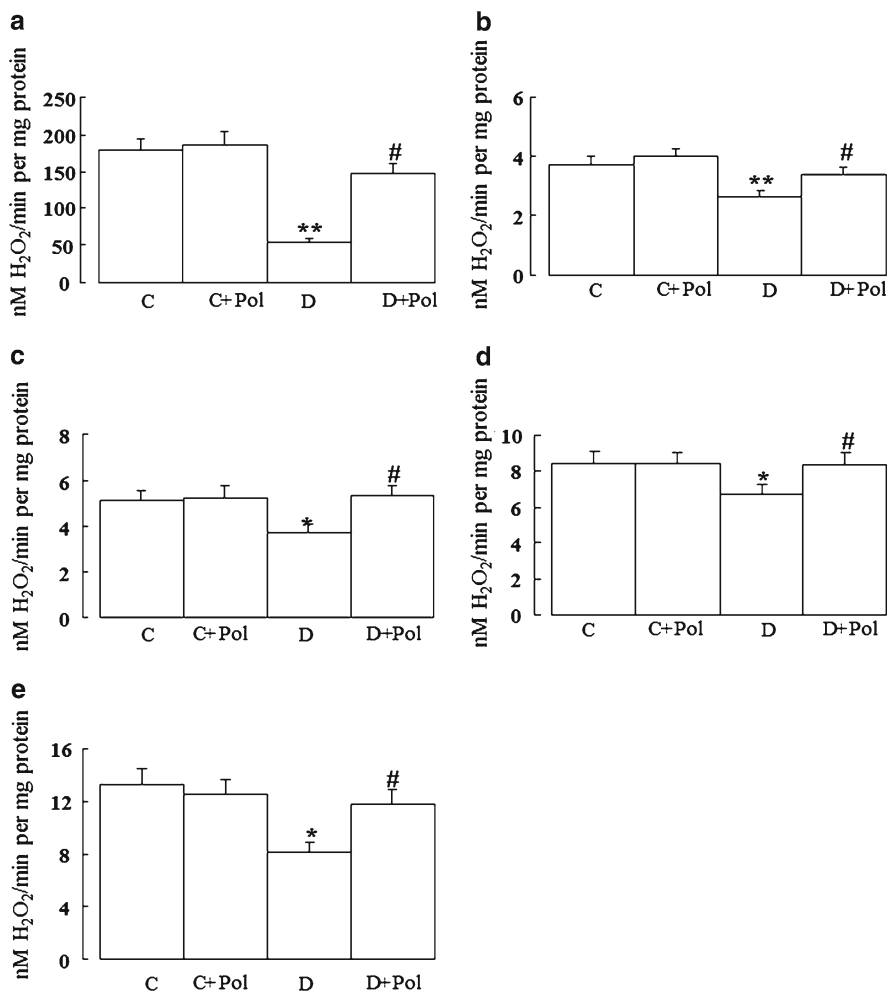
Fig. 12.9 The activity of catalase in the kidney (**a**), retinae (**b**), sciatic nerve (**c**), spinal cord (**d**) and DRG (**e**). *C* control, *C + Pol* control + polyphenol complexes of grape wine, *D* diabetic, *D + Pol* diabetic + polyphenol complexes of grape wine. Mean ± s.e.m., n = 5–7. *P < 0.05 and **P < 0.01 vs. controls. #P < 0.05 and ##P < 0.01 vs. untreated diabetic rats

During DM, catalase activity is also decreased in the sciatic nerve, DRG, spinal cord, kidney and retina by 27, 37, 21, 70 and 29 %, respectively (Fig. 12.9). A decrease in the activity of this enzyme can be explained by similar mechanisms as for SOD. Additionally, nitric oxide can directly come into contact with iron-porphyrine complexes of catalase, forming nitric derivatives. The appearance of heme-NO complexes prevents binding of H_2O_2 to the active center of catalase, and, hence, its expansion. Nitrite ions are also able to directly communicate with the iron component of the heme part of the enzyme, decreasing its activity [17].
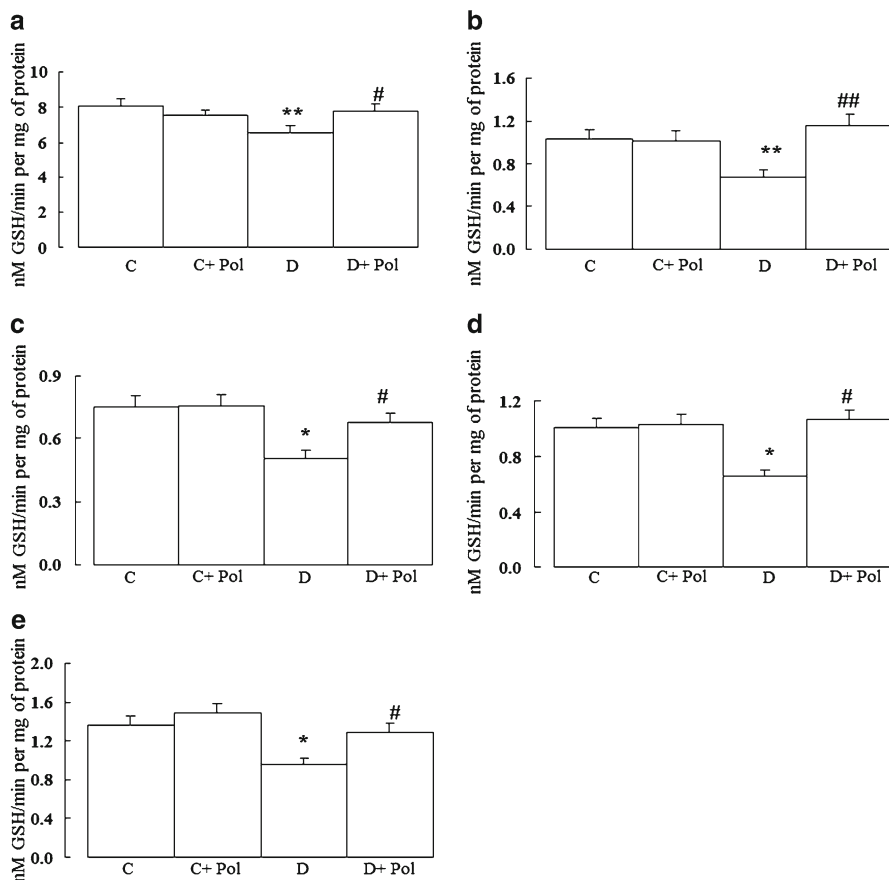
Fig. 12.10 The activity of glutathione peroxidase in the kidney (**a**), retinae (**b**), sciatic nerve (**c**), spinal cord (**d**) and DRG (**e**). *C* control, *C + Pol* control + polyphenol complexes of grape wine, *D* diabetic, *D + Pol* diabetic + polyphenol complexes of grape wine. Mean ± s.e.m., n = 5–7. *P < 0.05 and **P < 0.01 vs. controls; #P < 0.05 and ##P < 0.01 vs. untreated diabetic group

The glutathione system (GS) is especially important under oxidative-nitrosative stress (ONS). It provides effective protection to the cells against the effects of ONS and, therefore, when it is depleted, serious consequences for the organism can occur. Apart from CAT, the neutralization of hydrogen peroxide is also curried out by GPO. The affinity of GPO for H_2O_2 is significantly higher than for catalase.

During DM, the activity of GPO in the sciatic nerve, DRG and spinal cord, kidney and retina was reduced by 33, 30, 35, 19, 33 %, respectively, and the one of glutathione reductase (GR) was reduced by 37, 15, 30, 30 and 29 %, respectively (Figs. 12.10 and 12.11).

nM NADPH/min per mg of protein
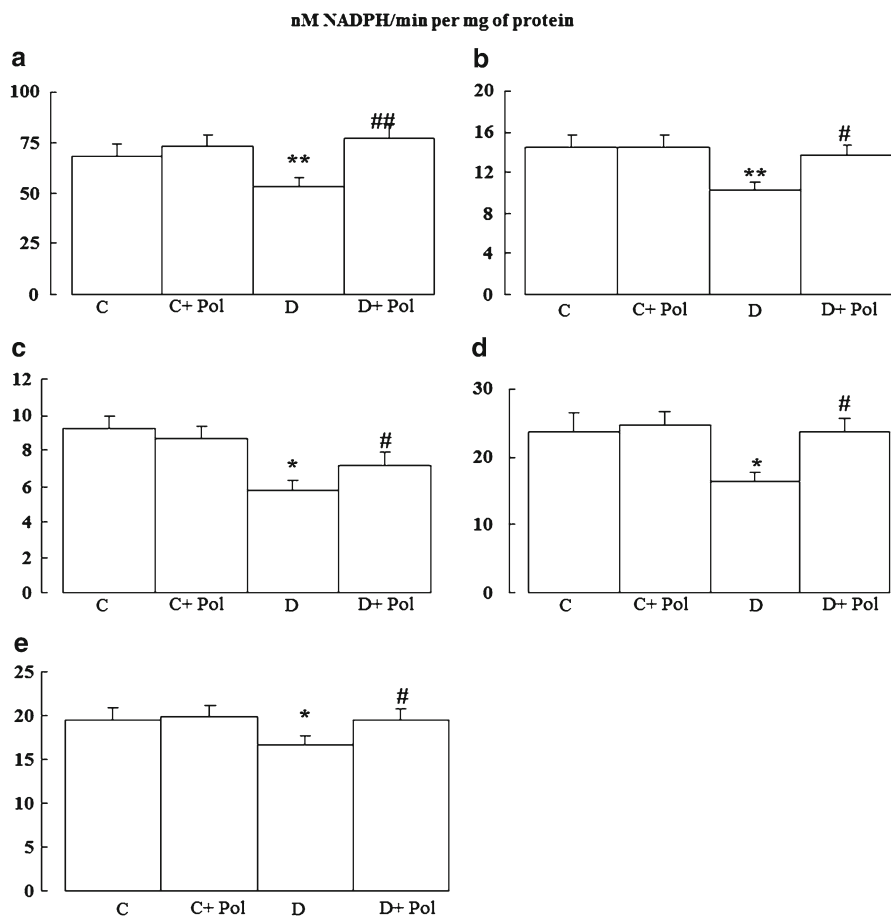
Fig. 12.11 The activity of glutathione reductase in the kidney (**a**), retinae (**b**), sciatic nerve (**c**), spinal cord (**d**) and DRG (**e**). *C* control, *C + Pol* control + polyphenol complexes of grape wine, *D* diabetic, *D + Pol* diabetic + polyphenol complexes of grape wine. Mean ± s.e.m., n = 5–7. *P < 0.05 and **P < 0.01 vs. controls; #P < 0.05 and ##P < 0.01 vs. untreated diabetic group

GPO activity depends on the content of reduced glutathione, the level of which is supported by the intracellular concentration of GR. GR activity is in turn determined by the level of reduced nicotinamide coenzymes.

A deficiency in energy substrates having a directly proportional effect on the efficiency of immune systems is taking place during DM. There is no effective protection without substrates with enough energy.

After consumption of the polyphenol complex, the activity of these enzymes was completely or partially restored to control values. The level of the
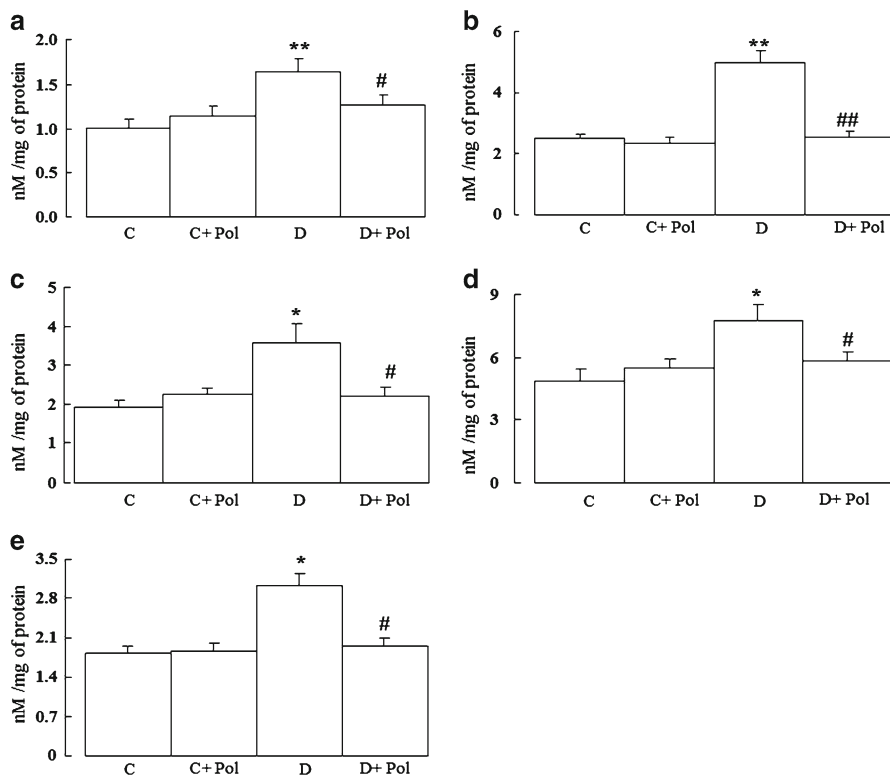
Fig. 12.12 The level of the sulfocarbanilide-positive content of LPO products in the kidney (**a**), retinae (**b**), sciatic nerve (**c**), spinal cord (**d**) and DRG (**e**). *C* control, *C + Pol* control + polyphenol complexes of grape wine, *D* diabetic, *D + Pol* diabetic + polyphenol complexes of grape wine. Mean ± s.e.m., n = 5–7. *P < 0.05 and **P < 0.01 vs. controls; #P < 0.05 and ##P < 0.01 vs. untreated diabetic group

sulfocarbanilide-positive content of LPO products in the kidney, retinae, sciatic nerve, spinal cord and DRG was also restored to control values (Fig. 12.12).

Thus, the natural polyphenol complex of grape wine has a significant anti-diabetic effect at the level of the whole organism. It protected it from dehydration and increased the activity of the antioxidant enzyme system in the sciatic nerve, DRG, spinal cord, kidney and retina. The levels of nitrosilative and PARylated proteins were almost restored to the control levels. The biochemical mechanisms of action of the natural polyphenol complex of grape wine are the subject of further research, but this natural complex can certainly be used in the treatment of complications of diabetes and the development of new anti-diabetic drugs.

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