Chapter 11

Health Status Improved by *Aronia Melanocarpa* Polyphenolic Extract

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Additional information is available at the end of the chapter

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Abstract

This chapter focuses on certain natural polyphenolic extracts from *Aronia melanocarpa* (Michx.) Elliott and also on their effects in insulin-dependent diabetes mellitus. The phenolic profile of berries ethanolic extract was characterized by HPLC/DAD/ESI-MS. HPLC/DAD/ESI-MS allowed identification of five phenolic compounds: chlorogenic acid, kuromanin, rutin, hyperoside, and quercetin. The results reveal that the glycosylated hemoglobin values are much higher in the diabetic group (DM) and they are significantly lower in the group protected by polyphenols (DM+P). It is found that due to the polyphenolic protection of the rats from the DM+P, the atherogen risk is preserved at normal limits. The serous activity of glutathione-peroxidase (GSH-Px) and superoxide-dismutase (SOD) has significantly lower values in the diabetic group as compared to the group protected by polyphenols. Renal function indicators like creatinine and blood-urea nitrogen (BUN) were also elevated in the streptozotocin diabetic rats when compared with control rats. When compared with the diabetic group the elevated levels of BUN was significantly ($p < 0.001$) reduced in animals treated with natural polyphenols. Through the hypoglycemiant, hypolipemiant, and antioxidant effects, *A. melanocarpa* represents a possible dietary adjunct for the treatment of diabetes and a potential source of active agents for the prevention of microvascular diabetes complications.

Keywords: Aronia melanocarpa, hypoglycemiant, hypolipemiant, antioxidant effects, renal function indicators

1. Introduction

Numerous population-based observational studies revealed that consumption of polyphenol-rich foods, principally fruits and vegetables, is beneficial to health, reducing mortality rates

and the incidence of the major diseases of modern civilization, cancer and cardiovascular disease [1, 2].

Antioxidants have been extensively investigated because of their ability to promote disease prevention and health maintenance by suppressing oxidative stress. From epidemiological and dietary intervention studies, it appears, however, that exogenous antioxidants at physiologic (nutritional) doses play an important role in the maintenance or re-establishment of redox homeostasis, an essential state in maintaining healthy biological systems [3, 4].

Experimental studies relating to the chemistry of *Aronia melanocarpa* refer to its berries being a rich source of pharmacologically relevant compounds. Polyphenols, especially anthocyanins and procyanidins, make up the main group of biologically active constituents in black chokeberry fruits [5].

It is known that natural polyphenols possesses complex biological properties such as antioxidant, antiinflammatory, cardioprotective, and antiplatelet activities [6–8]. Recently, interest has been focused on plant-based natural antioxidants such as tannins, polyphenols, and flavonoids to reduce the negative effect of oxidative stress and free radicals in diabetes patients and to prevent the destruction of β-cells [9]. The modulation of the enzymatic and nonenzymatic processes in diabetes mellitus, experimentally, via natural polyphenols, emphasizes the role of these vegetal extracts in metabolic diseases.

The chapter focuses on certain natural polyphenolic extracts from *A. melanocarpa* (Michx.) Elliott and also on their effects in insulin-dependent diabetes mellitus. *A. melanocarpa*, sometimes called black chokeberry, belongs to the Rosaceae family. Numerous health-promoting activities, such as antioxidative, cardioprotective, antidiabetic, antiinflammatory, antibacterian, antiviral, and immunomodulatory, have been demonstrated for *A. melanocarpa* extracts by both *in vitro* and *in vivo* studies [10–13].

2. The biochemical and morphological modifications of *A. melanocarpa* extract on experimental diabetes model

2.1. The biochemical modifications of *A. melanocarpa* extract on experimental diabetes model

Ripe berries of *A. melanocarpa* Elliott (Rosaceae, black chokeberry) were sampled in Botanical Garden, Iasi, Romania. Berries ethanolic extract contained 24.87 ± 0.54 mg total phenolics/g and 4.46 ± 0.06 μmol anthocyanin/g. Total phenolics quantification was performed by Folin-Ciocalteu method. The phenolic profile of berries ethanolic extract was characterized by HPLC/DAD/ESI-MS. HPLC/DAD/ESI-MS allowed identification of five phenolic compounds in berries ethanolic extract: chlorogenic acid, kuromanin, rutin, hyperoside, and quercetin (**Figure 1**). (+)-Catechin hydrate, chlorogenic acid, caffeic acid, rutin trihydrate, hyperoside, quercetin dihydrate, and kuromanin chloride were used as standards. Main phenolic constituents were identified by comparison of their retention times and mass spectral data to those of authentic standards.

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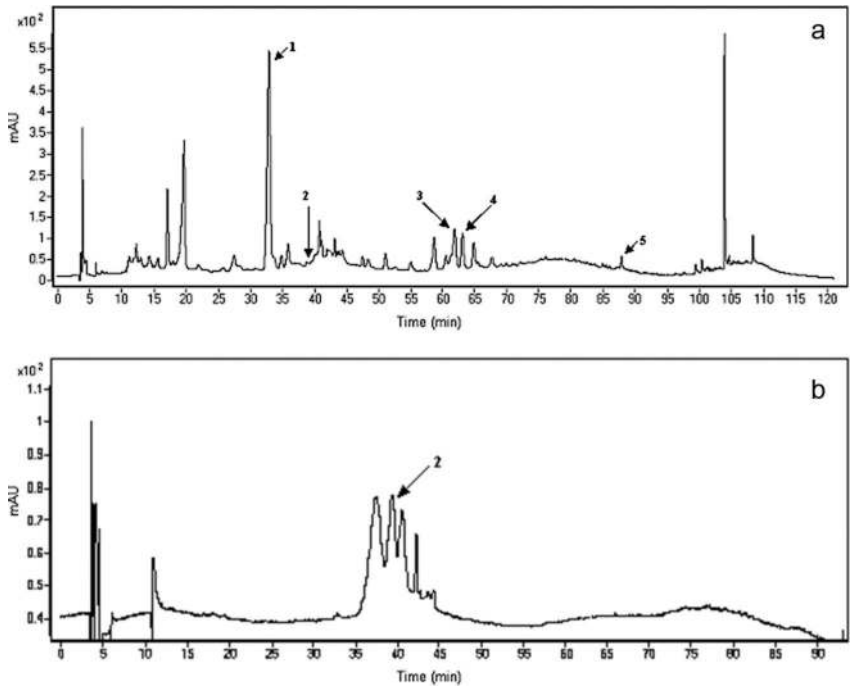

Figure 1. HPLC-DAD chromatograms of ethanolic extract of black chokeberry fruits. (a) Detection at 280 nm; (b) detection at 515 nm (1—chlorogenic acid, 2—kuromanin, 3—rutin+unknown compound, 4—hyperoside, 5—quercetin).

Chlorogenic acid has the capacity of radical trapping and singlet oxygen removal, and may prevent LDL oxidization and oxidative injury to nucleic acids [14, 15] and is reported to have effects associated with the prevention of diabetes. Chlorogenic acid inhibits α-glycosidase activity and postprandial elevation of blood glucose levels in sucrose- and maltose-treated rats [16].

α-Glucosidase, a membrane-bound enzyme located at the small intestine epithelium, is essential in carbohydrate digestion, catalyzing glucose cleavage from disaccharides and oligosaccharides. The onset of diabetes may be prevented by controlling postprandial hyperglycemia, inhibiting α-glucosidase, and α-amylase, which delays carbohydrate digestion to absorbable monosaccharide. Specialized literature shows that anthocyanins potentially inhibit intestinal α-glucosidase. Adisakwattana et al. [17] reports that cyanidin 3-rutinoside uses the same mechanism in delaying carbohydrate absorption.

All the requirements regarding the use of laboratory animals and biological preparations issued by the International Society of Pain Study and the European Council Committee (86/609/EEC) were followed during the experimental study, and it has been approved by the professional ethics committee of Grigore T. Popa University of Medicine and Pharmacy of Iasi (9803/12.09.2006).

The diabetes was obtained through the administration of STZ [2-deoxi-2(3-methyl-nitrozo-ureido)-p-glucopyranoza] in a single dose of 60 mg/kg body mass, 1% solution intraperitoneal (i.p.), after fasting for 18 hours.

The animals were kept in normal microclimate conditions. The clinical state of the animals was observed daily, the water and food ingestion, diuresis, glycosuria, and the possible presence of ketone bodies. The diet consisted of carbohydrates 59.12%, raw proteins 21.10%, raw lipids 5.08%, raw fibers 4%, minerals 5.14%, and humidity of 7.98%.

The research was performed on Wistar white rats, with an average weight of 250–280 g, which were divided into four groups of 10, namely: W Group = control, normal animals, that did not receive natural polyphenols; P Group = rats that were administered natural polyphenols 0.050 g/kg body every 2 days (by tube-feeding), for a period of 16 weeks; DM Group = rats with diabetes induced through streptozotocin (STZ) injection, 3 weeks after the beginning of the experiment; DM+P Group = rats that were administered a polyphenolic preparation for 3 weeks before and 13 weeks after the induction of diabetes mellitus.

The dry polyphenol extract was diluted in DMSO, 100 mL polyphenolic solution containing 840 mg natural polyphenols, 95 mL distilled water, and 5 mL DMSO. The experiment used *A. melanocarpa* active therapeutic doses, well-determined fractions of DL50 on an experimental model of diabetes mellitus. The dose representing 1/20 of DL50 was chosen, as it is the smallest dose that determined the pharmacodynamic effect that is being researched, without producing significant toxic effects. Polyphenols are able to penetrate tissues, particularly those in which they are metabolized, but their ability to accumulate within specific target tissues needs to be further investigated.

Mean hyperglycemia in rats suffering from diabetes mellitus and without polyphenolic protection increased progressively. This study indicates that streptozotocin-induced diabetes and subsequent glycemia level increase were reduced by the simultaneous administration of natural polyphenols. The hyperglycemia of the DM+P group was insignificantly reduced ($p < 0.05$) in comparison to the DM group. Thus, the polyphenol administration did not offer protection against the disease installation. The glycaemia evolution was reduced insignificantly (with only 29.5% at the diabetic rats with polyphenolic protection in comparison to the diabetic rats without protection).

Hyperglycemia activates glycolytic intermediates associated with injurious mechanisms such as hexosamine, polyol pathways, and advanced glycation end products formation. It had been reported that digestive enzymes such as lipase, α-amylase, and α-glucosidase were inhibited by proanthocyanidins and tannins in young chicks, which decreased the digestibility of protein, starch, and lipid [18].

The antidiabetic potential of *A. melanocarpa* may result from decreased mucosal maltase and sucrase activities in the small intestine. Yet other mechanisms may be involved, namely, stimulation of glucose uptake, increased insulin secretion, or reduction of oxidative stress [19, 20].

Specialized literature proposes multiple molecular mechanisms to explain hyperglycemia-induced diabetic complications. Such mechanisms include: increased polyol pathway,

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activation of the diacylglycerol (DAG)/protein kinase C (PKC) pathway, increased oxidative stress, formation and action of increased advanced glycation end products (AGE), and increased hexosamine pathway. Hyperglycemia is known to inhibit endogenous protective factors in vascular tissues, such as insulin, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and activated protein C (APC). These are crucial in maintaining vascular homeostasis and neutralizing hyperglycemia-induced toxic factors, such as oxidative stress, AGE, or activation of nuclear factor-jB (NF-jB) and prevent and delay the evolution of diabetic complications [21].

In diabetes mellitus, the prolonged excess of plasma glucose determines a glycosylation proportional with the severity and duration of the hyperglycemia, affecting numerous proteins: hemoglobin, albumin, lipoproteins, collagen, etc. [22]. The results reveal that the glycosylated hemoglobin values are much higher in the diabetic group (DM) and they are significantly lower in the group protected by polyphenols (DM+P). The photometric dosing of total hemoglobin and the colorimetric measurement of glycosylated hemoglobin (HbA_{1c}) were performed. Glycosylated hemoglobin values, considerably high in the diabetic group, diminish significantly in the protected group (**Table 1**).

The exploration of the lipid profile included the measurement by photocolorimetry, in the serum obtained after separation, of the concentration of total cholesterol (Ch-T), of triglycerides (TG) [23], of total lipids (TL) [the method with sulfovaniline], of high-density lipoproteins (HDL) [24], of low-density lipoproteins (LDL) [according to the Friedewald formula] for all the animals included in the experiment. The lipid profile obtained after the biochemical determinations is given in **Table 1**.

The delivery of plant polyphenols extracted from *A. melanocarpa* fruit significantly improves the dyslipidemia triggered by diabetes mellitus and the microangiopathic lesions. Following the perturbation of the lipid metabolism in the diabetic rats, atherogen risk has significantly increased values in comparison to the rats from the witness group (**Table 1**).

Experimental groups	W	P	DM	DM+P
Ch-T (mg/dL)	72.33 ± 4.81	66 ± 2.32	93.5 ± 7.89***	66.38 ± 3.02$^{\#\#\#}$
TG (mg/dL)	87.64 ± 6.88	70.38 ± 8.27*	149 ± 17.46***	92.83 ± 29.76$^{\#\#\#}$
HDL (mg/dL)	33.17 ± 4.24	31.17 ± 3.58	18.66 ± 5.33***	26.17 ± 3.01$^{\#\#}$
LDL (mg/dL)	22.07 ± 4.69	19.17 ± 3.81	44.43 ± 6.83***	25.24 ± 9.42$^{\#\#\#}$
Total Hb. (g/100 mL)	13.12 ± 1.13	13.66 ± 1.04	17.05 ± 2.48**	14.22 ± 1.85$^{\#\#}$
HbA_1c (% from Hb)	1.73 ± 0.04	1.61 ± 0.10	33.63 ± 5.68***	24.72 ± 2.21$^{\#\#}$
Adhesivity index	1.27 ± 0.04	1.46 ± 0.04	2.09 ± 0.07***	1.79 ± 0.08$^{\#\#}$

Note: Values are mean ± SEM. Statistical analyses *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs. W group. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ vs. DM group.

Table 1. Lipid profile, total hemoglobin, glycosylated hemoglobin, and adhesivity index values in the studied groups.

Chlorogenic acid administered to golden hamsters enhanced expression of PPARα in liver and total cholesterol, LDL, HDL, and glucose. Insulin levels in blood were reportedly lower than in the placebo group [25], showing that chlorogenic acid affects lipid metabolism through the activation of PPARα in liver.

A simple and reliable method for the evaluation of the atherogen risk is the calculation of the HDL-cholesterol/total cholesterol ratio. The calculus of the atherogenity index (AI) has shown a 57.14% AI decrease at the protected diabetics (DM+P) in comparison to the unprotected ones. The AI experienced an increase of 170.8% in DM group compared to W group and a 16.08% increase in DM+P group compared to W group. Taking into account the variability coefficient (%), the environments are representative for the respective series (medium dispersion). Depending on the values of the significance threshold (p), the statistical analysis shows insignificant differences ($p > 0.05$) for P and DM+P in comparison to W, and very strong significant differences ($p < 0.001$) for DM in comparison to W, and strong significant differences for DM+P in comparison to DM.

Following the perturbation of the lipid metabolism in the diabetic rats (DM), AI has significantly increased values in comparison to the rats from the witness group (W) and even from DM+P. It is found that due to the polyphenolic protection of the rats from the DM+P, the AI is preserved at normal limits. The administration of vegetal polyphenols in healthy rats (P) did not cause a significant change of AI. As a consequence, the administration of natural polyphenols determines a significant increase of HDL and a significant decrease of LDL in experimental diabetes mellitus.

Rovatti's method was employed to determine the adhesivity index, which is the ratio between the initial platelet number and the platelet number after thrombocyte adhesion to glass. Platelets were counted before and after thrombocyte adhesion to glass, using the visual EDTA solution method.

Diabetic platelet adhesion is considerably lowered by the polyphenols extracted from *A. melanocarpa*, as compared to the diabetic group. Statistical analysis reveals highly significant differences ($p < 0.001$) between DM and control groups, and significant differences between the DM+P and the DM groups. Platelets of nondiabetic yet hypercholesterolic rats had reportedly normal sensitivity to ADP, which shows that glycemia influences platelet activity by membrane protein glycation, both directly and indirectly (**Table 1**).

Understanding the mechanism through which the natural polyphenols have effects on the functionality of the endothelium cells, including on the membrane sensitivity and intracellular signaling, could represent a new way of therapeutically approaching the chronic metabolic diseases.

Insulin secretion, insulin resistance, and homeostasis are important factors in the onset of diabetes, a disease associated with pancreatic ß-cell dysfunction. Oxidative stress is thought to contribute to this dysfunction and antioxidant effects on diabetes onset were investigated. It is well-known that *A. melanocarpa* offers a strong antiradical activity resulting from high amount of natural antioxidants, especially polyphenols [26, 27]. The natural polyphenols compounds do reduce the lipids peroxides, do neutralize the lipid peroxil radicals, and do inhibit the LDL oxidation at the inner level of the blood vessels.

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The aim of the study was to assess the effectiveness of natural polyphenols supplementation on the antioxidant defense mechanisms of diabetic rats, in order to reduce damage caused by the peroxidation of membranes and other cell components. Reactive oxygen species oxidized GSH to GSSG, leading to a decrease in GSH and an increase in GSSG concentrations. Long-time oxidative stress can consume antioxidants, and reduce SOD, CAT, and glutathione-peroxidase (GSH-Px) levels in experimental diabetes.

The serous activity of GSH-Px and superoxide-dismutase (SOD) has significantly lower values in the diabetic group as compared to the group protected by polyphenols. The serum activity of SOD diminished by 18.10% in the DM group as compared to the W group. In the DM+P group, the serum activity of SOD reached normal values again (**Table 2**).

Group	SOD (U/gHb)	CAT (μmol/gHb)	GSH (γ/mL)	GSH-Px (μmol/gHb)
I – W	14.6 ± 1.03	8.5 ± 1.50	3.24 ± 0.29	63.6 ± 9.93
II – P	14.8 ± 1.07	8.0 ± 1.25	3.04 ± 0.49	62.8 ± 8.75
III – DM	9.7 ± 0.72	6.6 ± 1.37	2.06 ± 0.04	46.8 ± 4.9
IV – DM+P	16.4 ± 0.10	7.8 ± 1.75	2.36 ± 0.10	53.7 ± 15.9

Table 2. The effect of *A. melanocarpa* on antioxidant activity in normal and diabetic rats.

Polyphenols act as free radicals scavengers by donating hydrogen atoms or electrons from phenolic hydroxyls. This is the main mechanism by which polyphenols scavenge many reactive oxygen species (ROS) (i.e., superoxide anion radical, hydroxyl radical).

Oxidative stress is diminished in diabetic rats enjoying natural polyphenolic protection as compared to the rats in the control group. The plant polyphenols referred to increase the antioxidant *in vivo* action of the serum and provide protection against excessive oxygen-free radicals generated by the oxidative stress.

In the diabetic group, the SOD and GSH levels in the blood were significantly ($p < 0.001$) decreased when compared with normal group (**Figure 2**). In the diabetic group the GSH-Px level was significantly ($p < 0.001$) decreased when compared with the normal group. There was no significant change in the CAT values.

Our findings revealed that the antioxidant activity of catalase (CAT) in the liver decreased by 69.90% in the DM group as compared to the W group. The antioxidant activity of CAT increased significantly, i.e., by 97.46% ($p < 0.001$), in the animals in the DM+P group, unlike the same activity in the animals included in the DM group. This phenomenon is due to the polyphenol protection by direct mechanism of microsomal hepatic membranes and proteins against the destructive action of ROS.

The oxidative stress causes the SOD activity in the liver homogenate to decrease by 42.85% in the DM group against the W group. The DM+P group enjoys the normalization of the antioxidant activity of SOD, which increases by 75.26% as compared to the DM group (**Figure 3**).

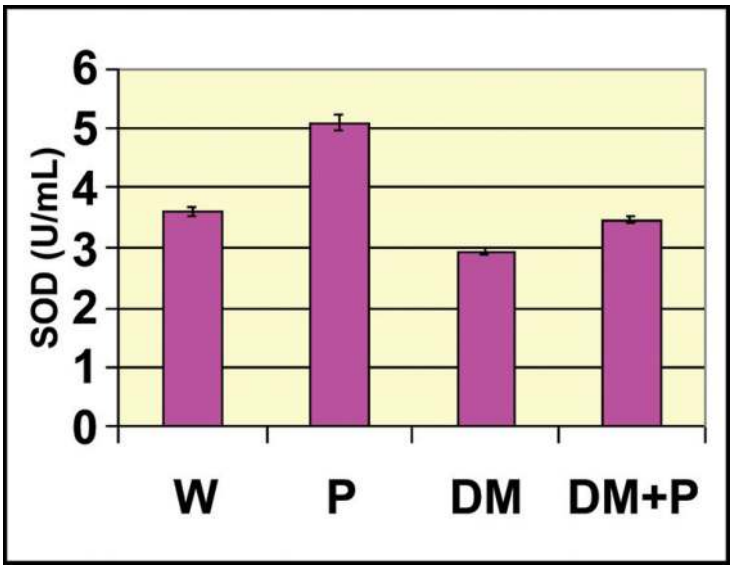

Figure 2. SOD blood activity in white Wistar rats.

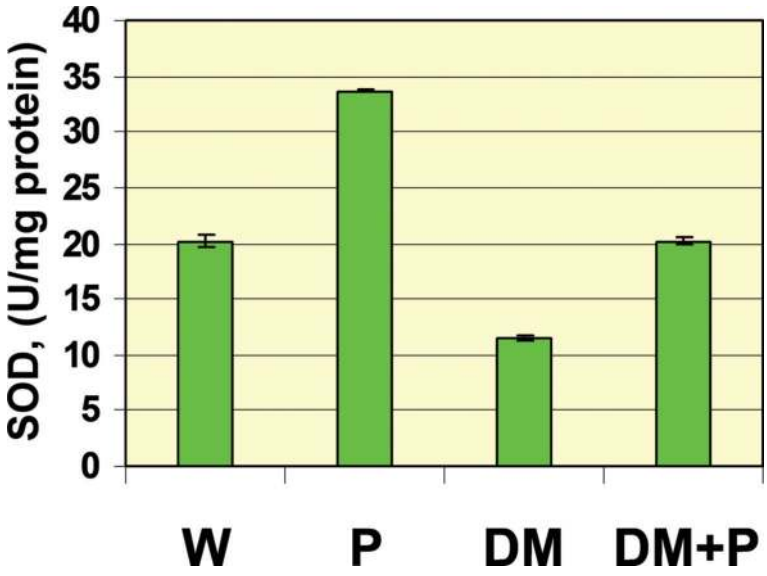

Figure 3. SOD hepatic activity in white Wistar rats.

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The analysis of our experimental findings in relation to the enzymatic systems involved in the antioxidant defense in the hepatic tissue and in the serum enabled us to conclude that the antioxidant defense increased significantly ($p < 0.001$) when diabetes mellitus was present.

The fact that the therapy with natural polyphenols administered to the diabetic group caused the recovery of the thiol groups up to normal limits in the liver tissue is suggestive of the protective effect that polyphenols have on reduced glutathione (GSH) as compared to the excessive peroxides developed in streptozotocin-induced diabetes. As a consequence of oxidative stress intensification, GSH has significantly lower values in the rats included in the DM group, than in those included in the W and DM+P groups, respectively. The decrease of GSH levels could be the result of decreased synthesis, or increased degradation of GSH by oxidative stress in diabetes. The GSH value in the liver increased by 66.22% in the DM+P group as compared to the DM group.

Polyphenols are secreted via the biliary route into the duodenum, where they undergo the action of bacterial enzymes, especially β-glucuronidase, in intestine distal segments, after which they may be reabsorbed, conducing to a longer presence of polyphenols within the body [28].

Liver function markers like *glutamate pyruvate transaminase* (GPT) and *alkaline phosphatase* (ALP) in serum were significantly ($p < 0.001$) elevated in STZ-induced diabetes when compared with normal rats. Animals treated with polyphenols showed significant ($p < 0.001$) reduction in the elevated level of ALP and GPT compared with diabetic rats (**Table 3**).

Group	GPT (U/mg prot)	ALP (KA/dL)
I – W	141.0 ± 11.92	33.1 ± 0.87
II – P	140.2 ± 10.86	32.7 ± 0.83
III – DM	307.1 ± 19.67	46.4 ± 1.78
IV – DM+P	225.8 ± 6.40	38.1 ± 0.68

Table 3. Liver function markers—GPT and ALP in the studied groups.

Aronia juice had a hepatoprotective effect in rats after acute exposure to carbon tetrachloride (CCl4) [29]. The liver cytotoxicity from CCl4 depends on its metabolism by cytochrome P450 in the presence of highly reactive trichloromethyl-free radicals. CCl3 radical reacts with oxygen and initiates lipid peroxidation, which results eventually in cell death. *Aronia* juice prevented the increase of lipid peroxidation induced by CCl4 as measured by the malondialdehyde content in rat liver and plasma.

The liver expresses batteries of cytoprotective genes that confer cellular resistance to oxidative stress and xenobiotic toxins, and protection against other stress-related diseases. These genes are mainly regulated by nuclear factor erythroid 2-related factor 2 (Nrf2), making this transcription factor a target for small molecule discovery to treat such diseases. Polyphenols may induce cellular defense genes by derepressing Nrf2 inhibition by Keap1 (Kelch ECH associating protein). This ability to derepress Nrf2 and reactivate its target genes may underlie the protection conferred by polyphenols against oxidative stress-related diseases [30].

Nrf2 is key to regulating GSH levels by upregulating GSH synthetic and regenerative enzymes, as well as enzymes using GSH as a cofactor [31]. Nrf2, which is a redox-sensitive transcription factor, is a highly protective factor, regulating multiple genes encoding antioxidant proteins and phase II detoxifying enzymes, thus regulating the physiological response to oxidative and electrophilic stress. Literature shows that natural compounds, including polyphenols, target Nrf2 and consequently can suppress oxidative stress and inflammation, and activate the antioxidant/electrophilic response element (ARE/ERE)-related cytoprotective genes [32].

Nrf2 is the transcription factor controlling ERE, which is normally bound to the sensory protein Keap1. Bound to an inducer, such as phytochemicals (i.e., curcumin), some polyphenols or sulforaphane release Nrf2, which activates ERE and the proteins it regulates [33].

ROS and oxidative stress increase as a result of diabetes, reacting with proteins-forming AGEs. AGEs interact with their receptors (RAGE) and activate the nuclear transcription factor kaba B (NF-κB) and its controlled genes like IL-6. AGE/RAGE interactions have been also shown to induce vascular oxidative stress through the activation of NADPH oxidase [34]. Blockade of the AGE/RAGE interaction by soluble RAGE has been shown to suppress atherosclerosis and neointimal formation [35] and nephropathy in diabetic animals [36].

Renal function indicators like creatinine and blood-urea nitrogen (BUN) were also elevated in the streptozotocin diabetic rats when compared with control rats. When compared with the diabetic group the elevated levels of BUN was significantly ($p < 0.001$) reduced in animals treated with natural polyphenols (**Table 4**).

Group	Creatinine (mg/dL prot)	BUN (mg/dL)
I – W	0.9 ± 0.13	9.4 ± 0.28
II – P	0.8 ± 0.13	9.1 ± 0.18
III – DM	2.3 ± 0.14	20.1 ± 0.97
IV – DM+P	1.9 ± 0.25	13.5 ± 0.39

Table 4. Renal function indicators—creatinine and blood urea nitrogen (BUN) in the studied groups.

When isolated rat kidney mitochondria were treated with quercetin, various changes consistent with access of quercetin to the interior of the mitochondria were observed, including increased mitochondrial membrane permeability and oxygen consumption, but decreased membrane potential and oxidative phosphorylation [37]. It thus appears that mitochondria would be easily able to absorb significant concentrations of polyphenols, provided the intracellular concentrations around them were high enough.

Mitochondrial adaptation, rather than antioxidant capacity, is emerging as the primary mode of action of the health benefits of dietary polyphenols. Possible mechanisms of action for polyphenols are direct activation of the components of the mitochondrial-biogenesis signaling pathway (e.g., sirtuin 1 and PPARγ); direct activation of the ERE via binding to its regulatory

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protein Keap1; stimulation of glycolysis and glucose uptake, which increases the supply of nutrients to the mitochondria; and stimulation of NO synthesis, which is a known signal for mitochondrial-biogenesis [31].

2.2. Morphological aspects related with polyphenols administration at the pancreas, kidney, and liver level in experimental diabetes mellitus

The morphological examination of the pancreas performed within the study showed a decrease in the number and volume of the Lagerhans islands (atrophy), as well as the decrease of the beta cells in the DM group. There was a major disturbance of the endocrine pancreas, with sclerosis and intense interstitial infiltrate. The inflammatory infiltrate was produced intra- and pericanalicular (**Figures 4** and **5**).

Atrophy was also revealed in the diabetic group under polyphenolic protection (DM+P). Nevertheless, the changes are much more discrete (**Figure 6**).

Diabetic nephropathy is a major cause of end-stage renal disease worldwide. Glomerular filtration rate progressively declines, associated to glomerular hyperfiltration, glomerular, and tubular epithelial hypertrophy, increased urinary albumin excretion, increased basement

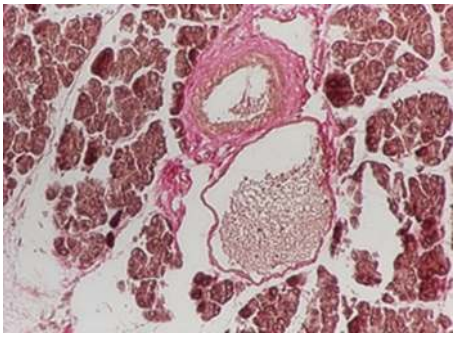

Figure 4. Pancreas, DM group: interstitial edema, perivascular, and interstitial fibrosis, vascular stasis (col.VG × 10).

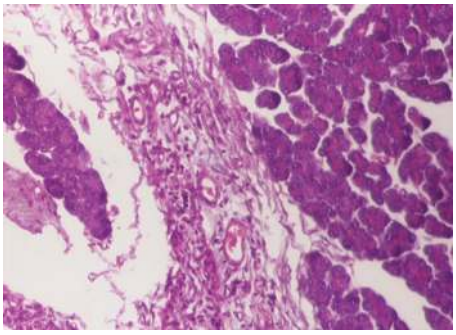

Figure 5. Pancreas, DM group: interstitial edema, fibrosis, dilated arterioles, and inflammatory infiltrate (col.HE × 10).

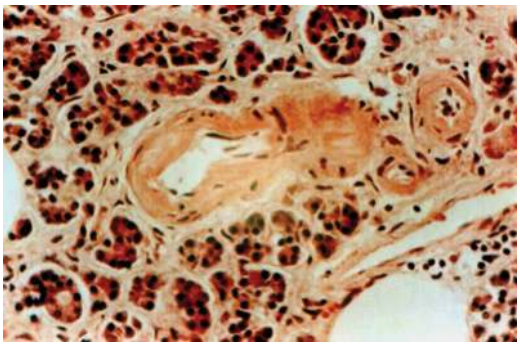

Figure 6. Pancreas, DM+P group: insulitis, edema, and a low level of fibrosis (col. HE × 10).

membrane thickness, and mesangial expansion with the accumulation of extracellular matrix proteins (ECM) [38].

The mechanisms which determined the production of renal alterations in the group with streptozotocin-induced diabetes could be: the formation of the nonenzymatic glycation products modifies the content in sialic acid of the glomerular filter with the alteration of the electric barrier; the accumulation of glycosphingolipids and glycosylceramides in the kidneys, as they represent secondary paths for glucose metabolizing process. There is a correlation between the accumulation of these substances in the kidneys and the renal hypertrophy in DM; and dyslipidemia secondary to the chronic hyperglycemia.

IL-6 has a strong association with the development of glomerular basement membrane thickening as well as possible relationships with increased endothelial permeability and mesangial cell proliferation. This reduction in the levels of IL-6 could play a major role in the attenuation of the progression of diabetic nephropathy and subsequently the significant reduction of the serum urea and creatinine in proanthocyanidin-treated group [39].

The histological images in the rat liver reveal granulo-vacuolar dystrophic lesions and inflammatory infiltrate in the portal spaces in the diabetic group, as well as glycogen spoliation in the pericentrolobular areas. In the animals included in the DM+P group, the degenerative and inflammatory phenomena are associated with the regeneration phenomena, which are probably stimulated by the administration of natural polyphenols (**Figures 7** and **8**).

The biologically active properties of flavonoids are closely related to their antioxidant and antiinflammatory capacity. Supplementing the alimentation with polyphenols may ameliorate the evolution of diabetes through: free radicals scavenger effect, reducing the glycation of proteins, and inhibiting the NMDA receptors (it may be considered as inhibitory of these receptors) [40–42].

Several studies and experimental research demonstrate that vegetal polyphenols increase the antioxidant capacity of the serum, *in vivo*, they have antiradical effect on oxygen-free radicals, produced in excess, under conditions of oxidative stress [43, 44].

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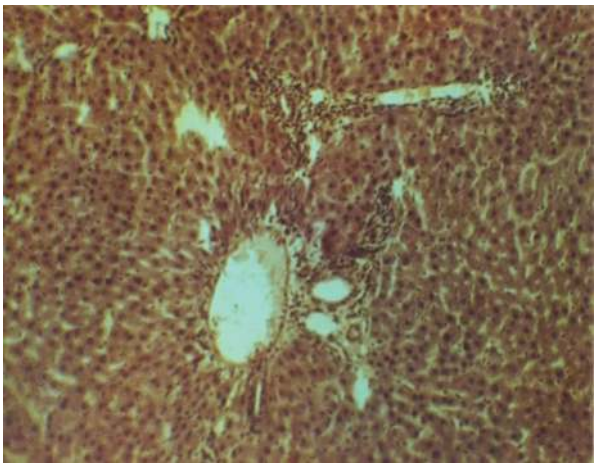

Figure 7. Liver, DM group. Distrophic granulo-vacuolare lesions and inflammatory infiltrate in the porte spaces.

The delivery of plant polyphenols extracted from *A. melanocarpa* fruit significantly improves the dyslipidemia triggered by diabetes mellitus and the microangiopathic lesions. Due to the health-promoting effects of *A. melanocarpa* extracts, it may constitute a valuable dietary supplement for people with risk factors of diabetes mellitus and cardiovascular diseases.

The biochemical parameters revealed the undeniable kidney protection achieved by polyphenolic extract delivery. The vascular protection effects of natural polyphenols on experimentally induced diabetes mellitus depend both on the administered dose of polyphenols, and on the length of their administration.

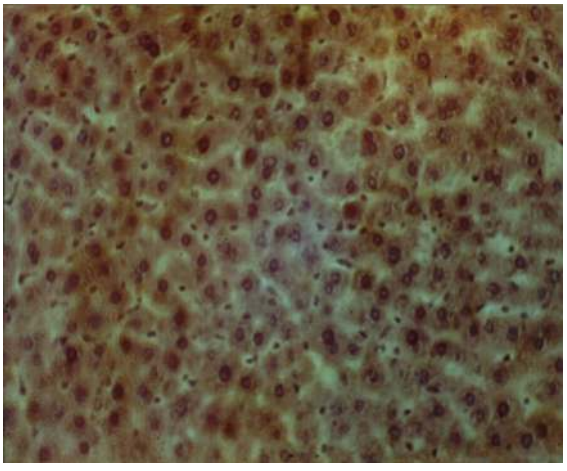

Figure 8. Liver, DM+P group. Degenerative and inflammatory phenomena, associated with regenerative phenomena, probably stimulated by the administration of natural polyphenols.

3. Conclusions

The low antioxidant enzyme levels found in the experimentally induced diabetes rats support the benefits of adding antioxidant supplements to the ordinary food intake. Through the hypoglycemiant, hypolipemiant, and antioxidant effects, *A. melanocarpa* represents a possible dietary adjunct for the treatment of diabetes and a potential source of active agents for the prevention of microvascular diabetes complications.

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