Original Research

Actinidia arguta supplementation protects aorta and liver in rats with induced hypercholesterolemia

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ABSTRACT

There are no published results focusing on the study of hardy kiwifruit as a supplementation to the atherogenic diet. We hypothesized that hardy kiwifruit (*Actinidia arguta* (*A. arguta*)) from Poland possess better pro-healthy action than two Asian varieties (Hayward and Bidan). We tested this hypothesis by measuring the metabolic reactions of rats loaded with 1% cholesterol and supplemented with 5% of hardy kiwifruit (*A. arguta*), Hayward, or Bidan in their diets. The experiment was performed on 71 male Wistar rats. Cholesterol showed a significant impact on the rise of liver somatic index, while lipid profile improved by decreasing the levels of TC, LDL-C, TC/HDL-C, AI, TG, and increasing HDL-C in the serum of rats ($P < .05$). Total plasma antioxidant capacity determined by ABTS, FRAP, and DPPH assays was increased. ALP in rat serum was higher in groups receiving cholesterol diets and kiwifruit. A decrease in fibrinogen as well as prolonged prothrombin time and a reduction of the MPO in serum were estimated. The smallest percentage of lesions in the aortic arch was in the ChGeneva, ChWeiki, and ChAnna. Similarly, the smallest fatty liver disease was recorded in the ChGeneva and ChAnna groups. The distribution of lipids in the liver from these groups had a character of "mosaic," in hardy/mini kiwifruit (Jumbo), Hayward, and Bidan was distributed uniformly. The longest villi were in ChWeiki, and

significantly lower in ChHayward and ChBidan. The present results support our hypothesis that *A. arguta* showed better pro-health impacts in rats loaded with cholesterol than Hayward and Bidan kiwifruit, and, for the first time, the positive nutritional effects of supplemented *A. arguta* for hypercholesterolemia are noted.

1. Introduction

Atherosclerosis is a multi-factorial disease in which hyperlipidemia plays a key role [1-4]. It is known that consumption of fruits and vegetables has great importance in the prevention of atherosclerosis [5-9]. Kiwifruit are a source of important bioactive compounds (polyphenols, ascorbic acid) and possess antioxidative properties [10-12]. The most popular among kiwis is *Actinidia deliciosa* (cultivar Hayward) while other species such as *A. eriantha* (cultivar Bidan) and *A. arguta* (mini kiwi, hardy kiwifruit) are less known [13]. A high correlation was found among the contents of total polyphenols, vitamin C, and antioxidant activity in kiwifruit *A. deliciosa* and *A. eriantha* [11,14-16]. Consumption of fruit plays a special role in the prevention of atherosclerosis and cardiovascular diseases [8,10,17,18], and exogenous cholesterol can lead to liver and heart damage in animals [19-22]. Atherosclerotic lesions were found in the aorta of rats [17,18], mice [19], and rabbits [20] fed a cholesterol-enriched diet. From our previous studies, we know that the Hayward variety of kiwifruit, given as part of an atherogenic diet, influenced the increase of plasma antioxidant capacity. Such supplementation had hypolipidemic, hypocholesterolemic, and anticoagulation effects; diminished liver enzyme activity; and influenced the RBC system in the blood [10]. Any observations concerning kiwifruit Bidan were not found. Likewise, there is no information concerning the impact of mini kiwis cultivated in Poland on the metabolic reaction of rats loaded with cholesterol in the diet. In this study, we assumed that the variable content of bioactive compounds in hardy kiwifruit from Polish plantations [12,16] and kiwifruit cultivated in Asia [10,15] can cause changes in the parameters of blood and organs in rats. We hypothesize that the tested varieties of hardy kiwifruit would better protect the aorta and liver of rats fed cholesterol. We tested this hypothesis by determining the effects of several cultivars of *A. arguta* in comparison with Hayward and Bidan cultivars on rats loaded with cholesterol. The metabolic answers were determined on the basis of blood parameters – lipids, TAC, enzymes activity, markers of inflammation, damage of atherosclerotic plaque and histological examination of the aorta, liver (lipids deployment), and ileum of rats. Based on the reactions of the rats, we selected the varieties of *A. arguta* that had the best health benefits. As far as we know, there are no previous published studies on these cultivars and cholesterol.

2. Methods and materials

2.1. *Actinidia arguta*, *mini kiwifruit*

Hardy kiwifruit (mini kiwifruit, *Actinidia arguta* (Siebold et. Zucc) Planch. ex. Miq.) was grown in 2013 on the ecological field of the Department of Environmental Protection at Warsaw University of Life Sciences (SGGW), Poland. Fruits of these cultivars – Bingo (hybrid *arguta* and *purpurea*), M1 (select *arguta*), Anna, Weiki, Jumbo, and Geneva – were harvested when ripe and then freeze-dried with the peel [10,11].

2.2. *Actinidia deliciosa*, Hayward and *Actinidia eriantha*, Bidan

In 2013, the kiwifruit cultivar, Hayward, was grown in organic conditions, while the cultivar, Bidan, was cultivated in conventional (conv) and organic (org) conditions in an orchard in Haenam County, Jeonnam Province, Korea. The freeze-dried fruits (without the peel) were added to the rats' diet and comprised 5% (50 g/kg) of it, similar to mini kiwis.

2.3. *Rats and diets*

Studies in rats have been accepted by The Animal Care Committee of Warsaw University of Life Sciences (SGGW), Poland. Male Wistar rats (n = 71), weight 113.7 ± 9.7 g, were divided into eleven groups. The rats were housed in plastic cages (TECNIPLAST S. p. A, 21 020, Italy) and had unrestricted access to drinking water. During the first five days of adaptation, all groups were fed the control diet. The control diet included (g/kg): casein (150), soybean oil (100), cellulose (10), vitamin (10) (AIN-93-MX Mineral mix Cat. No. 960 402), mineral mixtures (36.7) (AIN-93-MX mineral mix Cat. No. 960 400) of the American Institute of Nutrition for laboratory animals, choline (2) and wheat starch ad to 1 kg (691.3). After adaptation, the rats from the control group were fed the control diet over a period of 42 days. The diets of the other ten groups were supplemented with 1% of cholesterol (Ch) (10 g/kg) or 1% of Ch in addition to 5% (50 g/kg) of lyophilized kiwifruit: ChBingo, ChM1, ChAnna, ChWeiki, ChJumbo, ChGeneva, ChHayward, ChBidan org, and ChBidan conv. As mentioned previously, kiwifruit Bidan (as new one) were cultivated in organic (org) and conventional (conv) conditions for comparison. All rats were fed once a day at 10.00 h. The feed intake was controlled daily, and body weight was recorded once a week. At the end of the experiment (after 24 hours of starvation), rats were anesthetized using inhalation of halothane (Narcotan-Zentiva). The blood samples were taken from the left atrium of the heart. Blood was taken with EDTA, and plasma on sodium citrate and serum (without any coagulants) was used for a wide range of laboratory tests. The arch of the aorta was isolated, and the liver was also isolated, dried, and weighed, while the somatic index of liver (% of relative weight) was calculated. Samples from the intestine (ileum) were also taken.

2.4. *Determination of metabolic indices*

The contents of TC, LDL-C, HDL-C, and TG in serum were determined in the Roche analyzer (Cobas 6000), using the

reagent kits. Total antioxidant capacity (TAC) in plasma of rats was determined by different assays (DPPH, ABTS, and FRAP) which were described in detail in our previous study [10-12,14-16]. The activities of the liver enzymes—AST (aspartate aminotransferase), ALT (alanine aminotransferase), and ALP (alkaline phosphatase) in serum, as well as amylase, lipase, glucose, and albumin—were determined with utilization of Roche-Cobas 6000 analyzer. Hematologic parameters (RBC, WBC, PLT, HCT, and HGB) in full blood (taken for EDTA) were determined with utilization of analyzer Sysmex XE 2100D. Coagulation indices: prothrombin index, prothrombin time, INR, and fibrinogen were determined by chronometric method with the utilization of coagulation analyzer BCS XP Siemens and reagents of the company. The markers of inflammation: C-reactive protein (CRP) and interleukin-6 (IL-6) were determined by the Modular PPE analyzer by ELISA method in the EUROIMMUN analyzer. The marker of damage of atherosclerotic plaque: myeloperoxidase (MPO) was determined by the EUROIMMUN analyzer using the Mercodia MPO ELISA test, which is a solid phase two-site enzyme immunoassay.

2.5. Histological procedures

For the aortas' conservation, a formaldehyde-buffered bath was used, and cleaned aortas were cut lengthwise. The solutions of dyes Sudan III and Sudan IV were used, and the surface area of aortic athromatous lesions was measured by planimetry with a computer scanning system (Multi Scan Base 14.02) and expressed as a percentage of total intimal surface area.

Left lateral surface lobe was taken from the liver. Isolated specimens were dissected and fixed with buffered 10% formalin and cut in Criostat HM 505 N C on slicks of 10 μm thickness. The slicks were stained in 10% Red Oil to detect lipids and then placed in glycerol gelatin. The morphology of the liver lobules was evaluated by a light microscope BX-43 Olympus. The histopathological evaluation included the deployment of the lipid traits: in the liver tissue, in the lobule, in hepatocytes, and in the size of fat vacuoles. The fat content (μg/μm^3) in hepatocytes was also evaluated in 100 cells, and the integral density of the fat was measured using the computer program Lucia v.4x. (Nikson), at 100× magnification.

The intestinal samples (ileum) were taken for morphometric evaluation of villi length. The pieces of ileum that were obtained were fixed in buffered formalin and subsequently subjected to "routine histological methods" for light microscopy. The samples of 5 μm thickness were stained with hematoxylin and eosin. The tissue section images were obtained under a light microscope (OLYMPUS BX61), using Cell^P software (Olympus).

2.6. Statistical analyses

The data are expressed as means ±SD of n = 71, 6 or 7 per group. One–way analysis of variance (ANOVA) for statistical evaluation of results concerning blood analysis, lesions in the aortas, fat content of the liver, and the ileum parameter was used. This was followed by Duncan's new multiple range tests to assess differences between groups' means. $P < .05$ was considered significant.

3. Results

3.1. Performance parameters and lipids profile

Feed intake (from 16.5 to 17.4 g/d) and body weight (from 4.2 to 5.0 g/d) between groups did not show significant differences. FER values were also similar in all groups (3.67 ± 0.16) (data not shown). The somatic index of the liver was significantly higher ($P < .05$) in the Ch group than in the control group (3.45 vs 2.68, respectively) (Table 1). The supplementation of the Ch diet with hardy kiwifruit, Bidan, and Hayward cultivars lowered this value by an average of 2.8. An increase of TC in the serum of the rats fed a Ch diet versus control amounted to 26.4% ($P > .05$). The supplementation of kiwifruit (5%) to the atherogenic diet had a significant impact on the reduction of TC in serum of rats (with the exception of ChM1 and ChBidan conv). An increase of LDL-C was also obtained in the Ch versus the control by 346.2% ($P < .05$). All tested fruits significantly reduced the content of LDL-C. This decrease was the greatest in ChGeneva (0.29 mmol/L) and ChAnna (0.43 mmol/L) and the smallest in ChM1 (0.83 mmol/L) ($P > .05$). Cholesterol level significantly decreased (versus control group) the HDL-C level in the serum ($P < .05$). Bingo and Hayward cultivars led to an increase of cholesterol fraction, while the increase of HDL-C in the rat's serum in the other groups was not different. Changes of TG between the control and Ch group were not significant. All kiwifruit influenced the reduction of TG content, but this decrease was significant only in Jumbo, Geneva, Hayward, and Bidan conv (average of 42.1%). The value of the atherogenic indices (TC/HDL) increased from 1.29 (control) to 2.81 (Ch) ($P < .05$). Hardy kiwifruit, as well as Hayward and Bidan cultivars, significantly reduced ($P < .05$) the value of the atherogenic indices and the atherogenic index (AI), while the best values were obtained for Geneva, Bingo, and Bidan org.

3.2. Plasma TAC and enzymes activity

A decrease of total antioxidant activity (ABTS, FRAP, and DPPH assays) was registered in the Ch group compared to the control group ($P > .05$) (Table 2). Kiwifruit influenced an increase ($P < .05$) of TAC values in ChBingo, ChM1, ChAnna, ChBidan org, and ChBidan conv groups, as evaluated by the ABTS test. In other groups, this increase was negligible. The antioxidant activity in plasma determined by FRAP assay was significantly higher in ChM1, ChAnna, ChJumbo, ChGeneva, ChBidan org, and ChBidan conv than in the Ch group. For the DPPH test, a significant increase ($P < .05$) of antioxidant activity was noted in ChBingo, ChM1, ChBidan org, and ChBidan conv (versus Ch group); but, in the other groups, it was statistically not significant. The AST activity was higher in the Ch group than in the control (107.14 vs 90.00 IU/L) ($P > .05$). The significantly higher activity of this enzyme was recorded in ChBingo vs Ch ($P < .05$). Significant differences in AST activity were also shown between ChBingo and all groups of rats (except ChM1 and ChBidan org). In the case of ALT activity, the differences between groups were not significant ($P > .05$). The ALP activity was higher in the Ch group than in the control (177.10 vs 124.93 IU/L) ($P < .05$). Changes in ALP

Table 1 – Influence of hardy kiwifruit, Hayward and Bidan on lipids profile, atherogenic indices in serum of rats fed atherogenic diets and somatic index of liver

Indices	Control	Ch	ChBingo	ChM1	ChAnna	ChWeiki	ChJumbo	ChGeneva	ChHayward	ChBidan org	ChBidan conv
TC (mmol/L)	1.78 ± 0.35[a,b]	2.25 ± 1.07[a]	1.68 ± 0.30[b,c]	1.91 ± 0.42[a,b]	1.54 ± 0.21[b,c]	1.63 ± 0.49[b,c]	1.54 ± 0.18[b,c]	1.15 ± 0.13[c]	1.71 ± 0.28[b,c]	1.12 ± 0.35[b,c]	1.68 ± 0.41[a,b,c]
HDL-C (mmol/L)	1.39 ± 0.26[a]	0.90 ± 0.25[c]	1.27 ± 0.33[a,d]	1.07 ± 0.28[b,c]	1.12 ± 0.29[b,c]	0.91 ± 0.19[c]	1.05 ± 0.10[b,c]	0.86 ± 0.14[c]	1.21 ± 0.21[a,b]	0.86 ± 0.08[b,c,d]	1.01 ± 0.13[b,c]
LDL-C (mmol/L)	0.39 ± 0.09[a]	1.35 ± 1.22[b]	0.41 ± 0.26[a]	0.83 ± 0.47[a,b]	0.43 ± 0.24[a]	0.71 ± 0.31[a]	0.49 ± 0.20[a]	0.29 ± 0.07[a]	0.50 ± 0.28[a]	0.27 ± 0.28[a]	0.66 ± 0.29[a]
TG (mmol/L)	0.96 ± 0.38[a]	0.79 ± 0.31[a,b]	0.63 ± 0.23[b]	0.61 ± 0.16[b]	0.57 ± 0.12[b]	0.66 ± 0.28[b]	0.47 ± 0.05[c]	0.43 ± 0.07[c]	0.46 ± 0.18[c]	0.58 ± 0.20[a,b,c]	0.47 ± 0.11[c]
TC/HDL-C	1.29 ± 0.04[a]	2.81 ± 1.97[b]	1.37 ± 0.25[a]	1.85 ± 0.54[a]	1.43 ± 0.33[a]	1.76 ± 0.18[a]	1.49 ± 0.19[a]	1.35 ± 0.10[a]	1.44 ± 0.28[a]	1.30 ± 0.28[a]	1.63 ± 0.23[a]
AI	0.28 ± 0.03[a]	1.83 ± 1.98[b]	0.36 ± 0.26[a]	0.86 ± 0.56[a]	0.44 ± 0.33[a]	0.77 ± 0.20[a]	0.47 ± 0.21[a]	0.34 ± 0.10[a]	0.43 ± 0.28[a]	0.30 ± 0.30[a]	0.63 ± 0.23[a]
Liver (% BW)	2.68 ± 0.14[c]	3.45 ± 0.49[a]	3.16 ± 0.23[a,b,c]	3.20 ± 0.33[a,b,c]	3.18 ± 0.35[a,b,c]	2.92 ± 0.19[c]	2.87 ± 0.10[c]	3.08 ± 0.20[b,c]	3.17 ± 0.30[a,b,c]	3.45 ± 0.35[a,b]	3.00 ± 0.19[b,c]

Means ± SD (standard deviation) of 6 measurements. Averages in lines marked with different letters (a, b, c) differ significantly (P < .05). Lack of letters indicates no difference between the means (P > .05). Ch, atherogenic diet (1% cholesterol); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; TC/HDL-C, atherogenic indice; TC-HDL-C/HDL-C, atherogenic index (AI); BW, body weight; org, organic; conv, conventional.

Table 2 – Influence of hardy kiwifruit, Hayward and Bidan on TAC in plasma and enzymes activity in serum of rats fed atherogenic diets

Indices	Control	Ch	ChBingo	ChM1	ChAnna	ChWeiki	ChJumbo	ChGeneva	ChHayward	ChBidan org	ChBidan conv
ABTS (mmol TE/L)	1.59 ± 0.21[b,c,d,e]	1.51 ± 0.06[e]	1.65 ± 0.05[a,b,c]	1.68 ± 0.13[a,b]	1.65 ± 0.09[a,b,c]	1.53 ± 0.08[d,e]	1.62 ± 0.05[a,b,c,d,e]	1.5 ± 0.04[c,d,e]	1.55 ± 0.04[c,d,e]	1.68 ± 0.01[a,b,c,d]	1.71 ± 0.06[a]
FRAP (μmol TE/L)	239.82 ± 5.48[d,e]	230.87 ± 14.23[e]	250.15 ± 15.18[d,e]	290.24 ± 34.83[c]	266.31 ± 28.52[c,d]	244.51 ± 20.15[d,e]	264.47 ± 10.83[c,d]	263.70 ± 9.95[c,d]	256.89 ± 5.67[d,e]	345.33 ± 5.27[b]	393.44 ± 35.01[a]
DPPH (μmol TE/L)	279.86 ± 18.54[d,e]	277.13 ± 4.50[d,e]	286.81 ± 4.96[c,d]	296.04 ± 17.06[b,c]	281.83 ± 12.70[d,e]	272.89 ± 6.30[e]	283.82 ± 9.28[d,e]	282.34 ± 9.65[d,e]	276.75 ± 4.43[d,e]	309.83 ± 3.01[a,b]	325.36 ± 10.39[a]
AST (UI/L)	90.00 ± 8.57[a]	107.14 ± 29.21[a]	134.87 ± 13.08[b]	113.72 ± 9.44[a,b]	100.84 ± 14.74[a]	99.73 ± 9.77[a]	111.41 ± 25.43[a]	93.80 ± 8.45[a]	95.10 ± 8.38[a]	120.85 ± 36.27[a,b]	98.33 ± 13.64[a]
ALT (UI/L)	33.21 ± 6.92[a]	31.27 ± 7.03[a]	32.44 ± 3.58[a]	32.12 ± 6.34[a]	28.66 ± 5.99[a]	29.39 ± 6.33[a]	32.27 ± 5.00[a]	30.72 ± 3.01[a]	33.17 ± 7.49[a]	37.45 ± 3.32[a]	29.90 ± 2.63[a]
ALP (UI/L)	124.93 ± 16.67[a]	177.10 ± 24.16[b]	165.69 ± 26.42[a,b]	193.10 ± 37.79[b]	181.31 ± 30.28[b]	180.19 ± 30.15[b]	192.17 ± 64.11[b]	177.43 ± 26.47[b]	198.09 ± 38.05[b]	233.35 ± 79.27[b]	204.27 ± 71.63[b]
Lipase (UI/L)	7.88 ± 0.31[a,b]	7.74 ± 0.94[a,b]	8.31 ± 0.50[a,b]	8.04 ± 0.89[a,b]	8.00 ± 0.62[a,b]	7.63 ± 0.50[a,b]	8.27 ± 1.24[a,b]	7.39 ± 0.54[a]	8.67 ± 1.32[b]	7.83 ± 0.50[a,b]	7.66 ± 1.28[a,b]
Amylase, UI/L	1438 ± 149[a]	1472 ± 388[a]	1613 ± 279[a,b]	1363 ± 289[a]	1456 ± 267[a]	1664 ± 312[a,b]	1640 ± 276[a,b]	1824 ± 195[b]	1563 ± 295[a,b]	1869 ± 68[a,b]	1539 ± 215[a,b]

Means ± SD (standard deviation) of 6 measurements. Averages in lines marked with different letters (a, b, c, d, e) differ significantly (P < .05). Lack of letters indicates no difference between the means (P < .05). ABTS, 2,2_Azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; FRAP, Ferric-reducing/antioxidant power; DPPH, 1,1-diphenyl-2-picrylhydrazyl; RSA, radical scavenging activity; TE, trolox equivalent; TAC, total antioxidant capacity; AST, Aspartate aminotransferase; ALP, Alkaline phosphotase; Ul, Unit International.

activity between groups were not different ($P > .05$). The activity of lipase and amylase was similar in the control and Ch groups, while the lowest activity of lipase and the highest activity of amylase was noted in ChGeneva ($P < .05$). A significant difference concerning the activity of lipase in serum (7.39 vs 8.67 UI/L) ($P < .05$) was found between groups ChGeneva and ChHayward, while amylase activity in serum was significantly lower ($P < .05$) in groups ChM1, ChAnna, control, and Ch as compared to ChGeneva.

3.3. Blood hematological and coagulation parameters

The amount of RBC (Red Blood Cells) ranged from 7.28 to 8.20 T/L, WBC (White Blood Cells) were between 4.21–6.92 G/L, and PLT (Platelet) counts ran from 668.8 to 940 G/L, with no significant differences between groups (Table 3). Hemoglobin ranged from 13.30 (ChAnna) to 14.13 g/dL (ChBidan conv), while hematocrit values ranged from 40.12 (ChM1) to 42.63% (ChBidan conv) ($P > .05$). The differences in the values of prothrombin index, INR (International Normalized Ratio), prothrombin time, and fibrinogen content in blood were not statistically significant between the groups. Noteworthy was the reduction in prothrombin time in the Ch group (compared to control group) (17.30 vs 18.07 seconds) and the increase in the groups receiving kiwifruit (except for ChBingo and ChM1). Kiwifruit groups (except ChBidan conv and ChJumbo) caused a decrease (an average 30%) of fibrinogen content in blood ($P > .05$).

3.4. Serum inflammatory markers and glucose

The content of C-reactive protein increased in all groups of rats (except ChBidan org) compared to the control group. This increase was significant in ChBingo and ChM1 groups (Table 4). There were no changes in the amount of IL-6, all values were below linearity (<12 pg/mL). The activity of MPO increased in the groups fed the atherogenic diet compared to the Control group ($P > .05$). Groups fed diets with mini kiwis or kiwifruit Hayward and Bidan showed high individual variability between rats (especially in ChBingo, ChHayward, and ChBidan). It is worth stressing that the lowest MPO activity was in serum of groups ChGeneva, ChWeiki, ChJumbo, and ChAnna. An increase in glucose levels against Ch was found in groups ChWeiki (4.84 mmol/L) and ChGeneva (5.13 mmol/L) ($P < .05$). The lowest glucose level was recorded in ChBingo and ChM1 (an average of 2.65 mmol/L).

3.5. Histological changes in aortic arch, liver, and ileum

Changes in the aortic arch, liver, and ileum were smaller in the groups of rats receiving kiwifruit. The smallest number of atherosclerotic lesions in the aortic arch occurred in the ChGeneva and ChWeiki. The percentages of aortic lesions in arch of the aorta were: 0.07 (control), 6.2 (Ch), 4.5 (ChBingo), 4.2 (ChM1), 0.6 (ChAnna), 0.3 (ChWeiki), 3.8 (ChJumbo), 0.3 (ChGeneva), 1.9 (ChHayward), 3.95 (ChBidan org), and 3.8 (ChBidan conv) (Fig. 1). These changes were in the following order: ChGeneva = ChWeiki > ChAnna > ChHayward > ChBidan conv = ChJumbo > ChBidan org > ChM1 > ChBingo > Ch.

Morphological differences in the lipid content showed a pale orange color (low lipid content) in liver tissue taken from rats fed a control diet, while the red color (high lipid content) was evident in tissue from rats with a high cholesterol diet (Fig. 2). This was shown by the 1 to 10 μm of fat vacuoles in a compact system in the hepatocytes of rats fed with cholesterol. Similarly, evenly stained tissues were derived from rats fed the atherogenic diet with the addition of Bidan org, Bidan conv, Jumbo, or Hayward; the color of the tissue was uniform in these cases. For example, in the group of rats fed a diet that included cholesterol and fruits, the Bidan conv in hepatocytes revealed scattered vacuoles of 1 to 10 μm, and their color was orange (Fig. 3a). Liver tissue that was taken from rats fed the Ch diet with fruits Anna, Geneva, Weiki, and the M1 presents a picture of "mosaic." In this case, red stain was found next to the lobules of pale orange color. An example of this "mosaic" character is presented in Fig. 3b and c of liver tissue isolated from rats of ChGeneva group. "Mosaic" picture deployment of lipids in rats liver tissue shows that the distribution of lipids in each lobule had changed. The intensity of the color resulting from the lipid content was variable. It follows that the distribution of lipids in the liver tissue depends on the content of the bioactive compounds present in tested fruits. The "mosaic" picture of the liver in rats treated with atherogenic diets and supplemented with hardy kiwifruit is an important observation concerning fat deployment and indicates the presence of hepatocytes filled with fat (stained red). The hepatocytes which have already been "cleansed" of fat are light-colored. This is a positive protective effect of the tested hardy kiwifruit. As can be seen, the histological architecture of rat liver was acceptable and showed arranged hepatic cords radiating from the central venule. It is worth stressing that histological changes (numerous fat droplets) were observed inside hepatic cells of rats loaded with cholesterol. The fat integral density (μg/μm^3 of 100 hepatocytes) for groups C, Ch, ChBingo, ChM1, ChAnna, ChWeiki, ChJumbo, ChGeneva, ChHayward, ChBidan org, and ChBidan conv are as follows: 227, 823, 640, 656, 333, 403, 696, 253, 364, 502, 457, respectively. The results of fat integral density pointed toward a positive effect of the studied fruits Actinidia arguta (especially for cultivar Geneva and Anna), and their impact on changes in the liver and aorta was higher than for Hayward and Bidan, coming from organic and conventional cultivations.

Morphological studies of the small intestine of rats related also to other parameters, such as villus width and the thickness of the mucosa. The results given are only the length of the villi, as they were significantly correlated with the content of soluble dietary fiber (SDF) in kiwifruit. Kiwifruit significantly affected ($P < .05$) the length of villi in the ileum. The longest villi were in ChWeiki, and significantly lower ones estimated in ChHayward, ChBidan org, and ChBidan conv diet groups (Fig. 4A). A significant correlation ($R^2 = 0.8762$) between the SDF and length of intestinal villi (Fig. 4B) was found.

4. Discussion

The hypothesis of the present study was that hardy kiwifruit from Poland possess better pro-healthy action than other varieties from Asia. Therefore, for the first time, we evaluated the metabolic reaction of Wistar rats fed a diet for 42 days of 1% cholesterol and different cultivars of hardy kiwifruit and

Table 3 – Influence of hardy kiwifruit, Hayward and Bidan on hematological and coagulation parameters in blood of rats fed atherogenic diets

Indices	Control	Ch	ChBingo	ChM1	ChAnna	ChWeiki	ChJumbo	ChGeneva	ChHayward	ChBidan org	ChBidan conv
RBC, T/L	7.7 ± 0.49[a,b]	7.89 ± 0.39[a,b]	8.20 ± 0.25[a]	7.28 ± 1.44[b]	7.92 ± 0.56[a,b]	7.83 ± 0.24[a,b]	7.75 ± 0.37[a,b]	8.16 ± 0.25[a,b]	8.19 ± 0.20[a,b]	8.34 ± 0.12[a,b]	8.15 ± 0.28[a,b]
WBC,G/L	5.50 ± 2.18[a]	6.69 ± 2.07[a]	6.07 ± 1.68[a]	5.01 ± 2.70[a]	4.21 ± 1.85[a]	5.33 ± 1.18[a]	4.34 ± 1.20[a]	5.43 ± 1.39[a]	6.92 ± 3.70[a]	5.15 ± 0.21[a]	5.00 ± 0.77[a]
PLT, G/L	839.5 ± 77.8[a]	867.5 ± 59.4[a]	828.8 ± 294.0[a]	668.8 ± 154.9[a]	707.0 ± 395.0[a]	755.3 ± 34.9[a]	880.5 ± 251.1[a]	933.7 ± 306.2[a]	939.9 ± 30.4[a]	853.5 ± 33.2[a]	830.5 ± 221.5[a]
HGB, g/dL	13.62 ± 0.62[a]	13.48 ± 0.45[a]	13.83 ± 0.44[a]	13.80 ± 0.84[a]	13.30 ± 0.96[a]	13.40 ± 0.52[a]	13.78 ± 0.53[a]	13.57 ± 0.74[a]	13.87 ± 0.65[a]	13.90 ± 0.14[a]	14.13 ± 0.57[a]
Hematocrit,%	41.10 ± 2.33[a]	40.80 ± 1.17[a]	42.38 ± 1.44[a]	40.12 ± 5.58[a]	40.64 ± 2.59[a]	41.18 ± 1.89[a]	42.28 ± 1.21[a]	40.47 ± 1.06[a]	42.73 ± 2.30[a]	40.8 ± 0.28[a]	42.6 ± 1.50[a]
Prothrombin index, %	74.87 ± 8.66[a]	77.64 ± 4.37[a]	78.53 ± 7.44[a]	81.07 ± 1.21[a]	72.70 ± 9.02[a]	71.64 ± 4.28[a]	73.75 ± 7.14[a]	72.45 ± 5.94[a]	70.38 ± 7.92[a]	69.35 ± 9.55[a]	74.10 ± 5.41[a]
INR	1.47 ± 0.24[a]	1.38 ± 0.11[a]	1.37 ± 0.16[a]	1.30 ± 0.03[a]	1.53 ± 0.26[a]	1.54 ± 0.12[a]	1.50 ± 0.22[a]	1.52 ± 0.17[a]	1.59 ± 0.22[a]	1.62 ± 0.28[a]	1.48 ± 0.14[a]
Prothrombin time, sec	18.07 ± 2.21[a]	17.30 ± 1.01[a]	17.18 ± 1.57[a]	16.53 ± 0.25[a]	18.65 ± 2.41[a]	18.76 ± 1.11[a]	18.33 ± 2.01[a]	18.60 ± 1.56[a]	19.22 ± 2.05[a]	19.50 ± 2.69[a]	18.15 ± 1.32[a]
Fibrinogen, g/L	2.35 ± 0.72[a]	2.38 ± 0.58[a]	1.48 ± 1.16[a]	1.89 ± 1.11[a]	1.44 ± 1.37[a]	1.85 ± 0.80[a]	2.29 ± 0.81[a]	1.88 ± 0.87[a]	1.55 ± 0.94[a]	1.58 ± 1.46[a]	2.84 ± 0.66[a]

Means ± SD (standard deviation) of 6 measurements. Averages in lines marked with different letters differ significantly ($P < .05$).
Lack of letters indicates no difference between the means ($P > .05$). RBC, red blood cells; WBC, white blood cells; PTL, platelet; HBG, hemoglobin; INR, International Normalized Ratio; ch, cholesterol; org, organic; conv, conventional.

Table 4 – Influence of hardy kiwifruit, Hayward and Bidan on inflammatory markers and glucose in serum of rats fed atherogenic diets

Indices	Control	Ch	ChBingo	ChM1	ChAnna	ChWeiki	ChJumbo	ChGeneva	ChHayward	ChBidan org	ChBidan conv
Albumin (g/L)	35.86 ± 1.90[a]	35.43 ± 5.09[a]	37.31 ± 2.41[a]	35.73 ± 3.30[a]	36.11 ± 1.11[a]	34.63 ± 1.66[a]	37.27 ± 1.24[a]	37.12 ± 1.44[a]	36.97 ± 1.64[a]	42.85 ± 1.49[b]	36.82 ± 0.83[a]
MPO (μg/L)	0.65 ± 1.20[a]	1.83 ± 4.31[a]	2.31 ± 4.56[a]	1.45 ± 2.61[a]	0.57 ± 0.66[a]	0.2 ± 0.0[a]	0.2 ± 0.17[a]	0.20 ± 0.02[a]	1.40 ± 1.15[a]	1.27 ± 1.45[a]	2.01 ± 1.10[a]
IL-6 (pg/mL)	< 12	< 12	< 12	< 12	< 12	< 12	< 12	< 12	< 12	< 12	< 12
CRP (mg/L)	0.88 ± 0.18[b]	1.0 ± 0.18[a,b]	1.40 ± 0.29[a]	1.30 ± 0.29[a]	1.08 ± 0.82[a,b]	0.95 ± 0.35[b]	1.17 ± 0.39[a,b]	0.96 ± 0.29[b]	1.20 ± 0.36[a,b]	0.72 ± 0.18[b]	1.03 ± 0.22[a,b]
Glucose (mmol/L)	3.96 ± 0.85[a,b,c,d]	3.42 ± 1.02[b,c,d]	2.68 ± 0.78[d]	2.62 ± 0.52[d]	3.35 ± 1.10[c,d]	4.84 ± 1.89[a]	4.47 ± 0.85[a,b,c]	5.13 ± 1.07[a]	4.80 ± 1.74[a,b]	5.51 ± 0.62[a,b,c]	4.45 ± 1.04[a,b,c]

Means ± SD of 6 measurements. Averages in lines marked with different letters differ significantly ($P < .05$).
Lack of letters indicates no difference between the means ($P > .05$).
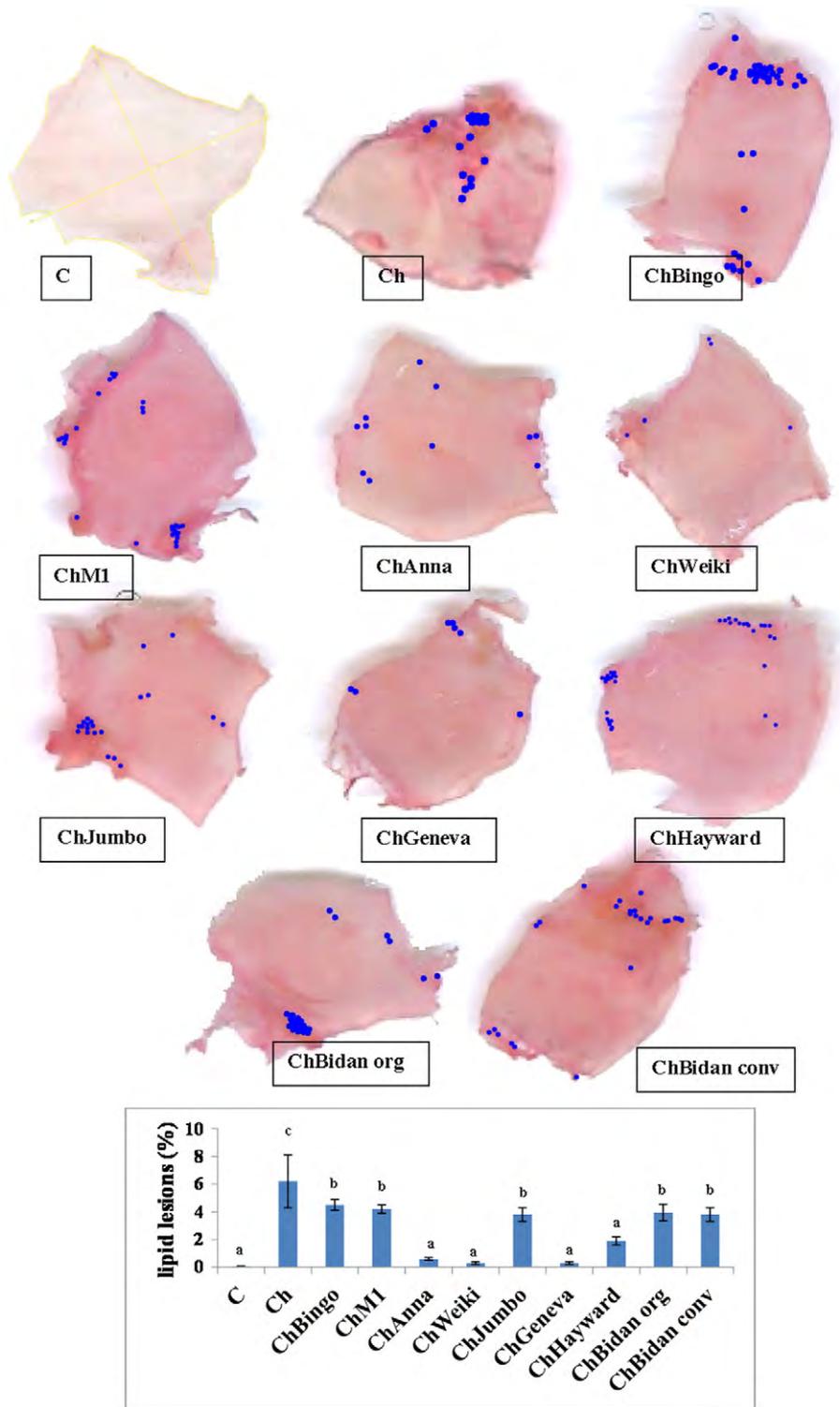MPO, myeloperoxidase; IL-6, interleukin-6; CRP, C-reactive protein; Ch, cholesterol; org, organic; conv, conventional.

Fig. 1 – (I) Changes in the histology of the aortic arch. (II) Lipid lesions in the aortic arch of rats fed different diets supplemented with kiwifruits (% of area). Footnotes: Abbreviations: C – control; Ch - cholesterol; org - organic cultivation; conv - conventional cultivation. Means ± SD (standard deviation) of n=6 measurements. Lack of symbols indicates no difference between the mean (P < .05).

Hayward and Bidan growing in organic and conventional conditions. Their composition and bioactivity had been published previously [16]. It has been shown that cholesterol slightly decreased (as compared to the control diet) the antioxidant activity of plasma, namely: 5, 4, and 1% for the

test ABTS, FRAP, and DPPH, respectively. However, in our previous studies, this decline was larger [10]. The supplementation of kiwifruit into the Ch diet caused an increase of the TAC in plasma of rats by 8% (ABTS), 24% (FRAP), and 5% (DPPH) (an average for all rats). The increase of total
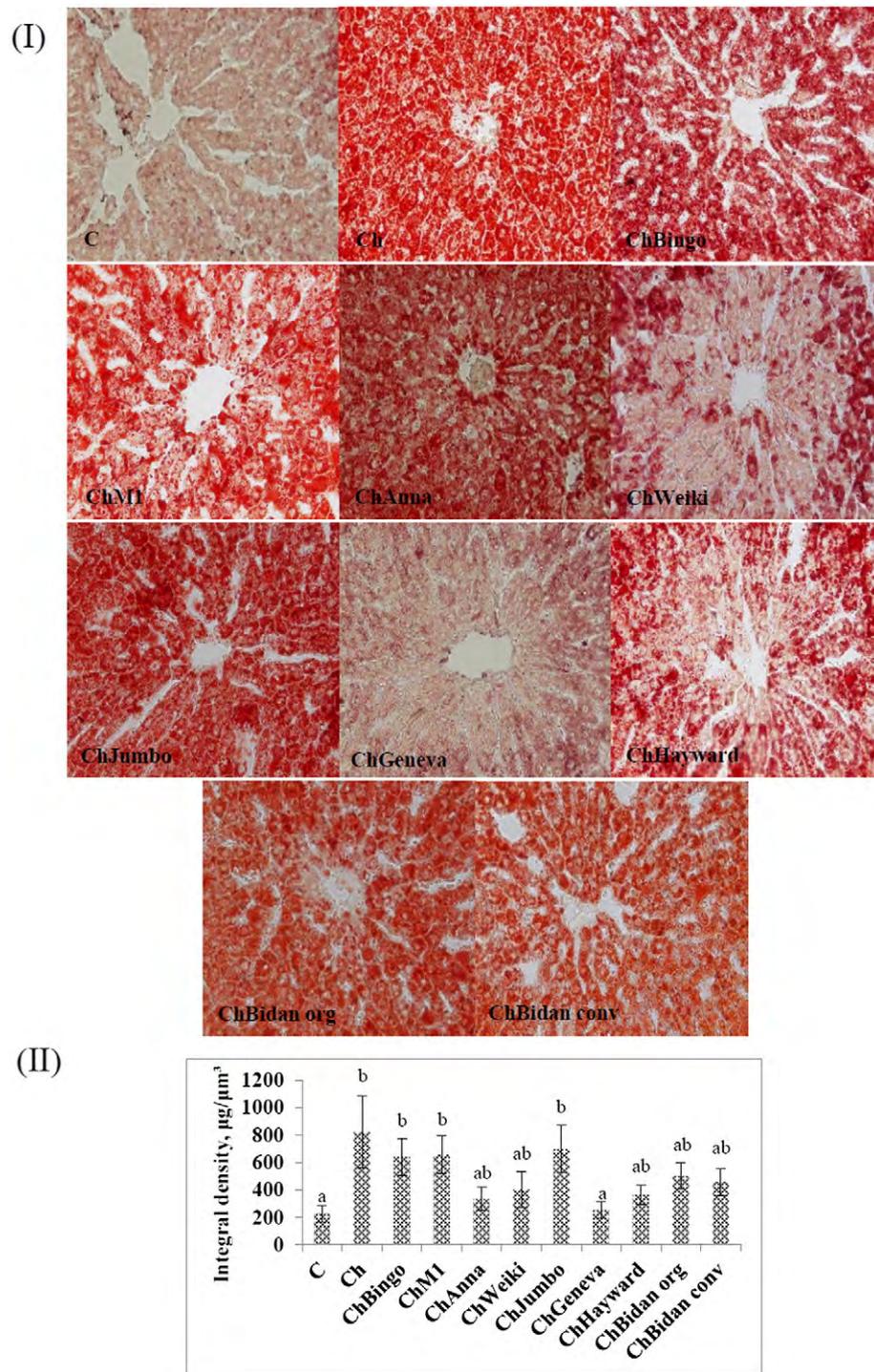
Fig. 2 – Hepatic tissue morphology of Wistar rats of the following diet groups: (I) C-Control. Ch-Control with cholesterol (intensive red coloring was the effect of high contents of fat inside hepatic cells). ChBingo, ChM1, ChAnna, ChWeiki, ChJumbo, ChGeneva, ChHayward, ChBidan org and ChBidan conv – groups with cholesterol and kiwifruits. Red Oil stain was used, magnification 40. (II). Changes in fat integral density ($\mu g/\mu m^3$) in liver. Footnotes: Values are the means ± SD of 6 measurements. Values in bars for every analysis bearing different letters are significantly different ($P < .05$).

antioxidant capacity in plasma of rats was the highest in ChM1 and ChBidan conv diet groups.

Polyphenols and other bioactive ingredients contained in fruits affect their antioxidant status and also total antioxidant capacity in plasma of rats [23], where low levels of plasma

TAC can cause mortality due to coronary atherosclerosis [24]. Supplementation of kiwifruit to an atherogenic diet significantly improved all lipid parameters. The tested fruits significantly influenced the decreased level of LDL-C. The largest decrease was recorded in ChGeneva, and in this group,
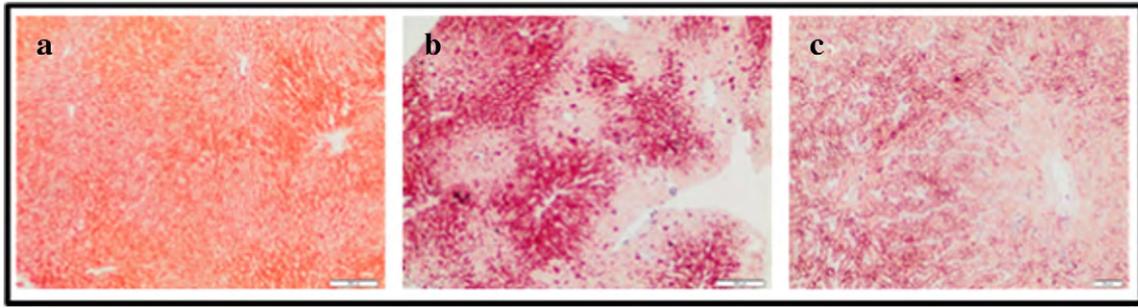
Fig. 3 – (a) An even deployment of fat in the liver tissue isolated from rats fed a diet with cholesterol and kiwifruit Bidan conv. Oil Red stain was used, magnification 20. (b and c) "Mosaic" character deployment of lipids in the liver tissue isolated from rats fed diets with cholesterol and hardy kiwifruit Geneva. Oil Red stain was used, magnification 20.

the lowest content of TC and TG in rat's serum was also noted. The content of HDL-C was the highest in ChBingo and ChHayward, and in other groups the values were comparable.

Our results are in line with a number of reports, where the positive effect of *A. deliciosa* Hayward was presented in a study on humans with hypercholesterolemia [25-27]. These authors showed an increase of HDL content in plasma and a decrease in the ratio of TC/HDL-C, as well as an improvement of

Fig. 4 – (A) Villi length in the intestine (ileum) of rats fed a diet with cholesterol and kiwifruit. (B) Correlation between villi length in the intestine (ileum) of rats and soluble dietary fiber (SDF) content in kiwifruit. Footnotes: Values are the means ± SD of 6 measurements. Values in bars for every analysis bearing different letters are significantly different (P < .05).

inflammatory status in subjects with elevated CRP levels who consumed kiwifruit. The increase of HDL level is associated with an increase in the content of apoA1 (the main structural HDL protein component) and with a reduction in plasma CRP and IL-6, thus suggesting a decrease in inflammation [26]. In the present study, a significant decrease in the values of the atherogenic indice TC/HDL-C in all groups of rats, with the addition of kiwifruit pointed to a valuable effect of these fruits in an atherogenic diet. Similar to kiwis, berries demonstrated a positive influence on HDL-C level [28,29]. The use of an atherogenic diet with kiwifruit reduced the content of TG, and it was significant in the ChGeneva, ChJumbo, and ChHayward groups. The reduction of 15% TG was estimated by others in humans [8] and also in our recent study [10]. The ratio of TC/HDL-C and TG/HDL-C, in all groups of rats receiving kiwifruit was lower. This is in line with other reports [30] that indicate that the consumption of one Hayward kiwifruit administration is associated with lower plasma triglycerides, fibrinogen content and higher HDL-C values in a large group of adults. This shows that this fruit mediates anti-inflammatory and hypolipidemic effects. Also, Stonehouse et al [31] underlined that fruits act in synergy to achieve multiple health benefits such as an increase of HDL-C and a decrease of TG and platelet aggregation.

These fruits improve poor iron status and protect the body from endogenous oxidative damage [31] while becoming part of our "daily prescription for health." Soluble dietary fiber (SDF) as well as insoluble dietary fiber (IDF) were higher in *A. arguta* than in Hayward and Bidan [16]. IDF, which resists digestion in the small intestine, becomes available as a substrate for microbial fermentation in the large intestine [5]. Dietary fiber and polyphenols present in fruits and vegetables contribute to health benefits in the gastrointestinal tract. SDF content in kiwis influence villi length in the small intestine (ileum). The important factors in atherosclerosis are disorders in coagulation and fibrinolysis. Duttaroy and Jorgensen [8] showed an 18% decrease in platelet aggregation in patients who ate kiwifruit.

In contrast to our earlier study [10], the present study showed not significant differences in blood platelets and in the values of coagulation parameters. However, it should be noted that there was a greater reduction of fibrinogen content (about 36%) in ChAnna, ChBingo, ChHayward, and ChBidan org groups. Lowering fibrinogen affected the prolongation of
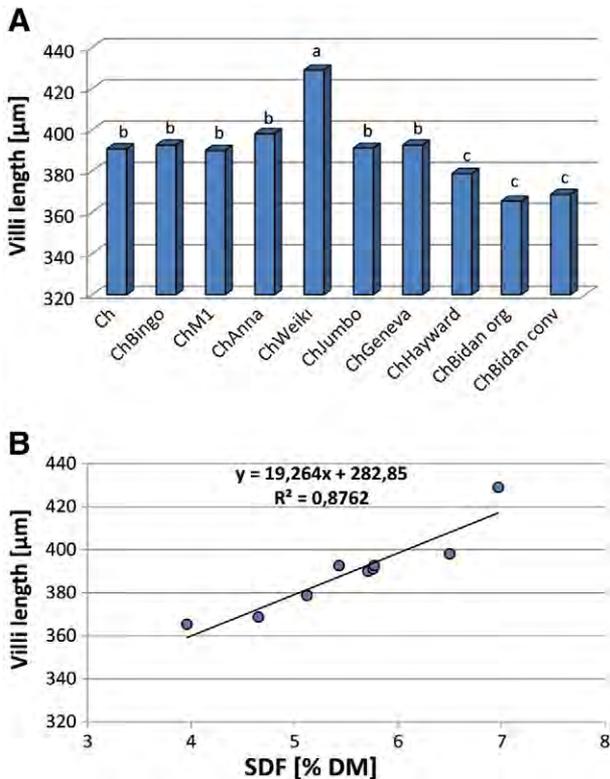
blood clotting time in rats fed kiwifruit, although not significantly. It is known [32-34] that vitamin C, polyphenols, and flavonoids (catechin, quercetin) can reduce the fibrinogen content. Results of Recio-Rodriques et al [30] underline the relationship between kiwifruit consumption and platelet aggregation. Elevated fibrinogen content, which is prominent acute-phase reactant, indicated cardiovascular risk.

The kiwifruit used herein had varying effects on glucose level in blood, and it may have been associated with the fruits' sugar content. According to Latocha et al [35], sucrose is the predominant sugar in mini kiwis, and the total amount of sugar in hardy kiwifruit may vary from year to year and differ between cultivars. Some authors pointed out the association between consumption of kiwis and insulin resistance [30].

Activity of liver enzymes was also evaluated in our study. Activity of ALP in blood increased in rats fed the diets with cholesterol in comparison to the control group, but did not change with supplementation of fruits. Similar results were obtained in another study on durian (5%) and kiwifruit Hayward (5%) in rats [10,17]. High-amylase activity affects the high glucose level in rat's blood from ChGeneva and other groups of rats (ChBidan org, ChJumbo). There were no signs of lipase activity in serum of rats in the Cholesterol group as compared to control. The level of interleukin-6 (IL-6) in serum was similar in all groups, and obtained results were below linearity (<12 pg/mL). CRP increased in all rat groups compared to the control group (except ChBidan org). The role of CRP in cardiovascular disease is controversial [36], as some authors claim that elevated CRP contents is a risk factor for CVD and coronary heart disease [37]. According to Ridker [38], CRP is a relatively moderate predictor of coronary heart disease, but it is stronger than LDL-C. The increase of CRP in the blood may promote pro-atherogenic effects [39]. In this connection, kiwifruit is a rich source of dietary compounds that is associated with beneficial effects on CRP, decreasing CRP and IL-6. Increased levels of quercetin and p-coumaric acid in plasma was present in subjects who consumed bilberry juice [40], while adult consumers of cranberry juice cocktail had lower CRP levels compared with nonconsumers [41].

In studies on human monocytic cell lines, it was demonstrated [42] that quercetin (also present in kiwis), resveratrol, and epicatechin inhibited the activation of nuclear factor-kappa B (NF-κB). This factor is a key regulator of pro-inflammatory signaling molecules, such as CRP and IL-6. Vitamins C and E in the kiwis inhibit NF-kB activation. Mieloperoxidase is a marker of damage of atherosclerotic plaque and plays a role in formation of cardiovascular diseases, including oxidation of LDL [43]. The activity of MPO was higher in the groups fed the Ch diet compared to the control group. Elevated levels of this enzyme are the factor of acute coronary syndrome [44,45]. The addition of hardy kiwifruit Anna, Weiki, Jumbo, and Geneva to the atherogenic diet decreased the MPO activity in serum. In our present and previous studies [17,18,22], we found atherosclerotic lesions in the aortas of rats. This is in accordance with other studies showing that the atherosclerotic lesions were found in mice [4,19,46] fed a diet with cholesterol.

We found that kiwifruit lowered the percent of atherogenic lesions in the aortic arch. The biggest changes were recorded for the hardy kiwifruits Geneva, Weiki, and Anna, and the smallest changes occurred with Bingo, M1, and Bidan. Our results showed reductions of atheromatous lesions in the total intimal surface area of aortic arch with ChGeneva, ChWeiki, and ChAnna. Similar reductions of aortic lesions in rats fed a diet with cholesterol and supplemented with fruits and vegetables was shown previously [17,22]. The positive effect of mini kiwis in rats given cholesterol revealed a difference in the distribution of fat in the liver tissue. ChGenewa, ChAnna, ChWeiki, and ChM1 groups had "mosaic" pattern while in the case of ChJumbo, ChHayward, and ChBidan showed uniform distribution of lipid, but the color intensity was lower than in the liver from the Ch group. The "mosaic" pattern demonstrates the positive impact of the ingredients contained in these fruits to partially limit the distribution of lipids in the liver tissue.

Somatic index of the liver was higher in all rats fed diets with the addition of cholesterol, in comparison with the control group. An increase of liver weight resulted from accumulation of lipids coming from the increase of lipogenesis in hepatocytes. The size of this accumulation was evaluated on the basis of the integral fat density in 100 hepatocytes. Our results correspond with those in a study by Nakayama et al [19], who found that young male mice fed a high-fat diet, had a markedly increased amount of fatty droplets in the liver cytoplasm when liver weight increased. The addition of cholesterol to a high-fat diet in mice caused liver damage by increasing the fat droplets in the liver [47]. Ramirez-Tortosa et al [20] reported that a study on rabbits found an increase of lipid levels both in plasma and in liver mitochondria and decreased concentrations of retinol and coenzyme Q-10 in plasma and mitochondria. Vinaixa et al [21] showed that dietary cholesterol increased the amount of hepatic cholesterol, triglycerides, and oleic acid, as well as the relative amount of long-chain polyunsaturated fatty acids in the liver. The supplementation of an atherogenic diet with durian or vegetables reduced the area of aortic atheromatous lesions and the fat integral density of 100 hepatocytes in the liver [17,22]. Based on our previous and present results, we can say that the changes in the aorta and deployment of lipids in the liver tissue depend on the content of the bioactive compounds present in tested fruits, and therefore, we accept our research hypothesis.

One of the limitations of the present investigation is the period of the rats' feeding. A longer period of investigation is more efficient in most cases because of the adaptation of the animals and their metabolic effects. The number of rats in each diet group was not sufficient. It would be preferable to increase the number of rats tested, but the Animal Care Committee of Warsaw only allowed seven rats per group. Additional control groups will help as well in comparing between the obtained results. The quality and the composition of varieties of kiwifruit depend on their collection from different geographic points and their harvest conditions, so 1 year to collect samples is limiting. However, since feed intake and performance parameters were similar, this, along with blood response and changes in aorta, liver, and ileum, let us draw the conclusions.

In conclusion, our results demonstrated that kiwifruit A. arguta, from Polish organic plantations, have antioxidative properties causing hypolipidemic and hypocholesterolemic

actions. Their anti-inflammatory properties protected the aortas and livers of rats loaded with exogenous cholesterol. Particularly noteworthy are the varieties of hardy kiwifruit Geneva, Anna, and Weiki, which significantly reduced the deposition of lesions in the aorta and caused a significant reduction of fat in the liver of rats. Their health-protective properties are better than in kiwifruit cultivars Hayward and Bidan. Hardy kiwifruit could be recommended for human health, since they appear to have the potential to reduce the risk of CVD. We believe that the results of this study are applicable to various fruit-supplemented diets.

Acknowledgment

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