„Najlepszy jest ten nauczyciel, który ucząc potrafi tchnąć przyjemność w dusze uczniów"

Platon

Serdeczne dziękuję moim Promotorom
Panu dr hab. Tomaszowi Sozańskiemu, profesorowi UMW
oraz Pani dr Agnieszce Matuszewskiej
za ich nieustające wsparcie, wyrozumiałość, poświęcony czas i cenne rady,
które były nieocenioną pomocą przy powstawaniu tej pracy.

SPIS TREŚCI

I. Wykaz publikacji stanowiących rozprawę doktorską

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2. Danielewski M, Matuszewska A, Szeląg A, Sozański T. The Impact of Anthocyanins and Iridoids on Transcription Factors Crucial for Lipid and Cholesterol Homeostasis. International Journal of Molecular Sciences. 2021; 22(11): 6074.
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3. Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (*Cornus mas* L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621.
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II. Streszczenie

Nieprawidłowe odżywianie jest jednym z najważniejszych problemów społecznych ostatnich lat. Zachodnia dieta, obfitująca w produkty wysokoprzetworzone, bogate w cholesterol i cukry proste, przyczynia się do obserwowanego stale trendu coraz powszechniej występującej nadwagi i otyłości oraz nierzadko powiązanych z nimi chorób metabolicznych, m.in. miażdżycy i cukrzycy typu II. W przebiegu tych stanów patologicznych często obserwuje się zaburzenia pracy układu sercowo-naczyniowego i wątroby, a także zmiany strukturalne (histologiczne), np. zgrubienie ścian tętnic. Profilaktyka i terapia chorób spowodowanych nieprawidłową dietą niejednokrotnie bywa nieskuteczna lub obarczona istotnymi działaniami niepożądanymi. Dlatego też stale poszukuje się nowych, potencjalnie skutecznych związków o prozdrowotnym wpływie na funkcje wątroby i układu krążenia, a jednocześnie bezpiecznych dla pacjenta. W takim przypadku obiecującą alternatywą pozostają naturalne związki pochodzenia roślinnego.

Do związków roślinnych, które mogą wykazywać korzystny wpływ na stan zdrowia w przypadku nieprawidłowej diety należą m.in. irydoidy i antocyjany. Wpływ ten wynika przede wszystkim z ich właściwości antyoksydacyjnych i przeciwzapalnych, a także zdolności do zmiany ekspresji niektórych czynników transkrypcyjnych. Irydoidy, substancje zaliczane do grupy monoterpenoidów, izolowane są głównie z części zielonych roślin, ale występują w dużym stężeniu także w niektórych owocach. Antocyjany, substancje z grupy polifenoli, to ciemne barwniki roślinne, pospolicie występujące w kwiatach i owocach. Źródłem związków z obu grup są m.in. owoce derenia jadalnego (*Cornus mas* L.).

Uwzględniając stosunkowo dużą zawartość irydoidów i antocyjanów w owocach derenia jadalnego, a także ich pozytywny wpływ na różnorakie parametry pracy układu sercowo-naczyniowego i wątroby, autor postanowił przeprowadzić szereg analiz, a ich wyniki i wnioski z nich wypływające przedstawić w niniejszej rozprawie. W tym celu dokonany został przegląd dostępnych źródeł literaturowych dotyczących prozdrowotnego wpływu związków czynnych z grup irydoidów i antocyjanów, ich mechanizmów działania i potencjalnych zastosowań w profilaktyce i leczeniu chorób układu krążenia i wątroby, a także zmian ekspresji najważniejszych czynników transkrypcyjnych istotnych dla przemian cholesterolu i lipidów. Natomiast celem badawczym przeprowadzonych analiz było określenie wpływu ekstraktu z owoców derenia jadalnego na ekspresję PPAR-α i PPAR-γ w aorcie oraz LXR-α w wątrobie, a także na stężenia wybranych markerów w surowicy krwi: cholesterolu (całkowitego, HDL, LDL), trójglicerydów, adipokin (leptyny, rezystyny, adiponektyny), apolipoprotein (A1, B100, E), glukozy i insuliny. Ponadto, ocenie poddano zmiany histopatologiczne

w ścianach aorty piersiowej i brzusznej – w warstwach intima i media oraz wyznaczono na ich podstawie wskaźnik I/M. W badaniach wykorzystano tkanki i krew pobraną od królików nowozelandzkich skarmianych przez 60 dni dietą bogatocholesterolową (1%). Zwierzęta w jednej grupie badawczej otrzymywały dodatkowo ekstrakt z owoców derenia jadalnego w dawce 10 mg/kg m.c., a w drugiej grupie badawczej w dawce 50 mg/kg m.c. Natomiast zwierzętom w grupie stanowiącej pozytywną kontrolę podawano dodatkowo simwastatynę w dawce 5 mg/kg m.c.

Zaobserwowano, że podawanie ekstraktu z owoców derenia jadalnego prowadzi do wzrostu ekspresji wszystkich trzech badanych czynników transkrypcyjnych. Przyczynia się także zwiększenia stężenia adiponektyny, jednocześnie zmniejszając stężenie leptyny, rezystyny i trójglicerydów. W ocenie histopatologicznej ścian tętnic odnotowano znaczący spadek wskaźnika I/M w obu grupach badawczych otrzymujących badany ekstrakt. Wszystkie powyższe zmiany wskazują na potencjalną skuteczność ekstraktu z owoców derenia jadalnego w profilaktyce i leczeniu chorób układu sercowo-naczyniowego i wątroby wynikających z nieprawidłowej diety. Irydoidy i antocyjany zawarte w ekstrakcie mogą przyczyniać się do ograniczenia ryzyka występowania, a także intensywności objawów związanych z otyłością chorób, takich jak miażdżyca, choroba wieńcowa czy zespół metaboliczny.

III. Summary

Improper nutrition is one of the most important social problems of recent years. The Western diet, rich in highly processed products abundant in cholesterol and simple sugars, contributes to the constantly observed trend of overweight and obesity, and frequently related to them metabolic diseases, including atherosclerosis and type II diabetes. In the course of these pathological conditions, disorders of the cardiovascular system and the liver, and also structural (histological) changes, e.g. thickening of the walls of the arteries, are commonly observed. Prevention and treatment of diseases caused by unbalanced diet are often ineffective or burdened with significant side effects. Therefore, new, potentially effective compounds with a health-promoting influence on the functions of the liver and cardiovascular system, and at the same time safe for the patient, are continuously searched for. In such case, natural compounds of plant origin remain a promising alternative.

Two groups of plant compounds that may possess a beneficial impact on health in case of an improper diet are iridoids and anthocyanins. This effect is mainly due to their antioxidant and anti-inflammatory properties, as well as their ability to alter the expression of certain transcription factors. Iridoids, substances classified to the group of monoterpenoids, are most often isolated from the green parts of plants, but are also found in high concentrations in some fruits. Anthocyanins, substances from the polyphenols group, are dark plant pigments commonly found in flowers and fruits. Cornelian cherry fruits (*Cornus mas* L.) are the source of compounds from both groups.

Taking into account the relatively high content of iridoids and anthocyanins in cornelian cherry fruits, as well as their positive impact on various parameters of the cardiovascular system and the liver, the author decided to conduct a number of analyzes and present their results and conclusions in this dissertation. For this purpose, a review of the available literature sources was made on the health-promoting effect of the active compounds from the groups of iridoids and anthocyanins, their mechanisms of action and potential applications in the prevention and treatment of cardiovascular and liver diseases, as well as changes in the expression of the most pivotal transcription factors crucial for cholesterol and lipid metabolism. The research goal of the conducted analyzes was to determine the effect of cornelian cherry extract on the expression of PPAR-α and PPAR-γ in the aorta and LXR-α in the liver, as well as on the concentration of selected markers in the blood serum: cholesterol (total, HDL, LDL), triglycerides, adipokines (leptin, resistin, adiponectin), apolipoproteins (A1, B100, E), glucose and insulin. Moreover, the histopathological changes in the walls of the thoracic and abdominal aorta were assessed - in the

intima and media layers, and the I/M ratio was determined on their basis. Tissues and blood collected from New Zealand rabbits fed for 60 days a cholesterol-rich diet (1%) were used in the experiment. One research group additionally received cornelian cherry extract at a dose of 10 mg/kg b.w., and the other research group at a dose of 50 mg/kg b.w. Animals in the positive control group received additionally simvastatin at a dose of 5 mg/kg b.w.

It was observed that the administration of cornelian cherry extract led to an increase in the expression of all three tested transcription factors. It also contributed to an enhancement in the concentration of adiponectin, while reducing the concentration of leptin, resistin and triglycerides. In the histopathological evaluation of the arterial walls, a significant decrease in the I/M ratio was noted in both research groups receiving the extract. All the above changes confirm the potential effectiveness of cornelian extract in the prevention and treatment of cardiovascular and liver diseases resulting from an improper diet. Iridoids and anthocyanins contained in the extract may contribute to the reduction of the occurrence risk and the intensity of symptoms of related to obesity diseases such as atherosclerosis, coronary artery disease or metabolic syndrome.

IV. Wstęp

Truizmem jest stwierdzenie, że odżywianie bezpośrednio wpływa na nasze zdrowie. Niezliczona liczba publikacji naukowych potwierdziła pozytywny lub negatywny wpływ, zarówno diety jako całości, jak i poszczególnych składników pokarmowych, na stan zdrowia człowieka, pracę poszczególnych organów czy układów. Daje się także zauważyć rosnącą świadomość konsumentów w kwestii rodzaju i jakości kupowanego pożywienia. I choć wciąż wiele popularnych produktów jest wysoko przetworzonych, bogatych w cukier i tłuszcze nasycone, to jednocześnie wzrasta dostępność wyrobów naturalnych i organicznych w powszechnej sprzedaży, nierzadko także producenci żywności starają się w pewnym stopniu modyfikować składy poszczególnych produktów w celu ograniczenia ich niekorzystnego wpływu na zdrowie człowieka, np. zmniejszając ilość użytego cukru czy zastępując olej palmowy olejem rzepakowym lub słonecznikowym.

Każdy człowiek ma swoje własne, unikalne nawyki żywieniowe, które kreuje wiele różnorakich czynników, jak chociażby aspekty kulturowe, ekonomiczne, geograficzne czy też panujące w danym momencie mody i trendy. Znane są różne rodzaje diet, jak np. podstawowa (zwyczajowa, określająca powszechny w danej społeczności sposób odżywiania się), alternatywna (np. wegańska, wegetariańska), terapeutyczna, eksperymentalna i wiele więcej. Za uniwersalną podstawę prawidłowego sposobu odżywania uważa się piramidy zdrowego żywienia, tworzone w różnych krajach m.in. według wytycznych Światowej Organizacji Zdrowia (WHO). Okresowo, w miarę postępu naukowego i coraz lepszego zrozumienia wpływu diety na stan zdrowia człowieka, piramidy są aktualizowane. W najnowszej obowiązującej w Polsce wersji, opublikowanej w 2016r. przez Instytut Żywności i Żywienia (obecnie część Narodowego Instytutu Zdrowia Publicznego), udział produktów roślinnych został zwiększony i to właśnie one powinny stanowić podstawę naszej diety i być spożywane w największej ilości [1].

Niewłaściwa dieta oraz niewielka aktywność fizyczna, prowadzące do nadwagi i otyłości, przyczyniają się do rozwoju chorób metabolicznych, takich jak miażdżyca, cukrzyca typu II czy zespół metaboliczny, w przebiegu których obserwuje się m.in. zaburzenia funkcji układu krążenia i wątroby [2-4]. Chorobom tym często towarzyszy przewlekły stan zapalny, w tym np. stan zapalny aorty, który prowadzi do zmian strukturalnych w jej ścianie. Zauważalne są również zmiany w stężeniach lub aktywności niektórych związków w surowicy krwi, np. adipokin, a także zmiany ekspresji czynników transkrypcyjnych kluczowych dla metabolizmu cholesterolu i lipidów, np. receptorów aktywowanych przez proliferatory

peroksysomów (*Peroxisome Proliferator Activated Receptor* - PPAR) czy receptorów wątrobowych X (*Liver X Receptor* - LXR) [5-9].

Wpływ diety na pracę układu sercowo-naczyniowego oraz wątroby został potwierdzony w licznych publikacjach naukowych. Stwierdzono m.in., że dieta obfitująca w produkty roślinne (owoce i warzywa) może zmniejszać ryzyko wystąpienia choroby wieńcowej, zmniejszać śmiertelność z powodu chorób układu krążenia, wykazywać właściwości hepatoprotekcyjne, a także korzystnie wpływać na poziomy lipidów krwi (różnych frakcji cholesterolu, trójglicerydów) czy enzymów wątrobowych (aminotransferazy alaninowej, ALT i asparaginianowej, AST) [10-12].

Wśród grup związków roślinnych, dla których potwierdzony został korzystny wpływ na stan zdrowia człowieka, wymienia się m.in. irydoidy i antocyjany. Są to dosyć powszechnie występujące substancje naturalne, których działania prozdrowotne są od dawna wykorzystywane w fitoterapii. Wciąż jednak trwają badania nad ich potencjalnie nowymi, nieznanymi dotąd właściwościami i zastosowaniami terapeutycznymi. Jednym z wartościowych źródeł zarówno irydoidów, jak i antocyjanów, są owoce derenia jadalnego [13].

Dereń jadalny (*Cornus mas* L.), zwany także czasem dereniem właściwym, z rodziny Dereniowatych (*Cornaceae*), to rozłożysty krzew lub małe drzewo, osiągające z reguły od 4 do 7 metrów wysokości. Owoce derenia mają wydłużony kształt o około 2 cm długości, dojrzewają na przełomie sierpnia i września, przyjmując intensywnie czerwoną, ciepłą barwę. Są bogate nie tylko w irydoidy i antocyjany, ale także m.in. fenolokwasy i kwasy organiczne [14, 15]. Jednak to właśnie ze względu na znaczną zawartość irydoidów i antocyjanów, owoce derenia jadalnego mogą odgrywać istotną rolę w profilaktyce i leczeniu wielu chorób układu krążenia i wątroby.

Irydoidy to organiczne związki chemiczne należące do grupy monoterpenoidów. Spotykane są w roślinach z powszechnie występujących rodzin, m.in. Jasnotowatych czy Trędownikowatych. Występują także w roślinach wykorzystywanych jako środki goryczowe, m.in. z rodziny Goryczkowatych. Związki irydoidowe izolowane są przede wszystkim z zielonych części roślin (głównie liści i młodych pędów), ale mogą też występować w dużych stężeniach w owocach. Do najważniejszych związków czynnych z grupy irydoidów zalicza się m.in. gencjopikrozyd, kwas loganowy, katalpol czy amarogentynę [16, 17].

Irydoidy wykazują bardzo szerokie spektrum działania terapeutycznego. Ich wpływ dotyczy przede wszystkim układu krążenia i wątroby (w tym przemian i poziomu glukozy i lipidów we krwi), ale opisano także na przykład działanie przeciwnowotworowe i immunomodulujące irydoidów. Korzystne efekty lecznicze związków irydoidowych wynikają głównie z ich właściwości antyoksydacyjnych i przeciwzapalnych. Dodatkowo wykazano

również, że irydoidy mogą zwiększać ekspresję czynników transkrypcyjnych, m.in. zaangażowanych w regulację metabolizmu lipidów i cholesterolu – PPAR, LXR oraz SREBP (*Sterol Regulatory Element Binding Protein*) [16, 18-20].

Antocyjany to ciemne barwniki roślinne należące do grupy polifenoli. Spotykane są w roślinach klasyfikowanych m.in. do rodzin Ślazowatych, Wrzosowatych czy Astrowatych. Z racji swej podstawowej roli – barwników – związki te izolowane są przede wszystkim z kwiatów i owoców. Antocyjany występują w roślinach głównie w postaci glikozydowej, jednak to przede wszystkim ich aglikony, nazywane antocyjanidynami, są właściwymi związkami czynnymi. Najważniejsze z nich to cyjanidyna, pelargonidyna i delfinidyna [21, 22].

Związki z grupy polifenoli to jedne z najczęściej wspominanych w kontekście działania prozdrowotnego związków naturalnych. Jest to bardzo szeroka grupa, która charakteryzuje się różnorakim spektrum działania na organizm na człowieka. Nie inaczej jest w przypadku antocyjanów, które podobnie jak irydoidy, mogą wpływać pozytywnie na funkcje układu sercowo-naczyniowego oraz wątroby, a także wielu innych układów i narządów, głównie dzięki właściwościom antyoksydacyjnym i przeciwzapalnym. Natomiast unikalną właściwością antocyjanów, odróżniającą je od innych polifenoli, jest zdolność do zmniejszania wytwarzania reaktywnych form tlenu (ROS) bez indukowania biogenezy mitochondrialnej lub ekspresji dysmutazy ponadtlenkowej [23]. Istnieje również wiele publikacji naukowych potwierdzających korzystny wpływ antocyjanów na ekspresję czynników transkrypcyjnych istotnych w metabolizmie lipidów i cholesterolu [24-26].

Procesy replikacji, transkrypcji i translacji odgrywają podstawową rolę w regulacji procesów namnażania, rozwoju i różnicowania komórek oraz utrzymania procesów życiowych wszystkich organizmów. Proces transkrypcji nigdy nie obejmuje całej nici DNA, a jedynie fragment, który koduje białko strukturalne lub funkcyjne, na które w danym momencie pojawia się zapotrzebowanie. Zjawisko to jest pośrednio kontrolowane przez specyficzną grupę białek i receptorów jądrowych, określanych mianem czynników transkrypcyjnych. Ich zadaniem, oprócz indukcji transkrypcji, jest skierowanie właściwego sygnału do sekwencji promotorowej genu kodującego pożądane białko [27, 28]. Do czynników transkrypcyjnych, które odgrywają kluczową rolę w metabolizmie cholesterolu i lipidów zalicza się: LXR-α, PPAR-α, PPAR-γ, C/EBPα (*CCAAT/enhancer-binding protein alpha*) oraz SREBP-1c. Zarówno irydoidy, jak i antocyjany, mogą zmieniać ekspresję wszystkich wymienionych powyżej czynników transkrypcyjnych.

Tkanka tłuszczowa wydziela mediatory zwane adipokinami. Wykazują one działanie autokrynne, parakrynne i endokrynne na różnorakie tkanki i narządy. Adipokiny odgrywają

istotną rolę regulatorową wielu procesów metabolicznych, m.in. przemian glukozy czy procesów energetycznych. Adipokiny są kluczowymi związkami wpływającymi na wrażliwość tkanek na insulinę, wśród nich można wyróżnić substancje zarówno o działaniu zmniejszającym insulinowrażliwość (np. rezystyna), jak i ją zwiększające (np. adiponektyna). Jedną z najważniejszych adipokin jest także leptyna, która nazywana jest hormonem sytości, bowiem oddziaływując na ośrodek głodu i sytości w podwzgórzu powoduje zmniejszenie łaknienia. Zaburzenia poziomu adipokin mogą przyczyniać się do rozwoju chorób metabolicznych, m.in. cukrzycy typu II czy chorób układu sercowo-naczyniowego, są także obserwowane w przebiegu otyłości [29-31]. Adipokiny mogą zatem pełnić jednocześnie rolę ważnych markerów niekorzystnych zmian zachodzących w organizmie pod wpływem nieprawidłowej diety. Dowiedziono, choć liczba publikacji na ten temat jest dotąd stosunkowo niewielka, że związki irydoidowe i antocyjanowe mogą wpływać na stężenia opisanych adipokin, tym samym pełnić potencjalnie wartościową rolę w profilaktyce i leczeniu chorób, w przebiegu których obserwuje się zaburzenia poziomu adipokin.

Białkowe części lipoprotein odpowiedzialne za wiązanie lipidów nazywane są apolipoproteinami. Wyróżnia się pięć klas apolipoprotein (oznaczonych literami od A do E), wśród których istnieje też wiele wariantów poszczególnych związków. Na stężenie i aktywność apolipoprotein w organizmie ludzkim wpływa wiele czynników, w tym m.in. dieta, a także aktywność hormonów, w tym insuliny [32, 33]. Dlatego też, podobnie jak w przypadku adipokin, zaburzenia poziomu apolipoprotein są obserwowane w przebiegu wielu chorób układu krążenia i wątroby, a oznaczenie i modyfikacje ich stężenia mogą pełnić istotną rolę diagnostyczną i terapeutyczną.

Zachodnia dieta (Western diet) charakteryzuje się wysokim udziałem produktów wysokoprzetworzonych, obfitujących w cholesterol i cukry proste, w tym napojów wysokosłodzonych. Wiąże się ją także ze stosunkowo szybkim powstawaniem nieprawidłowych zwyczajów żywieniowych, które mogą doprowadzić nawet do zmian mózgowych o charakterze przypominającym uzależnienie, a objawiających się objadaniem się produktami typu Fast-food i słodyczami [34, 35]. W odwracaniu tej niekorzystnej tendencji – modyfikacjach stosowanej diety, zwiększaniu aktywności fizycznej czy ograniczaniu zmian o charakterze metabolicznym – kluczową rolę pełni odpowiednia edukacja pacjentów, jednak rola farmakoterapii również jest nie do przecenienia. Niestety, stosowanie wielu leków syntetycznych w terapii miażdżycy czy otyłości wiąże się z występowaniem niekorzystnych działań niepożądanych. Z tego powodu stale poszukuje się nowych rozwiązań, środków które okazałyby się nie tylko skuteczne terapeutycznie czy profilaktycznie, ale także bezpieczne dla pacjenta. Alternatywą dla

farmaceutyków syntetycznych pozostają w takiej sytuacji związki pochodzenia roślinnego. Przykładem takiego środka jest ekstrakt z owoców derenia jadalnego, który dzięki stosunkowo wysokiej zawartości substancji czynnych z grup irydoidów i antocyjanów, może okazać się wartościowym preparatem stosowanym w zapobieganiu i leczeniu chorób układu sercowo-naczyniowego i wątroby, wynikających z nieprawidłowej diety.

Ewaluacja potencjalnego działania ekstraktu z owoców derenia jadalnego na organizm, poprzez wpływ na wszystkie opisane powyżej parametry, jest przedmiotem niniejszej rozprawy. W badaniu zastosowano model króliczy, w którym zwierzęta przez 60 dni skarmiano dietą bogatocholesterolową (1%). Jest to uznany model hipercholesterolemii – po raz pierwszy króliki zostały wykorzystane w badaniach nad miażdżycą przez Ignatowskiego w 1908 roku [36], a skarmianie królików dietą wzbogaconą o cholesterol (najczęściej w stężeniach wahających się w granicach od 0,05% do nawet 2% i więcej – w zależności od długości modelu badawczego oraz celu jaki ma w nim zostać osiągnięty), to obecnie jeden z trzech podstawowych modeli króliczych wykorzystywanych w badaniach nad hipercholesterolemią [37].

V. Założenia i cele pracy

Współczesna, zachodnia dieta może prowadzić do wystąpienia poważnych chorób metabolicznych, wynikających m.in. z zaburzeń pracy układu krążenia i wątroby. Związki naturalne z grup irydoidów i antocyjanów mogą wykazywać prozdrowotny wpływ na funkcje układu sercowo-naczyniowego i wątroby. Właściwości te mogą wynikać z wpływu na stężenie różnorakich związków endogennych istotnych w patogenezie chorób metabolicznych, z wpływu na ekspresję niektórych czynników transkrypcyjnych, a także pośredniego przeciwdziałania zmianom histologicznym zachodzącym w organizmie w następstwie nieprawidłowej diety. Owoce derenia jadalnego (*Cornus mas* L.) obfitują w składniki czynne z grup irydoidów i antocyjanów.

Podstawowym problemem badawczym podjętym w niniejszej rozprawie jest określenie czy ekstrakt z owoców derenia jadalnego może okazać się potencjalnie wartościowym terapeutycznie farmaceutykiem, który może być bezpiecznie stosowany w profilaktyce i leczeniu chorób wynikających z diety bogatej w cholesterol. W tym celu autor dokonał przeglądu, zestawienia i podsumowania dostępnych źródeł dotyczących wpływu związków z grup irydoidów i antocyjanów na różnorakie parametry funkcji wątroby i układu sercowo-naczyniowego – mechanizmów działania i ich efektów, a także potencjalnego zastosowania irydoidów i antocyjanów w konkretnych rodzajach zaburzeń i stanów patologicznych wątroby i układu krążenia (Publikacja nr 1 – udział autora rozprawy: opracowanie pomysłu i tematu artykułu, przegląd literatury, napisanie manuskryptu i stworzenie wizualizacji w postaci rycin). Następnie autor dokonał przeglądu, zestawienia i podsumowania dostępnych źródeł dotyczących wpływu związków z grup irydoidów i antocyjanów na najważniejsze czynniki transkrypcyjne odgrywające kluczową rolę w metabolizmie cholesterolu i lipidów (Publikacja nr 2 – udział autora rozprawy: opracowanie pomysłu i tematu artykułu, przegląd literatury, napisanie manuskryptu i stworzenie wizualizacji w postaci rycin i tabel). Na podstawie zdobytych informacji autor zaplanował badania i analizy, których celem było jak najdokładniejsze i jak najszersze określenie wpływu ekstraktu z owoców derenia jadalnego na wybrane parametry surowicy krwi, na ekspresję wybranych czynników transkrypcyjnych oraz na zmiany histologiczne zachodzące pod wpływem diety bogatej w cholesterol (Publikacja nr 3 – udział autora rozprawy: opracowanie pomysłu i tematu artykułu, wybór parametrów poddanych badaniom i metod ich pomiaru, wykonanie analiz metodą Elisa oraz Western Blot, analiza statystyczna wszystkich zebranych danych, przegląd literatury, napisanie manuskryptu i stworzenie wizualizacji w postaci rycin i tabel).

Konkretne cele badawcze niniejszej rozprawy zakładały ustalenie czy i w jakim stopniu dwie różne dawki ekstraktu z owoców derenia jadalnego (10 mg na kilogram masy ciała lub 50 mg na kilogram masy ciała), zastosowane w modelu króliczym, mają wpływ na:

- ekspresję receptora aktywowanego przez proliferatory peroksysomów-α (PPAR-α) i receptora aktywowanego przez proliferatory peroksysomów-γ (PPAR-γ) w aorcie,
- ekspresję receptora wątrobowego X-α (LXR-α) w wątrobie,
- stężenie leptyny, adiponektyny, rezystyny, glukozy, insuliny oraz wybranych lipoprotein i apolipoprotein w surowicy krwi,
- wartość wskaźnika intima/media aorty brzusznej i piersiowej (zmiany histopatologiczne).

Celem było również porównanie zmian w markerach surowicy krwi – tych, które odgrywają istotną rolę w praktyce klinicznej w przewidywaniu i ocenie ryzyka i progresji chorób sercowo-naczyniowych i metabolicznych – ze zmianami molekularnymi i histopatologicznymi w narządach docelowych.

VI. Materiał i metody

1. Materiał

Materiałem badań był ekstrakt z owoców derenia jadalnego (*Cornus mas* L.). Owoce derenia jadalnego zebrano w Arboretum i Instytucie Fizjografii w Bolestraszycach, w Polsce. Przed analizą owoce były przechowywane w probówkach typu Falcon w temperaturze -20°C. Okaz zielnikowy – *voucher specimen* (BDPA 3967) został uwierzytelniony i zdeponowany w Zielniku Arboretum i Instytutu Fizjografii w Bolestraszycach. Przygotowanie ekstraktu z owoców derenia jadalnego (EXT) zostało wykonane wg przepisu opisanego przez profesor Alicję Kucharską [38].

Zamrożone dojrzałe owoce derenia rozdrobniono i ogrzewano przez 5 minut w 95°C przy użyciu urządzenia Thermomix (Vorwerk, Wuppertal, Niemcy). Miazgę schłodzono do 40°C i poddano depektynizacji w temperaturze 50°C przez 2 godziny przez dodanie 0,5 ml Panzym Be XXL (Begerow GmbH & Co., Darmstadt, Niemcy) na 1 kg. Po depektynizacji i usunięciu pestek miazga została sprasowana w laboratoryjnej prasie hydraulicznej (Zodiak, SRSE, Warszawa, Polska). Wyciśnięty sok przefiltrowano i przepuszczono przez kolumnę z żywicą Amberlite XAD-16 (Rohm and Haas, Chauny Cedex, Francja). Zanieczyszczenia zostały spłukane wodą destylowaną, natomiast pigmenty i irydoidy eluowano 80% etanolem. Eluat poddano procesowi zagęszczania pod próżnią w 40°C. Rozpuszczalnik odparowano stosując Rotavapor (Unipan, Warszawa, Polska). Skoncentrowany ekstrakt barwników i irydoidów oczyszczono za pomocą octanu etylu w celu usunięcia niepolarnych zanieczyszczeń i innych flawonoidów. Procedurę oczyszczania powtórzono trzykrotnie. Próbka oczyszczonych związków została zatężona pod próżnią w temperaturze 40°C i liofilizowana (Alpha 1–4 LSC, Christ, Germany).

W doświadczeniu wykorzystano pięćdziesiąt dojrzałych płciowo królików nowozelandzkich płci męskiej, w wieku od 8 do 12 miesięcy. Zwierzęta były trzymane w indywidualnych klatkach w temperaturze w zakresie 21-23°C. Króliki poddano aklimatyzacji, ważeniu i obserwacji przez 4 tygodnie przed rozpoczęciem 60-dniowego badania. Następnie w sposób randomizowany zostały one podzielone na 5 grup po 10 zwierząt. Zwierzęta w grupie P były karmione standardową kompletną karmą dla królików. Zwierzęta w pozostałych grupach: CHOL, EXT 10, EXT 50 i SIMV 5 były karmione powyższą mieszanką wzbogaconą o 1% cholesterol. Podczas eksperymentu, króliki miały nieograniczony dostęp do wody i otrzymywały taką samą dzienną porcję karmy (40 g/kg). Raz dziennie, rano, przez kolejnych 60 dni doświadczenia, królikom podawano doustnie następujące substancje: grupa P

i CHOL – 0,9% r-r NaCl, grupa EXT 10 – ekstrakt z owoców derenia jadalnego 10 mg/kg m.c., grupa EXT 50 – ekstrakt z owoców derenia jadalnego 50 mg/kg m.c., grupa SIMV 5 – simwastatyna 5 mg/kg m.c. Próbki krwi zostały pobrane od każdego zwierzęcia na początku i końcu doświadczenia, z żyły brzeżnej ucha lub żyły odpiszczelowej. Na zakończenie eksperymentu, króliki zostały poddane terminalnej anestezji. Aorty i wątroby zostały następnie pobrane i oczyszczone, a później zamrożone i przechowywane w temperaturze -70°C do czasu wykonania dalszych analiz.

2. Metody badawcze

- Oznaczenie związków metodą HPLC-PDA

Oznaczenie irydoidów i antocyjanów przeprowadzono z wykorzystaniem wysokosprawnej chromatografii cieczowej (HPLC, *high-performance liquid chromatography*) z detektorem z matrycą diodową (HPLC-PDA). Metoda HPLC jest odmianą cieczowej chromatografii kolumnowej, która wyróżnia się wysokim ciśnieniem pod jakim podawany jest eluent na kolumny. Pozwala ona na separację, oczyszczenie i identyfikację związków chemicznych. Do oznaczenia wykorzystano system Dionex HPLC (Germering, Niemcy) wyposażony w detektor z matrycą diodową Ultimate 3000. Zastosowano kolumnę Cadenza Imtakt CD-C18 (75 x 4,6 mm, 5 μm). Faza ruchoma była mieszaniną 4,5% kwasu mrówkowego v/v (A) i 100% acetonitrylu (B), płynącą 1,0 ml/min w elucji gradientowej o następujących warunkach: 0–1 min 5% B w A, 1–20 min 25% B w A, 20–26 min 100% B, 26–30 min 5% B w A. Irydoidy monitorowano przy długości fali 245 nm, kwas elagowy przy 254 nm, fenolokwasy przy 320 nm, flawonole przy 360 nm i antocyjany przy 520 nm. Irydoidy zostały określone ilościowo jako kwas loganowy, antocyjany jako cyjanidyno-3-O-glukozyd, kwasy fenolowe jako kwas p-kumarowy, kwas kawowy i kwas elagowy, flawonole jako kwercetyno-3-O-glukozyd i kemferolo-3-O-glukozyd.

- Pomiar parametrów fizycznych i biochemicznych

Masę ciała królików oznaczono za pomocą wagi precyzyjnej (Radwag, Radom, Polska). Do oznaczeń poziomów lipidów surowicy krwi i glukozy wykorzystano metody kolorymetryczne - metody opierające się na określaniu stężenia roztworów barwnych poprzez porównanie intensywności barw roztworów badanych i odpowiednich wzorców. Poziomy

cholesterolu całkowitego (TC) i trójglicerydów (TG) w surowicy zostały określone z wykorzystaniem kolorymetrycznych metod enzymatycznych. Poziomy lipoprotein o dużej gęstości (HDL) i lipoprotein o małej gęstości (LDL) zmierzono bezpośrednimi metodami kolorymetrycznymi (ABX Pentra 400, Horiba Ltd., Kioto, Japonia). Stężenie glukozy określono przy użyciu testu aktywności glukozy Trindera (ABX Pentra 400, Horiba Ltd., Kioto, Japonia).

- Oznaczenie stężeń adiponektyny, leptyny, rezystyny, apolipoprotein (A1, B100, E) i insuliny metodą testu immunoenzymatycznego (ELISA)

Metoda ELISA to jeden z najczęściej stosowanych w diagnostyce biomedycznej testów immunoenzymatycznych fazy stałej. Służy do wykrywania i ilościowego oznaczania białek z wykorzystaniem przeciwciał mono- lub poliklonalnych, skoniugowanych z odpowiednim enzymem. Metoda ta opiera się na wytworzeniu wiązania pomiędzy antygenem a przeciwciałem, co uwidacznia reakcja barwna, której intensywność jest ilościowo zależna.

Następujące zestawy ELISA zostały wykorzystane do przeprowadzenia oznaczeń: adiponektyna (ADP/Acrp30, ERB0002, Fine Test, Wuhan Fine Biotech Corp., Wuhan, Chiny), leptyna (CSB-E06971Rb, Cusabio Technology LLC, Houston, Teksas, USA), rezystyna (ERB0105, Fine Test, Wuhan Fine Biotech Corp., Wuhan, Chiny), apolipoproteina A1 (ERB0009, Fine Test, Wuhan Fine Biotech Corp., Wuhan, Chiny), apolipoproteina B100 (ERB0010, Fine Test, Wuhan Fine Biotech Corp., Wuhan, Chiny), apolipoproteina E (ERB0014, Fine Test, Wuhan Fine Biotech Corp., Wuhan, Chiny), insulina (ELISA 90186, Crystal Chem Inc., Elk Grove Village, Illinois, USA). Badania przeprowadzono zgodnie z instrukcjami producenta. Wszystkie stężenia wyrażono w ng/ml lub mmol/l.

- Ocena histopatologiczna ścian aorty piersiowej i brzusznej

Materiał utrwalony w buforowanej 7% formalinie zatopiono w parafinie i pocięto na 4 µm sekcje, które wybarwiono standardową metodą hematoksylina-eozyna. Próbki oglądano bez znajomości podziału na grupę kontrolną i grupy eksperymentalne. Analizę mikroskopową przeprowadzono przy użyciu mikroskopu świetlnego Olympus BX53, połączonego z aparatem Olympus UC90. Pomiar grubości warstw intima i media wykonano przy użyciu standardowego oprogramowania cellSens V1. Zdjęcia zostały wykonane w powiększeniu 100× lub 200×, skala pokazana na zdjęciach to 50 µm lub 100 µm.

- Izolacja RNA, odwrotna transkrypcja i Real-Time PCR

Metoda PCR, czyli łańcuchowa reakcja polimerazy, to technika powielania łańcuchów DNA w wyniku wielokrotnego podgrzewania i oziębiania próbki. Znalazła szerokie zastosowanie w analizie ekspresji genów, diagnostyce klinicznej czy badaniach nad genomem. Real-time PCR, czyli metoda PCR w czasie rzeczywistym, inaczej nazywana PCR ilościowym, pozwala na określenie danej sekwencji w próbie za pomocą pomiarów fluorescencji. Charakteryzuje się znacznie lepszą czułością i precyzją w porównaniu do standardowego PCR, umożliwiając analizę jakościową i ilościową nawet przy niewielkim stężeniu badanego materiału, w stosunkowo krótkim czasie.

Całkowite RNA wyizolowano z badanych próbek tkanek za pomocą zestawu RNeasy Fibrous Mini Kit (Qiagen, Hilden, Niemcy) zgodnie z protokołem producenta. Aby wyeliminować ryzyko zanieczyszczenia genomicznego DNA, przeprowadzono trawienie DNazą na kolumnie przy użyciu zestawu RNase-Free DNase Set (Qiagen, Hilden, Niemcy). Ilość i czystość próbek RNA oceniono przez pomiar absorbancji przy 260 i 280 nm za pomocą spektrofotometru NanoDrop1000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). First-strand cDNA zsyntetyzowano przy użyciu zestawu High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, Kalifornia, USA) zgodnie z instrukcją producenta. Ekspresja mRNA PPAR-α i PPAR-γ została określona przez ilościową reakcję PCR w czasie rzeczywistym z wykorzystaniem systemu 7500 Real-Time PCR System i Power SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, Kalifornia, USA). Jako gen referencyjny zastosowano dehydrogenazę 3-fosforanową aldehydu glicerynowego (GAPDH). Reakcje przeprowadzono z wykorzystaniem zestawu primerów RT2qPCR dla króliczego PPAR-α (PPN13311A, Qiagen, Hilden, Niemcy), PPAR-γ (PPN05175A, Qiagen, Hilden, Niemcy) i GAPDH (PPN00377A, Qiagen, Hilden, Niemcy). Wszystkie reakcje przeprowadzono w trzech powtórzeniach w następujących warunkach: aktywacja polimerazy w 50°C przez 2 min, początkowa denaturacja w 94°C przez 10 min i 40 cykli denaturacji w 94°C przez 15 s, a następnie wyżarzanie i wydłużanie w 60°C przez 1 min. Swoistość reakcji PCR określono za pomocą analizy krzywej topnienia dla każdej reakcji. Względną ekspresję mRNA badanych czynników obliczono za pomocą metody ΔΔCt.

- Ocena ekspresji LXR-α metodą Western Blot

Metoda Western Blot znalazła szerokie zastosowanie w diagnostyce medycznej. Technika ta, inaczej zwana immunoblottingiem, to metoda badawcza biologii molekularnej, która pozwala na wykrycie konkretnych białek w analizowanym materiale, którym jest homogenat tkankowy lub ekstrakt białkowy. Podstawą działania tego testu jest reakcja antygen-przeciwciało. Western Blot składa się z dwóch głównych etapów: rozdziału elektroforetycznego oraz transferu rozdzielonych białek na membranę.

Wątroby królicze zhomogenizowano w buforze zawierającym 25 mM Tris pH 7,5, 50 mM NaCl, 1% NP-40 i zestaw inhibitorów proteazy. Po odwirowaniu, klarowny supernatant został zmieszany z buforem do próbek SDS, gotowany w 95°C przez 5 min i dodany do SDS-PAGE na 12% żelu. Rozdzielone białka przeniesiono na membranę PVDF (Thermo Fisher Scientific, Waltham, Massachusetts, USA) przy użyciu transferu półsuchego. Po przeniesieniu, membranę zablokowano 1% kazeiną w TBS w 4°C przez noc, a następnie inkubowano z 1 µg/ml przeciwciałem anty-LXR-α (NR1H3/LXR Alpha Antibody LS-B3526-50, LifeSpan Biosciences Inc., Seattle, Waszyngton, USA) i beta-aktyną C-04 (Santa Cruz Biotechnology Inc., Dallas, Teksas, USA) w temperaturze pokojowej przez 1 h, a następnie drugorzędowym przeciwciałem znakowanym peroksydazą chrzanową (Dako, Agilent, Santa Clara, Kalifornia, USA). Związane przeciwciała zwizualizowano za pomocą systemu detekcji West-Pico blotting (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Bloty zeskanowano, a gęstość optyczną prążków zanalizowano za pomocą programu Image J.

3. Metody statystyczne

Dane parametryczne wyrażono jako średnią ± odchylenie standardowe (średnia ± OS). Analizę statystyczną przeprowadzono przy użyciu oprogramowania Statistica v. 13.3 (TIBCO Software Inc., Palo Alto, Kalifornia, USA). Rozkład normalny wszystkich zmiennych ciągłych sprawdzono za pomocą testu Shapiro–Wilka - standardowego testu wykorzystywanego do określania normalności danych. Jednoczynnikowa analiza wariancji (ANOVA), służąca do porównywania średnich w wielu populacjach, z testem najmniejszych istotnych różnic (NIR) post hoc Fishera została przeprowadzona dla porównania obejmującego badane grupy. Za statystycznie istotne uznano wartości $p < 0,05$. Graficzne przedstawienia danych statystycznych zostały utworzone przy użyciu programu Statistica v. 13.3 (TIBCO Software Inc., Palo Alto, Kalifornia, USA).

VII. Publikacje

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Review Article

The Effects of Natural Iridoids and Anthocyanins on Selected Parameters of Liver and Cardiovascular System Functions

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The old adage says, "you are what you eat." And although it is a banality repeated by many with a grain of salt, it also has quite a bit of truth in it, as the products we eat have a considerable impact on our health. Unfortunately, humanity is eating worse from one year to another, both in terms of product quality and eating habits. At the same time, it is brought up frequently that plant products should form the basis of our diet. This issue was also reflected in the new version of the food pyramid. Iridoids and anthocyanins are groups of plant compounds with proven beneficial effects on health. Both groups affect the cardiovascular system and the liver functions. Although many mechanisms of action and the therapeutic effects of these compounds have already been learned, intensive animal and clinical research is still underway to explore their new curative mechanisms and effects or to broaden our knowledge of those previously described. In this article, we review the effects of natural iridoids and anthocyanins on selected parameters of liver and cardiovascular system functions.

1. Introduction

In the era of fast life and the constant pursuit of wealth, fame, and realization of various dreams, the time spent on preparing meals and caring for a proper, balanced diet has been reduced to a minimum by many people. This situation is being met by food producers, frequently large global food concerns, whose main motto, understandably from an economic point of view, is often to obtain only the highest possible financial profit. Highly processed products, containing many different chemical additives, e.g., flavor enhancers or preservatives; ready meals, which you only need to heat in a microwave; and finally fast food or sweetened drinks, are an increasing part of people's diet. This leads to global health and social consequences, which without exaggeration can be called an epidemic of unhealthy nutrition. Worldwide, diseases such as type II diabetes, atherosclerosis, hypertension, metabolic syndrome, and balance obesity are constantly observed.

Fortunately, opinions calling for a change of lifestyle, including paying more attention to the fact that plant products—fresh and low processed—should be the basis of diet, are becoming louder, including in the mass media. This belief has been confirmed by numerous, recent publications that have meta-analyzed previous studies and have shown significant correlations between increased consumption of fruits and vegetables and decreased risk of coronary artery disease (CAD), cardiovascular disease (CVD) mortality, and stroke [1, 2]. Similarly, a plant-based diet was reported to demonstrate a hepatoprotective capacity in nonalcoholic fatty liver disease (NAFLD) or alcoholic cirrhosis and to contribute to a reduction in cholesterol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels and an improvement in detoxifying processes [3–7].

The best example of a change in the global approach to nutrition is the fact that WHO has recently modified the multiyear guidelines for creating a healthy eating pyramid. In its new version, it is plant products that have a far dominant role. It seems that the simplest advice for those looking for a way to improve their eating habits is to eat more plants. There are many groups of plant compounds showing a confirmed beneficial effect on human health. Such groups are, e.g., iridoids and anthocyanins. These compounds have been known for many years, but their new, potentially valuable therapeutic and prophylactic properties are still being discovered. The aim of this article is to review the reports on the effects of natural iridoids and anthocyanins on selected parameters of liver and cardiovascular system functions.

2. Iridoids

Iridoids are a group of organic chemical compounds from the monoterpenoid group. They are found in many plant families, e.g., *Apocynaceae*, *Gentianaceae*, *Lamiaceae*, *Loganiaceae*, *Rubiaceae*, *Scrophulariaceae*, and *Verbenaceae* [8], usually as glycosides with a glucose moiety attached to C-1 in the pyrene ring. Large amounts of iridoids are also observed in herbs with bitter effects. Structurally, they are cyclopentano-(c)-pyran monoterpenoids, and biogenetically and chemotaxonomically, they determine a structural link between terpenes and alkaloids [9]. The basic structural feature of iridoids is a bicyclic H-5/H-9β, β-*cis*-fused cyclopentanopyran ring system; also, several enantiomeric forms of iridoids exist in nature, e.g., secoiridoids in which the cyclopentane ring is torn between C-7 and C-8 [10]. Iridoids are found in the green parts of plants, mainly in the leaves and young stems, and sometimes in fruits and sprouts. Among the most commonly mentioned iridoids in the therapeutic context, the following stand out above all: gentiopicroside, geniposide, sweroside, loganin, loganic acid, catalpol, and amarogentin (Figure 1) [8, 11–25].

2.1. Iridoid Mechanisms of Action and Potential Curative Effects. During numerous studies on the biological activity of iridoids, a very wide range of action on the human body was found. Those confirmed so far include cardiovascular, hypoglycemic, hypolipidemic, antihepatotoxic, choleretic, anti-inflammatory, antispasmodic, antitumor, antiviral, immunomodulatory, and purgative activities [11]. The health-improving properties of iridoids are majorly due to their antioxidative and anti-inflammatory activity. The anti-inflammatory effects of iridoids are attributed to the inhibition of cyclooxygenase 2 (COX-2), proinflammatory cytokines, prostaglandin E2 (PGE2), and thromboxane-B2 (TBX2) [12]. Some iridoids, like the hydrolyzed derivative of harpagide, have a similar chemical structure to PGE2 and celecoxib—a selective COX-2 inhibitor [12]. Previous studies have also shown that iridoids may increase the expression of transcription factors involved in the regulation of lipid metabolism, like peroxisome proliferator-activated receptors (PPARs) (Figure 2) and sterol regulatory element-binding proteins (SREBPs) [26, 27]. Among different iridoids possessing anti-inflammatory properties, particularly interesting, due to its possible therapeutic potential with high efficiency of COX inhibition, is loganic acid. Loganic acid has a greater affinity for inducible COX-2 than constitutive COX-1 [28], which means that its therapeutic use does not have to entail severe side effects, such as the risk of gastrointestinal ulceration. In addition to the aforementioned high affinity for COX-2, it reduces the expression of some proinflammatory cytokines, i.a., TNF-α, interleukin-1β (IL-1β), and interleukin-6 (Il-6). It also exerts an inhibitory effect on fMLP-induced superoxide generation in human neutrophils [29]. Confirmed and proposed effects and parameters altered by iridoids in humans are summarized in Figure 3.

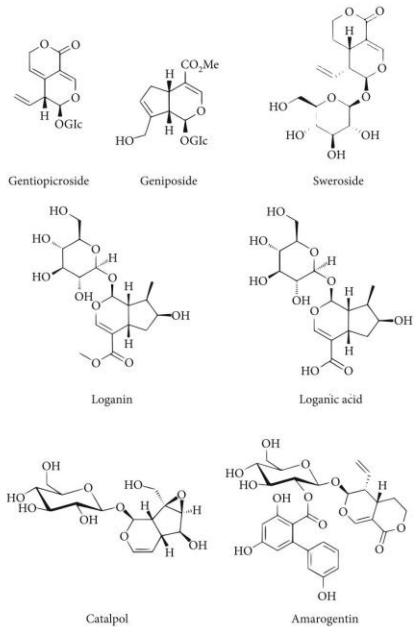

Figure 1: Chemical structures of common iridoids.

2.2. Iridoids in Liver Injury. The liver is a key organ responsible for the metabolism, excretion, and detoxification processes of the body. Due to its role in the body, it is exposed to injuries from various types of metabolism products, toxins, endotoxins, viruses, or drugs [30]. It is estimated that even about 1,000 commonly used drugs are hepatotoxic. Many cases of liver diseases have been noted every year in the world, and they are also one of the most common causes of death [31]. Oxidative stress is considered the major mechanism contributing to the appearance and progression of liver diseases. The excess of reactive oxygen or nitrogen forms, as well as the deficiency of antioxidant compounds, is the main factor responsible for liver damage [32]. Highly reactive metabolites lead to the depletion of reduced glutathione (GSH) which is needed for the detoxification reaction of glutathione-S-transferase, and also lead to a rise in the levels of

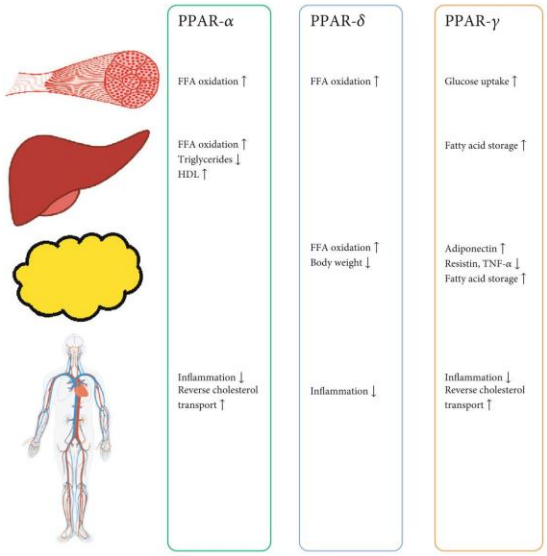

Figure 2: Influence of PPARs on muscles, liver, adipose tissue, and vessel walls.

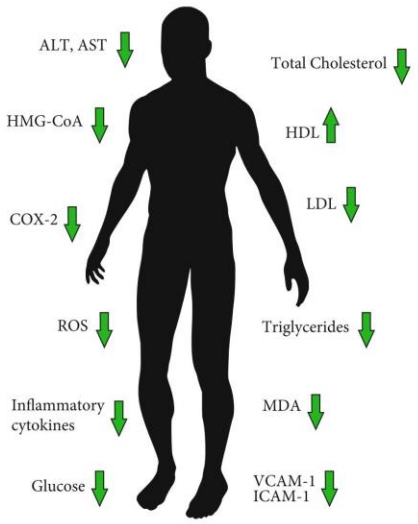

Figure 3: Confirmed and proposed effects and parameters altered by iridoids in humans.

lipid peroxides in the liver. This induces, through the nuclear factor-kappa B (NF-κB), the production of proinflammatory cytokines and chemokines, and the activation of cyclooxygenase 2 and inducible nitric oxide synthase (iNOS). Inflammation in the liver is most often mediated by tumor necrosis factor-alpha (TNF-α), interleukin-12 (IL-12), monocyte-1 chemotactic protein (MCP-1), and macrophage-2 inflammatory protein (MIP-2) (Figure 4). An increase in TNF-α leads to apoptosis and cell death [33, 34].

The hepatoprotective effect of iridoids is mainly attributed to their antioxidant activity. This action is both indirect, through the stimulation of the antioxidant defense system, and direct, through the removal of reactive oxygen species (ROS) [6]. Therefore, the use of plant substances with antioxidant properties, as ready-made preparations or intermediates to obtain drugs with hepatoprotective effects, seems to be justified and desirable. There are many such substances, e.g., silymarin, curcumin, ellagic acid, or exactly iridoids. An example of a mixture of the latter is Picroliv. Picroliv is a standardized mixture of iridoid glycosides isolated from the roots and rhizomes of the *Picrorhiza kurroa*. It contains at least 60% of a mixture of picroside I and kutkoside in a 1 : 1.5 ratio; the remainder (40%) is a mixture of iridoid and cucurbitacin glycosides [35]. Picroliv has antioxidant properties which appear to be mediated through the activity of superoxide dismutase, metal ion chelators, and xanthine oxidase inhibitors [36]. Moreover, Picroliv restores malondialdehyde (MDA) levels to normal in a carbon tetrachloride-(CCl_4-) induced liver injury model in mice, thereby pointing to antilipid peroxidative properties. It works on the principle of an anaerobic free radical scavenger, limiting lipid peroxidation involved in cell membrane damage caused by hepatotoxins [34, 35].

Tan et al. conducted an interesting study assessing the hepatoprotective ability of iridoids isolated from *Veronica ciliata* in an acetaminophen-induced acute liver injury murine model. Acetaminophen is one of the most popular analgesic and antipyretic drugs globally, commonly given to adults and children. It turned out that treatment with an isolated iridoid fraction significantly reduced the level of liver

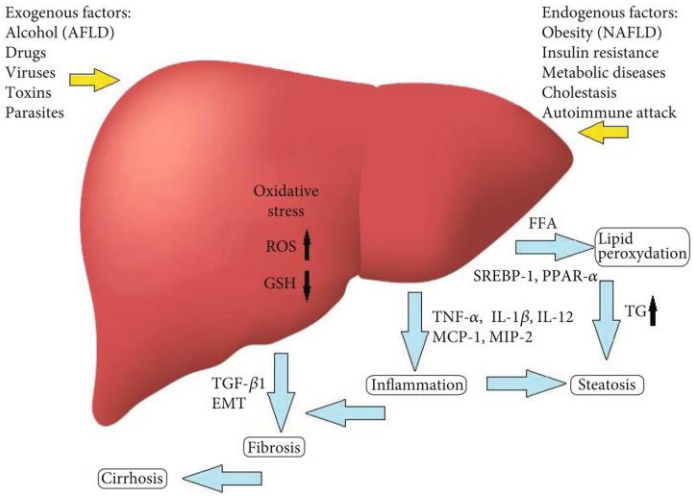

FIGURE 4: Oxidative stress in the liver, risk factors, follow-up compounds, and mechanisms that are the point of curative action of iridoids and anthocyanins.

enzymes alanine aminotransferase, aspartate aminotransferase, and tumor necrosis factor-alpha in the serum. Decreased malondialdehyde formation and expression of proinflammatory factors, along with elevated superoxide dismutase and glutathione activity in the liver, was also found [37].

One of the basic factors of liver damage, regardless of the cause, is its fibrosis as a result of the deposition of type I collagen in the extracellular space of the liver. Transforming growth factor-β1 (TGF-β1) plays a key role in the pathogenesis of liver fibrosis (Figure 4). Another important factor is epithelial-mesenchymal transition (EMT) which accompanies the pathological processes and leads to the transformation of the cellular phenotype, including changes in the activity of collagens (I-V) and elastin [13]. Following iridoid compounds, geniposide (present, i.a., in the fruit of *Gardenia jasminoides*) may inhibit TGF-β1-induced EMT in hepatic fibrosis by suppressing the TGF-β/Smad and ERK-mitogen-activated protein kinase (MAPK) signaling pathways [14]. Catalpol is another iridoid that protects the liver against fibrosis as well as steatosis and necrosis. Catalpol has been reported to reduce serum alkaline phosphatase (ALP), serum ALT, AST, and bilirubin, as well as the liver/-body weight ratio. In addition, catalpol limited the fibrosis process by reducing collagen deposition in the liver as well as reduced inflammation by lowering the levels of proinflammatory IL-1β, TNF-α, IL-18, IL-6, and COX-2 in a dose-dependent manner *in vivo* [15].

In 2018, Dai et al. published the results of a study in which they examined the effect of iridoids, secoiridoids, and their glycosides on acontine-induced hepatotoxicity. The effect of 53 active substances on the human CYP3A4 enzyme was tested. Researchers particularly focused on six popular iridoid compounds: gentiopicroside, sweroside, swertiamarin, loganic acid, 6-O-β-d-glucosyl-gentiopicroside, and amarogentin. In particular, the latter showed a significant inductive effect on CYP3A4 mRNA levels in HepG2 cells, as well as in the case of acontine-induced toxicity [16]. These results are consistent with the previously published work of other researchers who confirmed the effectiveness of amarogentin on carbon tetrachloride-induced liver fibrosis in mice. They showed that amarogentin delayed the formation of liver fibrosis and decreased ALT, AST, MDA, and hydroxyproline levels. At the same time, it increased the levels of albumin, cyclic guanosine monophosphate (cGMP), glutathione peroxidase, and superoxide dismutase. In addition, a decrease in the level of α-smooth muscle actin, TGF-β1, and protein kinases has been observed [17]. Based on the above results, a conclusion that the hepatoprotective effects of iridoids are caused by the facilitation of drug metabolism, amelioration of mitochondrial dysfunction, reduction of oxidative stress, and suppression of the mitogen-activated protein kinase signaling pathway can be drawn.

2.3. Iridoids in Fatty Liver Disease. Fatty liver disease is one of the first stages of alcoholic liver damage. Importantly, it is usually reversible. Consumption of large amounts of alcohol contributes to the increase of fatty acid production and impairment of their beta-oxidation in mitochondria, which consequently leads to lipid accumulation in the liver and inflammatory changes within hepatocytes. Sterol regulatory element-binding proteins, primarily SREBP-1c, as well as the PPAR-alpha transcriptional factors, play a major role in controlling fatty acid synthesis and their oxidation. In turn, AMP-activated protein kinase (AMPK), a key factor in controlling cellular energy homeostasis, affects hepatic lipid metabolism through modulating the downstream acetyl-CoA carboxylase (ACC) and carnitine palmitoyltransferase-1 (CPT-1) pathway [18]. Kupffer cells (hepatic resident

macrophages) play a pivotal role in the pathogenesis of these inflammatory changes. They express a range of chemokines and proinflammatory cytokines, including IL-1β [19]. The release of IL-1β requires caspase-1 activation by NOD-like receptors (NLRs), mainly NLRP3, which forms an inflammatory complex. Activation of Kupffer cells leads to the upregulation of inflammatory mediators through toll-like receptor 4 (TLR4). Additionally, too high uric acid or ATP level also plays an important role in the development of inflammation in the course of alcoholic fatty liver disease (AFLD) [20]. High ATP concentration activates the P2X7 receptor, an ATP-gated ion channel, which is considered to be a secondary signal important in the pathogenesis of liver steatosis [21].

Gentiopicroside is an effective and safe therapeutic option in the case of alcoholic fatty liver disease. Li et al. reported that gentiopicroside administered in both acute and chronic alcohol-induced liver impairments in mice reduces the level of serum liver enzymes, as well as the accumulation of triglycerides (TGs). Moreover, it modulates the expression of SREBP-1, PPAR-α, and phosphorylated acetyl-CoA carboxylase. Researchers also observed P2X7 receptor suppression and reduction of IL-1β production [21]. In turn, geniposide can rebalance a wide range of metabolic disorders due to alcohol-induced liver injury. Zhang et al. identified 48 AFLD-specific biomarkers. Geniposide regulated up to 32 of them. It modified abnormal liver metabolism resulting in the regulation of amino acid metabolism disorders and lessening oxidative stress [22].

The previously mentioned balance obesity associated with improper diet and deficiency of physical activity can lead to nonalcoholic fatty liver disease (NAFLD). Among the factors that may affect the development of NAFLD are, e.g., insulin resistance, oxidative stress, and the action of adipokines (e.g., adiponectin), cytokines, and other inflammatory mediators. Insulin resistance contributes to an increase in lipolysis and concentration of free fatty acids in hepatocytes. This phenomenon together with emerging oxidative stress and mitochondrial dysfunction results in the development of inflammation in the liver and its steatosis [23]. Moreover, overexpression of uncoupling protein-2 (UCP2) in the liver can cause acute liver injury. Genipin (an aglycone derived from geniposide) inhibits UCP2-mediated pyroptosis and reverses liver damage caused by a high-fat diet [24]. Genipin also protects against ischemia and reperfusion- (IR-) induced hepatic injury via improving mitochondrial quality control. It ameliorates hepatocellular oxidative damage and mitochondrial dysfunction triggered by IR [25].

2.4. Iridoids in Cholestasis. Liver damage and failure are also commonly caused by cholestasis. Analysis of proteomic and metabonomic studies showed altered pathways in cholestasis-induced liver injury involving increased activity of farnesoid X receptor (FXR)/retinoid X receptor (RXR), bile acid biosynthesis, and peroxisome proliferator-activated receptor-α/retinoid X receptor-α. Gentiopicroside normalizes metabolic, protein, and blood biochemical markers, as well as alleviates liver damage, so it can be a useful therapeutic alternative in the treatment of cholestasis [38]. It was shown that gentiopicroside modulates bile acid metabolism, which results in a decrease of the intracellular bile acid pool back to basal levels. It upregulates hepatic mRNA levels of synthesis enzymes, transporters, and also ileal bile acid circulation mediators. This effect leads to a decrease in serum and hepatic bile acid levels and a further increase in urinary and fecal bile acid levels [39]. Similar findings were established with the use of another iridoid glycoside—sweroside [40]. It is worth remembering, however, that the hepatoprotective effect of most iridoids is dose-dependent and high doses may cause harmful effects. It has been proven that geniposide at a dose of 300 mg/kg can induce liver injury with accompanying changes in bile acid regulating genes, leading to an accumulation of taurine conjugates in the rat liver [41].

2.5. Iridoids in Hyperlipidemia. Hyperlipidemia is a set of metabolic disorders manifested by elevated total cholesterol (often including elevated LDL-C and decreased HDL-C) and triglyceride serum levels. It is most frequently caused by improper nutrition or lack of physical activity or due to genetic predispositions. It evokes excessive fat accumulation in peripheral tissues, especially in arterial blood vessels. It is a direct risk factor for atherosclerosis, cardiovascular diseases, hypertension, diabetes mellitus, nonalcoholic fatty liver disease, dyslipidemia, osteoarthritis, and cancer [42, 43]. Among others, microRNAs (miRNAs) have been reported to play a crucial role in regulating lipid and lipoprotein metabolism. MicroRNAs are short, noncoding, single-stranded RNA molecules which regulate the expression of target genes by partial sequence-specific base-pairing to the targeted mRNA 3′UTR, blocking its translation and promoting its degradation or its sequestration into processing bodies. In the course of hyperlipidemia, increased miRNA levels in circulation are observed [44]. For example, miR-122, through targeting SREBP-1c, regulates fatty acid metabolism, and upregulation of miR-34a results in the downregulation of hepatic PPAR-α. Zhong et al. revealed that genipin decreases body weight, lipid serum levels, and hepatic lipid accumulation in high-fat diet mice. It increases the expression of miR-142a-5p, which bounds to SREBP-1c and thus leads to the inhibition of lipogenesis [45]. Another extensive analysis of the curative impact of the iridoid fraction was carried out by Zhu et al. Rats fed a high-fat diet received three different doses of iridoid fraction isolated from *Valeriana jatamansi*. Regardless of the dose, a decrease in weight gain was observed in rats compared to the model group, as well as a decrease in triglyceride and an increase in HDL-C serum levels. In addition, all doses enhanced the expression of apolipoprotein A5 (ApoA5) and the PPAR-α receptor and reduced the expression of the SREBP-1c protein in the liver. Moreover, depending on the dose, a different influence of the iridoid fraction was observed, among others, on AST and ALT levels, lipoprotein lipase (LPL) and hepatic lipase (HL) activities, or liver X receptor-α (LXR-α) expression. These results suggest the positive effect of iridoids on the functioning of the liver and circulatory system in the course of hyperlipidemia. It also indicates the possibility of differentiating some of the desired therapeutic effects depending on the used dose of these substances [46]. Iridoids have also been found

to reduce the expression of vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) as well as the MCP-1 protein, thereby contributing to the reduction of inflammation within the blood vessels. Iridoid glycosides increase the expression of PPAR-alpha and PPAR-gamma, which act as modulators of both acute and chronic inflammation. They also play a key role in regulating energy metabolism, and their agonist may be used in the treatment of dyslipidemia and CVDs [29].

2.6. Iridoids in Cardiovascular Disease. One of the most important factors causing cardiovascular disease is type 2 diabetes. At the same time, CVDs are a common cause of mortality in patients with diabetes, mainly due to macrovascular complications such as atherosclerosis. Platelets and shear stress play a major pathophysiological role in the development of atherosclerosis. Atherosclerosis is also a condition directly associated with hyperlipidemia and inflammation in blood vessels. Natural iridoids seem to possess properties that can prevent and reduce the severity of CVDs. *Cornus mas* L. (cornelian cherry) fruits are an interesting, rich source of iridoids, as well as flavonoids including anthocyanins. Sozański et al. conducted a series of studies examining the influence of cornelian cherry on selected parameters of liver and circulatory system functions in various animal models. They proved that administering cornelian cherry significantly reduces serum triglyceride and LDL-cholesterol and increases HDL-cholesterol levels. Moreover, it decreases intima thickness and the intima/media ratio in the thoracic aorta. Cornelian cherry compounds have a substantial protective effect on oxidative stress in the liver and causes a vital enhancement of PPAR-alpha and PPAR-gamma expression in the liver [47–50]. In turn, clinical trials in diabetic type 2 and hyperlipidemic patients showed a significant improvement of the sugar level and insulin secretion in diabetic patients and an amelioration of the lipid profile, apolipoprotein status, and vascular inflammation in hyperlipidemic patients. It was also proven that *Cornus mas* fruits are safe on acute toxicity studies in rat and human models [7].

Catalpol is another iridoid compound the administration of which resulted in significant attenuation of atherosclerotic lesions. It is the main active ingredient of *Rehmannia glutinosa*, *Katalpa officinalis*, or *Euphrasia rostkoviana*. As in the examples quoted earlier, catalpol lowers the levels of total cholesterol, triglycerides, and low-density lipoproteins in blood serum and boosts the level of high-density lipoproteins. Furthermore, the reduction of TNF-α, Il-6, MCP-1, soluble VCAM-1, and soluble ICAM-1 levels in the serum was observed in a rabbit study [51]. Meanwhile, the depletion of VCAM-1, MCP-1, TNF-α, iNOS, matrix metalloproteinase-9, and NF-κB expression levels in the aortic arch was noticed. The lessening of the lipid peroxidation levels and the enhancement of the antioxidant capacity were also established during the catalpol treatment [51, 52]. Catalpol may be a particularly valuable therapeutic agent in diabetes therapy. It has been proven that it reduces fasting blood glucose (FBG) and random blood glucose (RBG) in a dose-dependent manner, with the reduction of RBG being even greater than in the case of the basic antidiabetic drug—metformin. Moreover, catalpol significantly improves glucose tolerance via increasing insulin sensitivity. The administration of catalpol to the db/db mice significantly increases the expression of 287 genes involved mainly in lipid metabolism, response to stress, energy metabolism, and cellular processes and significantly decreases the expression of 520 genes involved in cell growth and death, in the immune system, and in the response to stress [53]. Catalpol ameliorates hepatic insulin resistance in type 2 diabetes through acting on the AMPK/NOX4/PI3-K/AKT pathway [54]. It exerts a renal protective effect in diabetic db/db mice [55] and restores balance between oxidative and antioxidative enzymes in the course of diabetes [56].

One of the suggested mechanisms of the antiatherosclerotic action of iridoid compounds is the inhibition of the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase. It is a liver enzyme located in the cytoplasm of hepatocytes, responsible for regulating the amount of synthesized cholesterol and thus also affecting its plasma level. The HMG-CoA-inhibiting properties of iridoids, as well as their capacity for lowering the level of total cholesterol, triglycerides, low-density lipoproteins, and very low-density lipoproteins in serum, have been confirmed, i.a., in swertiamarin [57]. Moreover, swertiamarin presents similar signaling pathways as serotonin 5-HT2 receptor modulators. A peripheral increase in serotonin levels is considered to be one of the markers of diabetes. Changing the level of this autacoid implies alterations in the expression of 5-HT receptors which are involved in the pathogenesis of diabetes [58].

Guo et al. reported that geniposide modulates ATP production and glucose-stimulated insulin secretion (GSIS) and increases GLUT2 protein levels in high-glucose concentration in rat INS-1 pancreatic β cells [59]. It also reverses glucose-induced impairment of insulin release, induces phosphorylation of AMPK, and inhibits hepatic glucose production. It appears that AMPK plays a key role in geniposide-regulated GSIS in pancreatic β cells and glucose production in HepG2 cells [60]. These results are consistent with outcomes of other studies [61, 62]. Moreover, geniposide protects rat insulinoma cells from apoptosis in high-glucose concentrations [63] and regulates endoplasmic reticulum (ER) stress, which might be an important factor of dysfunction and death of pancreatic β cells [64]. The antioxidative properties of geniposide eventuates from either the inhibition of numerous pathological processes (e.g., production of some inflammatory cytokines, LPS-induction of nitric oxide, and blocking upstream of TLR4, NF-κB, and MAPK pathways) or the activation of various proteins associated with cell survival, e.g., heme oxygenase-1 (HO-1) and B-cell lymphoma-2 (Bcl-2) or a combination of both. It was proven that geniposide may be useful in the prophylaxis or treatment of not only diabetes mellitus but also cardiac fibrosis, cardiac hypertrophy, myocardium I/R, obesity-related cardiac injury, atherosclerosis, ischemic stroke, and diabetic nephropathy [65].

3. Anthocyanins

Besides iridoids, the other group of natural substances that have garnered increasing interest in recent years are anthocyanins. Anthocyanins are polyphenolic glycosidic plant dyes

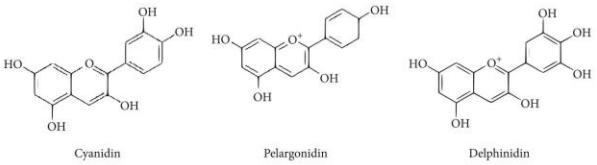

Figure 5: Chemical structures of common anthocyanidins.

(mainly red, blue, or purple) of primarily fruits and flowers, which are biochemically related to flavonoids. Structurally, anthocyanins are aliphatic or aromatic three-ring compounds with one or more sugar molecules and sometimes with a sugar-attached aryl group. The colored aglycons are the anthocyanidins (usually cyanidin, pelargonidin, or delphinidin) (Figure 5).

3.1. Anthocyanin Mechanisms of Action and Potential Curative Effects. As in the case of iridoids, the overall health-improving properties of anthocyanins seem to result significantly from its antioxidative and anti-inflammatory activity. What distinguishes anthocyanins from other polyphenol compounds is that they are unique in decreasing ROS generation without inducing mitochondrial biogenesis or manganese superoxide dismutase expression [66]. To date, the broad effect of anthocyanins on the liver and cardiovascular function parameters has been reported. The intake of especially anthocyanidins was associated with a statistically significant reduction of cardiovascular disease risk [67, 68]. Anthocyanin's ability to reduce insulin resistance, improve glycemic control, decrease lipid accumulation in the liver, constrain inflammation (manifested as a lessening of inflammatory markers such as TNF-α), decrease oxidative stress in the liver (including stress markers such as MDA), and decrease levels of liver enzymes (such as ALT and AST) was also confirmed [69–71].

The following mechanisms of anthocyanin activity have been proposed, i.a., reduction of cholesterol synthesis through the downregulation of HMG-CoA reductase expression; inhibition of cholesteryl ester transfer protein (CEPT) which lowers the concentration of LDL; reduction of apolipoprotein B and apolipoprotein C-III levels which results in decreasing the concentration of TG in serum; and lessening the levels of various inflammatory cytokines such as IL-6 and IL-1β, TNF-α, iNOS, and NF-κB in HepG2 cells [70–72]. Anthocyanins were also reported to slow ageing-related deterioration of liver function and structure by inhibiting DNA damage [73]. As anthocyanins potentially inhibit platelet function (e.g., decrease the number of activated platelets and their aggregation), they may represent a useful adjunct in patients treated with typical antiplatelet drugs and be exploited as a complementary therapy to reduce CVD in diabetes [71, 74]. The modulation of the redox state and inflammation by anthocyanins is mediated through various pathways. One of them is the upregulation of the nuclear factor erythroid 2-related factor 2 (Nrf2) [75]. This inducible transcriptional factor is a key regulator of antioxidant responses and contributes to inflammatory tissue injuries.

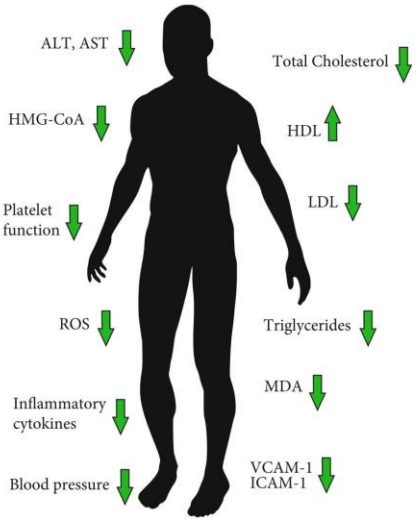

Figure 6: Confirmed and proposed effects and parameters altered by anthocyanins in humans.

Nrf2 is also responsible for the downregulation of endothelial monocyte chemoattractant protein-1, which is one of the pivotal chemokines regulating the migration and infiltration of monocytes and macrophages [76]. Other proposed mechanisms include the ability to capture free radicals and anions; inhibition of xanthine oxidase—a superoxide-producing enzyme; chelating metal ions, which leads to the formation of stable anthocyanin-metal complexes; and the inhibition of the oxidation of LDL-cholesterol, as well as the modulation of cyclooxygenases and lipooxygenases—the key enzymes in the synthesis of prostaglandins and leukotrienes [71]. Moreover, anthocyanins may protect the heart from ischemia/reperfusion-induced injury by activating signal transduction pathways and sustaining mitochondrial functions [77]. Confirmed and proposed effects and parameters altered by anthocyanins in humans are summarized in Figure 6.

3.2. Anthocyanins in Liver Injury. Anthocyanins, like iridoids, may prove effective in the treatment of liver fibrosis. They reduced ALT and AST levels in blood serum; MDA and protein carbonyl content of liver homogenate; and MCP-1, IL-1β, MIP-2, collagen III, and α-SMA in a mice study [78]. Similar results were obtained in a rat liver fibrosis study [79].

One of the most common anthocyanidin compounds is cyanidin. It occurs in the fruits of hawthorn, hibiscus, elderberry, or blueberry. It exhibits potent antiatherogenic activity *in vitro* and *in vivo* by changing the expression and binding to all three PPAR subtypes, mostly to PPAR-α[80]. It was proven that cyanidin-3-O-β-glucoside (C3G) possesses the ability of enhancing cellular AMPK activity and ACC phosphorylation and stimulation of carnitine palmitoyltransferase-1 CPT-1 expression, which leads to a significant increase of fatty acid oxidation in HepG2 cells [18]. Similar results were obtained in other studies on anthocyanins [81, 82]. Thereto, C3G lowered fasting glucose levels and improved the insulin sensitivity in both the high-fat diet-fed mice and the db/db mice model. Furthermore, depletion of white adipose tissue messenger RNA levels of MCP-1, TNF-α, and IL-6; serum concentrations of TNF-α, IL-6, and MCP-1; macrophage infiltration in adipose tissue; and attenuation of liver steatosis were observed. It has been reported that C3G attenuated oxidative stress by activating the GSH synthesis antioxidant defense mechanism against excessive intracellular ROS production, contributing to the prevention of hyperglycemia-induced hepatic oxidative damage [83]. C3G was also shown to regulate the thermogenic and secretory functions of brown adipose tissue (BAT) [84].

3.3. Anthocyanins in Cardiovascular Disease. Although there has been quite a lot of research on anthocyanins, their mechanisms of action are not fully known and it has not been thoroughly determined which compounds and in what doses are the most beneficial; therefore, it is worth noting any reports that broaden our knowledge on this issue. Habanova et al. have shown that the regular intake of bilberries (*Vaccinium myrtillus* L.), fruits very rich in anthocyanins, both in women and men causes decreasing LDL-C/TG and increasing HDL-C levels and thus may contribute to reducing the risk of CVDs [85]. Cassidy et al. conducted a series of clinical trials examining the influence of higher intake of fruit-based anthocyanins and flavonoids on some cardiovascular diseases. They are potentially useful in the prevention of hypertension, in the reduction of myocardial infarction (MI) risk in men and young women, and in the reduction of ischemic stroke risk in men. Researchers suggested that a key component underlying the reduction in that risk may be an anti-inflammatory effect of anthocyanins and flavonoids [86–89].

However, it should be taken into consideration that low plasma concentrations and rapid clearance kinetics of anthocyanins suggest that it is their phenolic metabolites which are responsible for their biological activity *in vivo*. The most common theory of anthocyanin metabolism leans on the assumption that their degradation is a result of their chemical instability and the impact of bacterial catabolism, resulting in a number of circulating phenolic metabolites. Warner et al. investigated the influence of physiologically relevant anthocyanin metabolite signatures, derived from C3G, on soluble VCAM-1 and IL-6 in human endothelial cells. It turned out that signatures of anthocyanin metabolites, identified post-consumption of dietary achievable levels of anthocyanins, had inhibitory effects on inflammatory protein secretion, with maximal effects observed for the 6 and 24 h profiles. Researchers also noticed that the greatest inhibition of VCAM-1 occurred in response to the 24 h metabolite signature, which may suggest that the metabolites of lower intestinal microbial origin are responsible for fasting or chronic anti-inflammatory impact [90]. In another study, it was shown that anthocyanin metabolites modify vascular reactivity by inducing HO-1 and modulating NADPH oxidase (NOX) activity in the endothelium, which resulted in decreased superoxide production and ameliorated NO bioavailability [91]. These results were confirmed in a subsequent study, which additionally proved that the bioactivity of common phenolic metabolites of anthocyanins is increased when in combination, indicating their additive or synergistic effects [92].

4. Concomitant Intake of Iridoids and Anthocyanins

Using the knowledge obtained, it is interesting to put forth the hypothesis that anthocyanins and iridoids consumed concomitantly may exert positive additive or synergistic effects on dyslipidemia and atherosclerosis. Thus far, only several fruits containing both anthocyanins and iridoids are known. They are, mainly, the cornelian cherry (*Cornus mas* L.), *Cornus officinalis* Sieb. et Zucc., and other *Cornaceae*, honeysuckle berry (*Lonicera caerulea* L.), lingonberry (*Vaccinum vitis-idaea* L.), blueberry (*Vaccinium corymbosum* L.), and cranberry (*Vaccinium oxycoccos* L.). Researchers found that oral administration of lyophilisated cornelian cherry fruits (with substantial amounts of anthocyanins and iridoids, mainly loganic acid) in feed-induced atherosclerotic rabbits prevented the development of dyslipidemia and atherosclerosis through the activation of PPAR-alpha receptors in the liver. Anti-inflammatory properties of whole fruits, measured as a decrease in proinflammatory cytokines were also confirmed [49]. Then, they found that oral administration of cornelian cherry may modulate vascular NO balance by increasing the L-arginine and L-arginine/asymmetric dimethylarginine (ADMA) ratio and decreasing endogenous inhibitors of endothelial nitric oxide synthase- (eNOS-) ADMA and symmetric dimethylarginine (SDMA). These effects were accompanied by a decrease in intima thickness and the intima/media ratio and an increase in GSH level in the thoracic aorta [47]. In a subsequent study done with the same animal model, Sozański et al. demonstrated that both loganic acid and anthocyanins isolated from cornelian cherry fruits diminished dyslipidemia, increased the expression of PPAR-alpha and PPAR-gamma receptors (Figure 2), and decreased intima thickness and the intima/media ratio in the thoracic aorta. Although the degree of these effects differed between anthocyanins and iridoids, the trend in changes was similar. The only significant difference between anthocyanins and iridoids was that it was mainly loganic acid exerting anti-inflammatory effects [48]. Further analyses are required to indicate which iridoids and anthocyanins and at what doses have the most beneficial anti-inflammatory effect.

5. Future Perspectives

Incorrect eating habits can be called a peculiar epidemic of the 21st century. Fortunately, thanks to the louder calls for

change and the slow but steadily increasing public awareness of healthy nutrition, the share of plant products in the diet of many people is increasing. Thanks to this, compounds with proven therapeutic effects on the body, such as iridoids or anthocyanins, may play an increasingly important role in the prevention and adjunctive or combined treatment of many civilization diseases. The undoubted advantages of using iridoids and anthocyanins in medicine include the fact that many of their effects and mechanisms of action are relatively well known and have been confirmed by many studies. Unfortunately, those were largely animal studies and certainly one of the most important goals for the near future is to conduct more clinical studies to confirm these effects in humans. New applications for the abovementioned compounds should also be sought, as they are potentially a valuable option for usage in, e.g., metabolic syndrome. However, it should be remembered that as natural products, iridoids and anthocyanins will prove best in the prevention of early stages of liver and cardiovascular diseases. Moreover, their complete effect will depend on properly selected doses with confirmed pharmacological effects of a single compound or a mixture of these compounds, the length of therapy, and the regularity of use by patients.

6. Conclusion

In conclusion, current knowledge clearly points to the benefits of consuming foods comprising both iridoids and anthocyanins. These compounds exert many curative ascendancies, including pleiotropic positive effects, modulation of transcription factors regulating lipid and glucose metabolism, redox stress and inflammation, and impact on NO vascular balance. The abovementioned findings justify future studies that are aimed at better understanding the properties of currently known iridoids and anthocyanins or creating new phytopharmaceuticals containing both groups of substances.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Review

The Impact of Anthocyanins and Iridoids on Transcription Factors Crucial for Lipid and Cholesterol Homeostasis

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Abstract: Nutrition determines our health, both directly and indirectly. Consumed foods affect the functioning of individual organs as well as entire systems, e.g., the cardiovascular system. There are many different diets, but universal guidelines for proper nutrition are provided in the WHO healthy eating pyramid. According to the latest version, plant products should form the basis of our diet. Many groups of plant compounds with a beneficial effect on human health have been described. Such groups include anthocyanins and iridoids, for which it has been proven that their consumption may lead to, inter alia, antioxidant, cholesterol and lipid-lowering, anti-obesity and anti-diabetic effects. Transcription factors directly affect a number of parameters of cell functions and cellular metabolism. In the context of lipid and cholesterol metabolism, five particularly important transcription factors can be distinguished: liver X receptor (LXR), peroxisome proliferator-activated receptor-α (PPAR-α), peroxisome proliferator-activated receptor-γ (PPAR-γ), CCAAT/enhancer binding protein α (C/EBPα) and sterol regulatory element-binding protein 1c (SREBP-1c). Both anthocyanins and iridoids may alter the expression of these transcription factors. The aim of this review is to collect and systematize knowledge about the impact of anthocyanins and iridoids on transcription factors crucial for lipid and cholesterol homeostasis.

Keywords: anthocyanins; iridoids; transcription factors; lipids; cholesterol

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1. Introduction

It is a truism to say that nutrition directly reflects our health. Many scientific publications have proven the beneficial effects of a proper diet on, among others, blood parameters, skin condition or the work of key metabolic organs such as the pancreas, liver or kidneys. Moreover, although, globally, store shelves are filled with highly processed, heavily sweetened and high-saturated-fat products, natural and organic ingredients are becoming more and more popular. Various campaigns are organized—sometimes national or nationwide—calling for populations to adopt an appropriate dietary regimen. Furthermore, the mass media, celebrities and public figures often promote a healthy lifestyle, frequently by setting an example.

Each individual has their own unique dietary habits. Countless factors influence our ways of eating. An important role is played by cultural, financial or geographic aspects. Various types of diets are known, such as basic, alternative, therapeutic, experimental and many more. Occasionally, new diets are proposed. They are based on, for example, including or excluding a certain dietary group from everyday consumption, a specific caloric balance or the content of basic nutrients—proteins, fats or carbohydrates. Regardless of the scientific basis for creating a given diet or the effectiveness of achieving its goal, the WHO pyramid of healthy eating remains a universal determinant of proper nutrition. According to its new version, plant products should form the basis of our diet and be consumed in the largest quantities.

Transcription factors are proteins that possess the ability to bind DNA in the promoter or enhancer sequence at a specific site or region, where they regulate the transcription process. Transcription factors are essentially classified by three different aspects: mechanism of action, regulatory function and sequence homology in their DNA-binding domains. Due to the fact that they help to "turn on" or "turn off" individual genes, they directly affect a number of parameters of cell functions and cellular metabolism. Thus, in a broader context, transcription factors affect the work of different organs, e.g., the liver, or entire systems, e.g., cardiovascular [1–5].

Five transcription factors play a crucial role in lipid and cholesterol metabolism: liver X receptor (LXR), peroxisome proliferator-activated receptor-α (PPAR-α), peroxisome proliferator-activated receptor-γ (PPAR-γ), CCAAT/enhancer binding protein α (C/EBPα) and sterol regulatory element-binding protein 1c (SREBP-1c). There are many groups of plant substances that influence the expression of these factors, and thus also the genes whose functioning they regulate. This impact was confirmed in numerous studies—both tissue and animal as well as human models. Among the most frequently mentioned compounds exhibiting such effects, the following are worth mentioning: polyphenols, phytoestrogens, soy proteins, mono- and polyunsaturated fatty acids, alkaloids, saponins, and also anthocyanins and iridoids. The latter two are the subject of our particular interest. Studies summarizing the current knowledge on the effect of, e.g., polyphenols or unsaturated fatty acids, on the transcription factors associated with lipid and cholesterol turnovers are quite common. However, to the best of our knowledge, there is no publication collecting data on this subject in the case of anthocyanins and iridoids. Therefore, we decided to assemble and systematize information on the impact of anthocyanins and iridoids on transcription factors crucial for lipid and cholesterol metabolism.

2. Major Transcription Factors Involved in Lipid and Cholesterol Metabolism

Replication, transcription and translation—at the cellular level—play a key role in the reproduction, development and maintenance of life processes in all organisms. The genome contains all information regarding the structure and function of bodily proteins. The process of protein formation is indirectly controlled by a specific group of proteins and nuclear receptors, described as transcription factors (TFs). The transcription process never enfolds the whole DNA strand, but only the fragment that encrypts a protein that is currently required by the body. This phenomenon is determined, among others, by transcription factors. Their task, apart from the transcription induction itself, is to direct the appropriate signal to the promoter site of the gene encoding the desired protein [6,7]. It was found that a dysfunction of transcriptional factors is related to a third of human developmental disorders. Moreover, TFs occur numerously among the group of oncogenes [3].

Transcription factors control initiation or elongation processes. Certain TFs may affect both of these processes. They typically bind cofactors—protein complexes that are associated with activation (coactivators) and repression (corepressors) but do not have DNA-binding capabilities of their own. Most TFs are thought to contribute to transcription initiation precisely by recruiting coactivators [2].

Transcription factors, as mentioned earlier, can be divided according to various criteria, but the simplest classification differentiates them into two basic groups: general transcription factors (GTFs) and specific transcription factors (STFs). GTFs, which always remain active, forming the pre-initiation complex, are responsible for inducing the transcription process. Six general factors can be distinguished: TFIIA, TFIIB, TFIID, TFIIE TFIIF, and TFIIH. In turn, specific factors accelerate or inhibit the transcription of target genes in response to signals sent to the cell nucleus. These factors are activated or suppressed depending on the demand for specific proteins [6–8].

STFs attach to the DNA fragment within the promoter, which leads to facilitating or hindering the recognition of the binding sequence by the pre-initiation complex. This conflation takes place by using small binding proteins, called either enhancers or silencers, i.e., short DNA sequences that are located even up to several thousand base pairs away

from the promoter. The attachment of the transcription factor to the enhancer results in accelerated transcription, while silencers cause the opposite effect [2,9]. This is why STFs are a very engaging research target and therapeutically promising factors for many substances, including various food compounds.

To date, over 1000 various transcription factors have been described in humans [3]. Five of them play a key role in lipid and cholesterol metabolism: liver X receptor (LXR), peroxisome proliferator-activated receptor-α (PPAR-α), peroxisome proliferator-activated receptor-γ (PPAR-γ), CCAAT/enhancer binding protein α (C/EBPα) and sterol regulatory element-binding protein 1c (SREBP-1c).

Liver X receptor is a ligand-activated transcription factor of the nuclear receptor superfamily, playing a critical role in the regulation of the expression of major genes involved in cholesterol, lipid and glucose homeostasis. Two isoforms of liver X receptor can be distinguished: LXRα and LXRβ. It was proven that LXRs' reverse the transportation of cholesterol to peripheral tissues and transport the excess cholesterol into the liver, reduce cholesterol absorption by intestinal epithelial cells and directly induce the expression of the essential transcription factor for lipid and cholesterol synthesis in the liver, sterol regulatory element-binding protein 1c [10,11]. One of the first direct LXR target genes identified was ATP-binding cassette transporter A1 (ABCA1). LXR activation leads to the robust upregulation of ABCA1 in macrophages, the intestine and in the liver, and the efflux of cholesterol to apolipoprotein A1, which results in the formation of high-density lipoproteins (HDL) [10,12]. LXRs are responsible for the stimulation of HDL synthesis [13].

Cholesterol is, above all, an essential constituent of the cell membrane but also represents a precursor for the synthesis of several hormones and bile acids that play significant roles in various physiological processes. Incorrect maintenance of cholesterol homeostasis, including absorption, biosynthesis, transmission and efflux, may contribute to severe disorders, such as atherosclerosis, metabolic syndrome, cardiovascular disease (CVD) or cholelithiasis [14]. Therefore, LXR as a cholesterol sensor play a critical role in its metabolism and protection against atherosclerosis [15–17]. It is worth highlighting that synthetic LXR agonists may exert undesirable effects on plasma and hepatic triglycerides through activating another transcriptional factor—hepatic sterol regulatory element-binding protein 1c [18]. For this reason, natural LXR agonists, which could be deprived of these side effects, are urgently needed.

Peroxisome proliferator-activated receptors (PPARs) are a superfamily of nuclear receptors functioning as ligand-activated transcription factors. PPARs include PPAR-α, PPAR-δ and PPAR-γ subtypes. PPARs play a relevant role in the regulation of glucose and lipid metabolism. PPAR-α, highly expressed in the liver, is closely related to fatty acid β-oxidation in the liver and in brown adipose tissue and also modulates both acute and chronic inflammation [11,19]. To a lesser extent, it occurs in the cardiovascular system, muscles and kidneys. PPAR-α regulates the transcription of multiple genes, including acyl-CoA-oxidase, carnitine palmitoyl transferase (CPT) and several CYP4As [20]. PPAR-δ is expressed in almost all the tissues of the body. PPAR-γ is a major regulator expressed in fat tissue that promotes fat storage in white adipose tissue and also, to a lesser extent, in the liver, muscles and cardiovascular system [11]. In the case of overweight or obesity, white adipose tissue plays a pivotal role in the development of oxidative stress, inflammation and depletion of n-3 long-chain polyunsaturated fatty acids [21].

Two essential transcription factors that adjust adipocyte differentiation and regulate the expression of adipogenic and lipogenic genes are PPAR-γ and CCAAT/enhancer binding protein α (C/EBPα) [22]. CCAAT/enhancer binding protein α is a member of a family of six transcription factors, named from C/EBPα to C/EBPζ. They interact with the CCAAT (cytosine–cytosine–adenosine–adenosine–thymidine) sequence, present in several gene promoters. C/EBPs are pivotal in adipogenesis (C/EBPβ and C/EBPδ are early adipogenic transcription factors, while isoform α regulates the final stages of adipogenesis) and the development of osteoporosis. C/EBPα is one of the inductors of PPAR-γ expression [23,24]. Importantly, from a potential therapeutic perspective, the

suppressor of PPAR-γ, C/EBPα, and also SREBP-1 expression, is AMP-activated protein kinase (AMPK) [25,26]. AMPK is a key factor controlling cellular energy homeostasis. It influences hepatic lipid metabolism through modulating the downstream acetyl-CoA carboxylase (ACC) and carnitine palmitoyl transferase-1 (CPT-1) pathway [27].

Another crucial transcription factor that enhances lipogenesis and adipogenesis is sterol regulatory element-binding protein 1c (SREBP-1c). There are two isoforms of sterol regulatory element-binding protein 1, SREBP-1a and SREBP-1c. SREBP-1a is expressed in the intestine and spleen, whereas SREBP-1c is mainly expressed in the liver, muscle and adipose tissue. SREBP-1c regulates genes required for glucose metabolism, fatty acid and lipid production and facilitates the storage of fatty acids as triglycerides [28–30]. Its expression is induced i.a. by insulin. A high-fat diet (HFD) is another key factor triggering the overexpression of both SREBP-1c and PPAR-γ in the liver and adipose tissue [31,32].

3. Impact of Anthocyanins

Anthocyanins are a group of natural substances that have garnered increasing interest in recent years. Anthocyanins are natural polyphenolic glycosidic phytopigments (mainly red, blue or purple), occurring primarily in fruits and flowers, that are biochemically related to flavonoids. Structurally, anthocyanins are aliphatic or aromatic three-ring compounds with one or more sugar molecules, and sometimes with a sugar attached aryl group. The colored aglycons are the anthocyanidins (usually cyanidin, pelargonidin or delphinidin) [33] (Figure 1). Fruits, which contain anthocyanins, are the sources of 90% of their habitual consumption [34]. Although anthocyanins are common in fruits, their daily intake by individual people in western countries is often relatively low [35]. The most common sources of these compounds are blueberries, blackberries, cranberries, acai berries, chokeberries, purple sweet potatoes, cherries, redcurrants or black soybeans. Anthocyanins possess potential anti-oxidative, antimicrobial, anti-inflammatory, anti-aging, lipid-lowering, anti-diabetic, anti-cancerous and anti-obesity bioactivities [36,37].

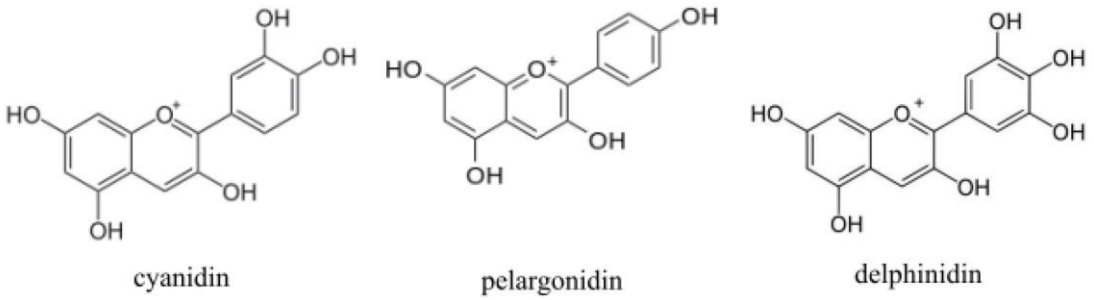

Figure 1. Chemical structures of main anthocyanins' aglycons.

Anthocyanins suppress lipid accumulation in adipocytes due to the broad inhibition of the transcription factors regulating lipogenesis. This may partially explain the mechanism by which anthocyanins exert their anti-obesity effect. Anthocyanins may also lessen the process of triglyceride and lipid accumulation during adipocyte differentiation [38]. They reduce the gene and protein expression levels of lipogenic transcription factors such as liver X receptor α, sterol regulatory element-binding protein 1c, peroxisome proliferator-activated receptor-γ and CCAAT/enhancer binding protein α. A fortiori, target genes' and proteins' expression of these TFs is also distinctly suppressed by anthocyanins [38].

One of the most common anthocyanidins (aglicons of anthocyanins) is cyanidin. Cyanidin is natural compound abundant in fruits and vegetables, i.a. hawthorn, hibiscus or blueberry [33]. Jia et al. [13] showed that cyanidin is a direct ligand for both LXRα and LXRβ. Moreover, cyanidin affects all three PPAR subtypes, with the greatest affinity to PPAR-α [39]. Cyanidin-3-*O*-*β*-glucoside (C3G) activates liver X receptor (LXR)-ATP-

binding cassette transporter-dependent cholesterol efflux [40,41] and phosphorylation of cellular AMPK in human hepatoma (HepG2) cells [27,42,43]. It was proven that C3G decreases the expression of adipogenic transcription factors involved in hepatic lipid metabolism, such as SREBP-1c, C/EBPα and PPAR-γ, both in the HepG2 cells and in the livers of supplemented mice [44]. The decreasing influence of anthocyanins on the gene expression of SREBP-1c and C/EBPα was also validated in other studies [43,45–47].

Nonetheless, the issue of anthocyanins' effects on PPAR-α activation still requires more research. There have been reports that anthocyanins have a strong affinity and a significant impact on the expression of this receptor [39,41,45,48], while Rimando et al.'s [49] results on the hamster model stand in opposition to the above findings.

Anthocyanins have been reported to have beneficial effects on obesity and obesity-related metabolic disorders (e.g., insulin resistance and dyslipidemia). Anthocyanins elicited from, e.g., purple corn, sweet cherry, hibiscus or black soybean, in mice or 3T3-L1 preadipocyte models, prevented adipocyte differentiation, lipid accumulation and low-density lipoprotein (LDL) oxidation and protected against diet-induced hepatic steatosis by reducing PPAR-γ transcriptional activity [50–53]. A very abundant source of anthocyanins is the fruits of *Aronia melanocarpa* (AM). Park et al. [54] conducted an interesting AM study both on cells and mice. It appeared that AM extract significantly reduced free fatty acid-induced lipid droplet accumulation in vitro. Moreover, the synthesis of PPAR-γ2 was inhibited in vivo, the transcriptional activity of PPAR-γ2 was downregulated in vitro and the mRNA expression of PPAR-γ2 and its target genes, adipocyte protein 2 and lipoprotein lipase, was lowered both in vitro and in vivo.

However, in a recently conducted human study, berry supplementation triggered an inhibition of nuclear factor-kappa B (NF-κB)-dependent gene expression, accompanied by an enhancement in PPAR-γ expression. Nevertheless, an improvement in selected features of metabolic syndrome and related cardiovascular risk factors was observed [55]. Therefore, it can be hypothesized that stimulation or inhibition of PPAR-γ expression by anthocyanins may have both positive and negative consequences. Stimulation of this transcription factor may contribute to the reduction of inflammation while acting as a lipogenic, whereas the inhibition of PPAR-γ expression brings beneficial effects in the context of lipid homeostasis. Overall, it may lead to a partial prevention of obesity-induced atherosclerosis by attenuating inflammatory responses. It is also worth remembering that there are differences in fat metabolism between rodents and humans, which also may translate into disparities in the results of individual research models.

In 2019, Park et al. [37] confirmed most of the properties attributed to anthocyanins in earlier studies. They proved that another common anthocyanin—Delphinidin-3-*O*-*β*-glucoside (D3G)—significantly inhibited the accumulation of lipids in a dose-dependent manner without displaying cytotoxicity. In the 3T3-L1 adipocytes, D3G lowered the expression of major adipogenic and lipogenic transcription factors, i.e., PPAR-γ, SREBP-1 and C/EBPα. In addition, D3G enhanced the phosphorylation of AMPK and ACC—a fatty acid oxidation enzyme. These results once again supported the previously presented hypothesis that anthocyanins attenuate adipogenesis and promote lipid metabolism and are potential therapeutic agents in the treatment of obesity. Similar findings were obtained with a sole aglicon, delphinidin [56].

Anthocyanins represent also an interesting therapeutic option in diabetic nephropathy. Renal damage occurring in this disorder is mainly caused by oxidative stress and lipotoxicity. Db/db mice treated with anthocyanins show decreased albuminuria, ameliorated intra-renal lipid concentrations and improvement of glomerular matrix expansion and inflammation related to an increase in AMPK phosphorylation, upregulation of PPAR-α and PPAR-γ and decrease in SREBP-1 activity [48]. The studies describing the impact of anthocyanins on the discussed transcription factors are summarized in Table 1.

Table 1. List of studies describing the impact of anthocyanins on discussed transcription factors.

Authors and Date of Publication	Research Model	Compounds Used in Study	Observed Changes
Aboonabi et al., 2020 [55]	human	berry anthocyanin supplements	PPAR-γ ⇩
Chang et al., 2013 [43]	HepG2 cells	mulberry anthocyanin extract	SREBP-1c ⇩ PPAR-α ⇧
de Sousa et al., 2018 [45]	rats	extruded sorghum flour	SREBP-1c ⇩ PPAR-α ⇧
Du et al., 2015 [41]	HK-2 cells	cyanidin-3-*O*-*β*-glucoside, cyanidin	LXRα ⇧ PPAR-α ⇧
Fu et al., 2014 [40]	mice, mice mammary epithelial cells	cyanidin-3-*O*-*β*-glucoside	LXRα ⇧
Hwang et al., 2011 [42]	mice	purple sweet potato anthocyanin fraction	SREBP-1c ⇩
Jia et al., 2013 [39]	HepG2 cells, CHO-K1 cells	cyanidin	PPAR-α ⇧ PPAR-δ ⇧ PPAR-γ ⇧
Jia et al., 2013 [13]	macrophages, hepatocytes	cyanidin	LXRα, LXRβ ⇧ SREBP-1c ⇧
Kao et al., 2009 [52]	mouse macrophage J774A.1 cells	hibiscus anthocyanin extract	PPAR-γ ⇩
Khan et al., 2018 [47]	3T3-L1 cells	*C. kousa* anthocyanin ethanolic leaf extract	PPAR-γ ⇩ C/EBPα ⇩
Kim et al., 2012 [53]	3T3-L1 cells	black soybean anthocyanin extract	PPAR-γ ⇩
Koh et al., 2015 [48]	mice	*Seoritae* anthocyanin extract	PPAR-α ⇧ PPAR-γ ⇧ SREBP-1c ⇩
Lee et al., 2014 [38]	3T3-L1 cells	grape anthocyanin isolate	LXRα ⇩ PPAR-γ ⇩ C/EBPα ⇩ SREBP-1c ⇩
Luna-Vital et al., 2017 [50]	3T3-L1 cells	purple corn pericarp anthocyanin extract, pure anthocyanins	PPAR-γ ⇩
Park et al., 2015 [46]	rats	unfermented and fermented black carrot extract	SREBP-1c ⇩ PPAR-α ⇩
Park et al., 2017 [54]	mice, FL83B cells	*A. melanocarpa* spray-dried ethanol extract	PPAR-γ ⇩
Park et al., 2019 [44]	HepG2 cells	honeyberry extract	SREBP-1c ⇩ PPAR-γ ⇩ C/EBPα ⇩ PPAR-α ⇧
Park et al., 2019 [37]	3T3-L1 cells, primary white adipocytes	delphinidin-3-*O*-*β*-glucoside	PPAR-γ ⇩ C/EBPα ⇩ SREBP-1c ⇩
Rahman et al., 2016 [56]	3T3-L1 cells	delphinidin	PPAR-γ ⇩ C/EBP ⇩
Rimando et al., 2016 [49]	hamsters	blueberry peel extract	⟺ PPAR-α
Song et al., 2016 [51]	mice	sweet cherry anthocyanins	PPAR-γ ⇩
Sozański et al., 2014 [12]	rabbits	cornelian cherry fruits lyophilisate	PPAR-α ⇧
Sozański et al., 2016 [36]	rabbits	mixture of anthocyanins	PPAR-α ⇧ PPAR-γ ⇧

⇧— up regulation, ⇩—down regulation, ⟺—unchanged.

4. Impact of Iridoids

Iridoids are a large group of organic monoterpenoids, occurring in plants usually as glycosides with a glucose moiety attached to C-1 in the pyrene ring. The basic structural feature of iridoids is a bicyclic H-5/H-9β, β-cis-fusedcyclopentanopyran ring system. Iridoids are found in the green parts of plants, mainly in the leaves and young stems, and sometimes in fruits and sprouts. They are found in many plant families, e.g., *Apocynaceae*, *Gentianaceae*, *Lamiaceae*, *Loganiaceae*, *Rubiaceae*, *Scrophulariaceae* and *Verbenaceae*. Among the most commonly mentioned iridoids in the therapeutic context, the following stand out above all: gentiopicroside, geniposide, sweroside, loganin, loganic acid, catalpol and amarogentin [33] (Figure 2). In foods consumed in western diets, they are rarely present. However, they can be found in some raw fruits (e.g., olives, cornelian cherry, honeysuckle berries and cranberries) and in products derived from these fruits [57–60]. Iridoids possess potential cardiovascular, hypoglycemic, hypolipidemic, antihepatotoxic, choleretic, anti-inflammatory, antispasmodic, antitumor, antiviral, immunomodulatory and purgative bioactivities [61].

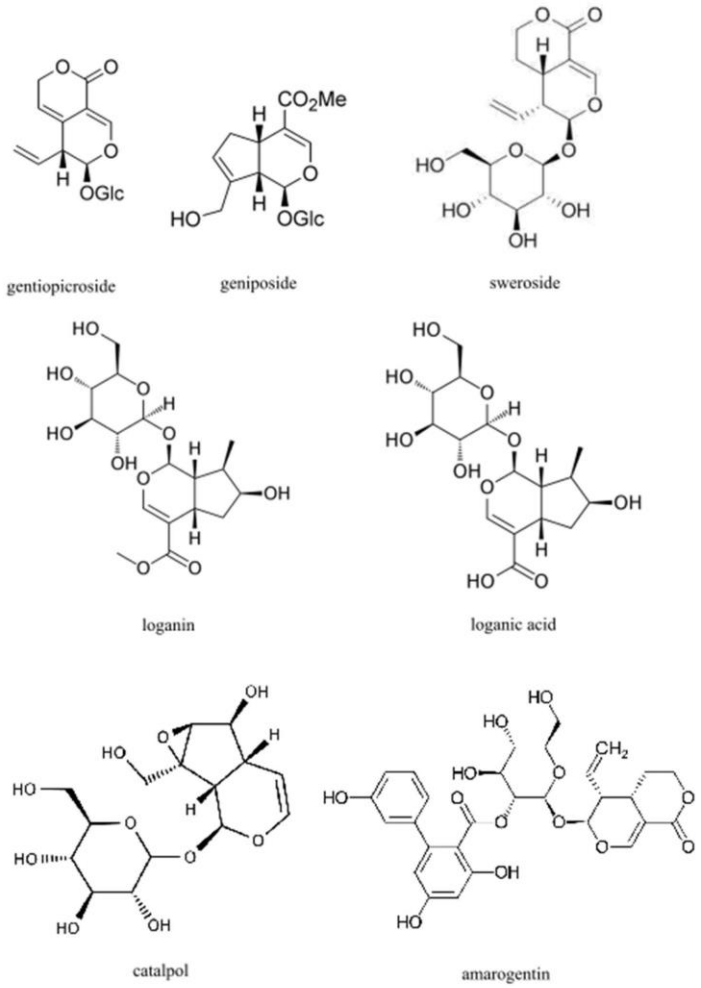

Figure 2. Chemical structures of main iridoids.

MicroRNAs are short, non-coding, single-stranded RNA molecules which have been reported to play a key role in the adjustment of lipid and lipoprotein metabolism. Increased levels of some miRNAs have been observed in the circulation in the course of hyperlipidemia [62]. It was proven that genipin upregulates the expression of miR-142a-5p, which binds to SREBP-1c and consequently leads to the inhibition of lipogenesis, which results in decreased body weight, lipid serum levels and hepatic lipid accumulation in HFD mice [63]. However, it is worth noting that there are a lot of miRNAs involved in fat metabolism, and some variants grow while others decline in the course of hyperlipidemia.

Zhu et al. [64] conducted an interesting study whose assumptions were based on providing HFD rats with three different doses of an iridoid fraction isolated from *Valeriana jatamansi*. Compared to the model group, a decrease in weight gain as well as a decrease in triglyceride and an increase in HDL-C serum levels were noticed regardless of the dose. All doses enhanced the expression of the PPAR-α receptor and reduced the expression of the SREBP-1c protein in the liver. In addition, depending on the dose, alterations in liver X receptor-α expression were observed. Hypolipidemic and antioxidant activity of iridoids through the stimulation of PPAR-α was noted also in different studies [36,65–68].

Cornus alternifolia (CA) is a tree that is common mainly in Eastern Asia and North America. Extracts of this plant have been used in traditional Chinese medicine as tonics, analgesics and diuretic drugs, while, in the United States, CA is grown as an ornamental plant. *Cornus alternifolia* leaves are a valuable source of iridoid compounds. It was shown that one of them—kaempferol-3-*O*-*β*-glucopyranoside—exhibited significant agonistic activities for PPAR-α, PPAR-γ and LXR dose-dependently in human hepatoma (HepG2) cells and Chinese hamster ovary cells (CHO) [69]. Several other iridoid substances isolated from CA leaves also influenced the expression of these transcription factors, but it is kaempferol-3-*O*-*β*-glucopyranoside which presents the most promising curative potential [69]. *Fraxinus* is a tree genus from which valuable quantities of iridoid compounds can also be obtained. *Fraxinus* compounds show significant inhibitory activity on adipocyte differentiation in the 3T3-L1 cells, reduce fat and triglyceride accumulation in differentiated 3T3-L1 cells without affecting cell viability and suppress the induction of C/EBPα, C/EBPβ and PPAR-γ transcription factors, concomitantly activating the PPAR-α receptor [70,71]. Similar effects were noticed in the case of iridoids of olive trees [72].

As with anthocyanins, iridoids, e.g., catalpol, may be a useful therapeutic option for the treatment of diabetes complications [73]. Gentiopicroside, a compound that belongs to the sub-group of seco-iridoids, ameliorated dyslipidemia and improved nerve blood flow through regulating the PPAR-γ/AMPK/ACC signal pathway in a diabetic peripheral neuropathy rat model [74]. Moreover, gentiopicroside in both acute and chronic alcohol-induced mouse hepatosteatosis (the latter stage of alcoholic liver disease, ALD) models mitigated the upregulation of SREBP-1 and downregulation of PPAR-α, among others, by activation of AMPK [75]. Alleviation of SREBP-1 expression was observed also in a nonalcoholic fatty liver disease model, namely by swertiamarin [76].

Iridoids and seco-iridoids such as catalpol, geniposide, harpagoside, loganin and oleuropein have been found to exhibit essential neuroprotective effects in Alzheimer's disease (AD) and Parkinson's disease (PD). One of the proposed mechanisms by which the process of neurogedeneration is slowed down is by increasing the PPAR-γ receptor expression, which contributes to the clearance of amyloid-β in the apolipoprotein E-mediated pathway in the brain [77].

For the prevention and treatment of obesity and dyslipidemia, a potentially useful iridoid compound is loganic acid (LA). Park et al. [78] demonstrated the antiadipogenic effects of LA in vitro and in vivo. Loganic acid treatment significantly decreased the adipocyte differentiation of 3T3-L1 preadipocytes in a dose-dependent manner and substantially lowered the expression of key adipogenesis transcription factors such as peroxisome proliferator-activated receptor-γ and CCAAT/enhancer-binding protein α. Moreover, a diminution of body weight gain, total fat increase, fatty hepatocyte deposition in the liver and adipocyte enlargement in the abdominal visceral fat tissues were noticed in an

ovariectomy-induced obesity mice model compared to the comparative group. Loganic acid is also an important active ingredient found in cornelian cherry fruits. *Cornus mas* fruits, abundant in substantial amounts of both anthocyanins and iridoids, may exert positive additive or synergistic effects on dyslipidemia and atherosclerosis. In a rabbit model of diet-induced dyslipidemia and atherosclerosis, Sozański et al. [36] demonstrated that both anthocyanins and, to a lesser extent, loganic acid increased the expression of PPAR-α and PPAR-γ receptors in the liver and decreased intima thickness and the intima/media ratio in the thoracic aorta. The studies describing the impact of iridoids on the discussed transcription factors are summarized in Table 2.

Table 2. List of studies describing the impact of iridoids on discussed transcription factors.

Authors and Date of Publication	Research Model	Compounds Used in Study	Observed Changes
Bai et al., 2010 [71]	3T3-L1 cells	aqueous extract and compounds isolated from the seeds of *F. excelsior*	PPAR-α ⇧
Choi et al., 2011 [70]	3T3-L1 cells	hydroxyframoside B	C/EBPα ⇩ C/EBPβ ⇩ PPAR-γ ⇩
Drira et al., 2011 [72]	3T3-L1 cells	oleuropein	PPAR-γ ⇩ C/EBPα ⇩ SREBP-1c ⇩
He et al., 2012 [69]	HepG2 cells, CHO cells	leaf extract of *C. alternifolia*, incl. Kaempferol-3-*O*-*β*-glucopyranoside	PPAR-α ⇧ PPAR-γ ⇧ LXRα ⇧
Li et al., 2018 [75]	mice, HepG2 cells, macrophages	gentiopicroside	SREBP-1c ⇩ PPAR-α ⇧
Lu et al., 2018 [74]	rats	gentiopicroside	PPAR-γ ⇧
Ma et al., 2011 [66]	rats	geniposide	PPAR-α ⇧
Malliou et al., 2018 [65]	mice	oleuropein	PPAR-α ⇧
Park et al., 2018 [78]	3T3-L1 cells, mice	loganic acid	PPAR-γ ⇩ C/EBPα ⇩
Patel et al., 2016 [67]	HepG2 cells	swertiamarin	SREBP-1c ⇩ PPAR-α ⇧
Sozański et al., 2014 [12]	rabbits	cornelian cherry fruits lyophilisate	PPAR-α ⇧
Sozański et al., 2016 [36]	rabbits	loganic acid	PPAR-α ⇧ PPAR-γ ⇧
Yang et al., 2019 [76]	mice	swertiamarin	SREBP-1c ⇩
Yang et al., 2020 [68]	mice	sweroside	PPAR-α ⇧
Zhong et al., 2018 [63]	mice, primary hepatocytes	genipin	SREBP-1c ⇩
Zhu et al., 2016 [64]	rats	iridoids rich fraction in *V. jatamansi*	LXRα ⇩ SREBP-1c ⇩ PPAR-α ⇧

⇧—up regulation, ⇩—down regulation.

5. Future Perspectives

Improper nutrition is a compelling problem nowadays. The share of plant products in the diets of many people should be much more extensive. In this context, compounds with proven therapeutic effects on the body, such as anthocyanins and iridoids, may play an increasingly important role in the prevention and adjunctive or combined treatment of many human diseases. Many studies confirmed the curative effects and mechanisms of action of these groups of substances. Unfortunately, the majority of these studies were performed on animals, and first and foremost, so the paramount goal for the near future is to conduct more clinical studies to confirm the positive impact of anthocyanins and

iridoids on the human body. New applications for the above-mentioned compounds should also be sought. Interestingly, most of the conducted research pertains to the influence of anthocyanins and iridoids on high-fat-diet models. It would certainly be a challenging option to consider a study, or a whole series, verifying the impact of these substances on multiple parameters in a low-fat diet.

6. Conclusions

In conclusion, the current knowledge clearly points to the benefits of consuming foods comprising anthocyanins and iridoids. Both groups of substances modulate the expression of crucial transcription factors in lipid and cholesterol homeostasis, i.e., liver X receptor, peroxisome proliferator-activated receptor-α, peroxisome proliferator-activated receptor-γ, CCAAT/enhancer binding protein α and sterol regulatory element-binding protein 1c and their target genes (Figure 3). Therefore, anthocyanins and iridoids present an essential therapeutic option in disorders proceeding with disturbances in lipid and cholesterol metabolism, such as obesity, atherosclerosis, diabetes, metabolic syndrome and many more.

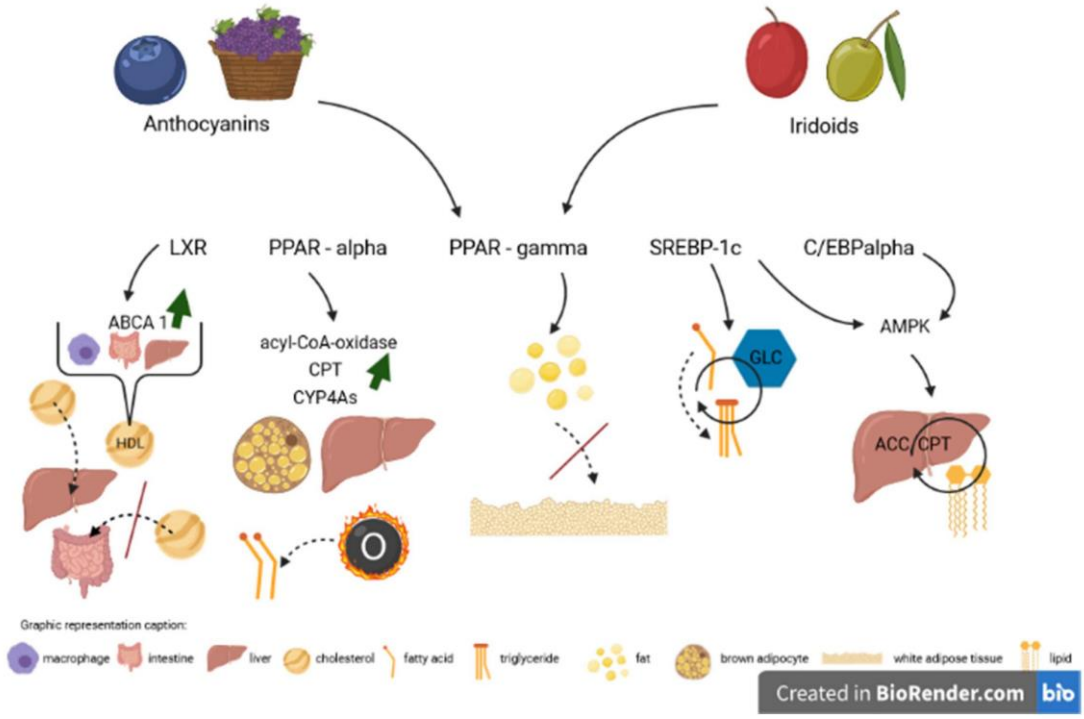

Figure 3. Influence of anthocyanins and iridoids on main transcription factors involved in cholesterol and lipid metabolism. Accessed on 28 May 2021.

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Abbreviations

ABCA1	ATP-binding cassette transporter A1
HDL	high-density lipoprotein
acyl-CoA-oxidase	acyl coenzyme A oxidase
CPT	carnitine palmitoyl transferase
CYP4As	cytochrome P450 4A subfamily enzymes
GLC	glucose
AMPK	AMP-activated protein kinase
ACC	acetyl coenzyme A carboxylase
O	β-oxidation

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Cornelian Cherry (*Cornus mas* L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model

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Abstract: Cornelian cherry (*Cornus mas* L.) fruits possess potential cardiovascular, lipid-lowering and hypoglycemic bioactivities. The aim of this study is to evaluate the influence of resin-purified cornelian cherry extract rich in iridoids and anthocyanins on several transcription factors, intima/media ratio in aorta and serum parameters, which determine or are valuable indicators of the adverse changes observed in the course of atherosclerosis, cardiovascular disease, and metabolic syndrome. For this purpose, male New Zealand rabbits were fed a diet enriched in 1% cholesterol for 60 days. Additionally, one group received 10 mg/kg b.w. of cornelian cherry extract and the second group 50 mg/kg b.w. of cornelian cherry extract. PPAR-α and PPAR-γ expression in the aorta, LXR-α expression in the liver; cholesterol, triglycerides, adipokines, apolipoproteins, glucose and insulin levels in serum; the intima and media diameter in the thoracic and abdominal aorta were determined. Administration of cornelian cherry extract resulted in an enhancement in the expression of all tested transcription factors, a decrease in triglycerides, leptin and resistin, and an increase in adiponectin levels. In addition, a significant reduction in the I/M ratio was observed for both the thoracic and abdominal aorta. The results we have obtained confirm the potential contribution of cornelian cherry extract to mitigation of the risk of developing and the intensity of symptoms of obesity-related cardiovascular diseases and metabolic disorders such as atherosclerosis or metabolic syndrome.

Keywords: cornelian cherry; iridoids; anthocyanins; transcription factors; adipokines; triglycerides; aorta; atherosclerosis; metabolic syndrome

1. Introduction

There are many groups of plant compounds with proven curative effects on the body. These include, among others, iridoids and anthocyanins. Both groups affect positively especially the function of the cardiovascular system and the liver. Up to this date cardiovascular, hypoglycemic, hypolipidemic, antihepatotoxic, choleretic, anti-inflammatory, antispasmodic, antitumor, antiviral, immunomodulatory and purgative activities of iridoids [1,2] and antioxidative, antimicrobial, anti-inflammatory, anti-aging, lipid-lowering, anti-diabetic, anticancerous, and anti-obesity activities of anthocyanins were reported [3–6].

One of the valuable sources of both iridoids and anthocyanins is the cornelian cherry fruits. *Cornus mas* L., from the *Cornaceae* family, is a branchy shrub or small tree, native to central and south-eastern Europe and western Asia. In addition to iridoids and anthocyanins, cornelian cherry fruits contain also other flavonoids (e.g., flavonols), phenolic acids, terpenoids (ursolic acid), carotenoids and organic acids [7–10]. Due to the significant content of the iridoids and anthocyanins, cornelian cherry fruits may play an important role in the prevention and treatment of many diseases of the cardiovascular system and the liver [11,12].

The excess level of cholesterol, often resulting from improper diet and lack of adequate physical activity contributes to the development of hyperlipidemia and then possible atherosclerosis, metabolic syndrome, hypertension or cardiovascular disease (CVD) [13–16]. Cholesterol overabundance, broaden by frequently accompanying chronic inflammation, can also lead to aortic dysfunctions, which in extreme cases may result in the aortic aneurysm or aortic valve insufficiency [17,18]. Noticeable alterations in the levels of compounds important for lipid metabolism and transport, e.g., adipokines, as well as changes in the expression of transcription factors, such as peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs) are also observed [19–21].

The previous findings [4,22–24] showed that cornelian cherry fruits are a prospectively valuable source of chemicals that can play an exploitable role in preventing adverse changes in the cardiovascular system, including the aorta and also the liver. However, the impact of cornelian cherry's iridoids and anthocyanins on transcription factors that play an important role in lipid and cholesterol metabolism, and also on many serum parameters crucial in the development and diagnostics of cardiovascular and liver diseases, is not fully explored.

The aim of this study is to determine and compare whether and to what extent two different doses of resin-purified cornelian cherry extract (10 mg per kilogram of body weight or 50 mg per kilogram of body weight) have an effect on the expression of peroxisome proliferator-activated receptor-α (PPAR-α), peroxisome proliferator-activated receptor-γ (PPAR-γ) in the aorta, and liver X receptor-α (LXR-α) in the liver; on serum levels of leptin, adiponectin, resistin, glucose, insulin and selected lipoproteins and apolipoproteins (Apos); and on aortal intima/media ratio (histopathological changes) in the rabbit model. We also try to compare changes in blood markers–those that play an important role in clinical practice in predicting and assessing the risk and progression of cardiovascular and metabolic diseases–with molecular and histopathological changes in target organs. So far, to our knowledge, no one has studied these features so thoroughly, and we believe that our results may constitute another important step towards full determination of the therapeutic value of cornelian cherry fruits and their potential use in the prophylaxis and therapy of cardiovascular diseases.

2. Materials and Methods

2.1. Animal Model

Fifty sexually mature male New Zealand rabbits aged 8 months to 1 year were used in the experiment. The animals were housed in individual chambers with temperatures maintained at 21–23 °C. Rabbits were acclimated, weighed and observed for four weeks prior to the beginning of the sixty-day study. Then, they were randomly divided into 5 groups of 10 animals. The animals in group P were fed the standard complete formula for rabbits. Animals in other groups: CHOL, EXT 10, EXT 50 and SIMV 5 were fed with the above-mentioned mixture enriched in 1% cholesterol. During the experiment, rabbits had *ad libitum*

water access and received the same daily portion of chow (40 g/kg). Once-daily, in the morning, for the consecutive 60 days of the study, the following substances were administered orally to the rabbits: groups P and CHOL–normal saline solution, group EXT 10–*Cornus mas* L. extract 10 mg per kg b.w., group EXT 50–*Cornus mas* L. extract 50 mg per kg b.w., group SIMV 5–simvastatin 5 mg per kg b.w. The feeding schema is presented in Table 1.

Table 1. Experimental groups, feeding plan and scheduled administration of tested substances.

Group	Chow	Tested Substance	Dose of Tested Substance
P	standard chow	none	none
CHOL	standard chow + 1% cholesterol	none	none
EXT 10	standard chow + 1% cholesterol	cornelian cherry extract	10 mg/kg b.w.
EXT 50	standard chow + 1% cholesterol	cornelian cherry extract	50 mg/kg b.w.
SIMV 5	standard chow + 1% cholesterol	simvastatin	5 mg/kg b.w.

Blood samples were collected before and after 60 days of the experiment from each animal, from the margin vein of the ear or the saphenous vein. At the end of a study, the rabbits were euthanized with terminal anesthesia, using Morbital® (Biowet, Puławy, Poland; 1 mL of the drug contains 133.3 mg of sodium pentobarbital and 26.7 mg of pentobarbital) at a dose of 2 mL/kg given intraperitoneally (i.p.). The aortas and livers were then harvested and cleaned, and afterward frozen and stored at the temperature of −70 °C for further examination.

2.2. Chemicals and Materials

Acetonitrile, methanol, and formic acid were purchased from Sigma-Aldrich (Steinheim, Germany). Loganic acid, *p*-coumaric acid, caffeic acid, ellagic acid, quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, and cyanidin 3-*O*-glucoside were purchased from Extrasynthese (Lyon Nord, France).

2.3. Plant Materials and Preparation of Extract

Cornelian cherry (*Cornus mas* L.) fruits were assembled in the Bolestraszyce Arboretum and Institute of Physiography, Poland. Before analysis, fruits were stored in a falcon tube at −20°C. A plant herbarium specimen (BDPA 3 967) was authenticated and deposited in the Herbarium of the Bolestraszyce Arboretum and Institute of Physiography, Poland. The preparation of a resin-purified cornelian cherry extract (EXT) was previously described by Nowak et al. [25]. The quantitative composition of iridoids and polyphenols in the EXT is shown in Figure 1.

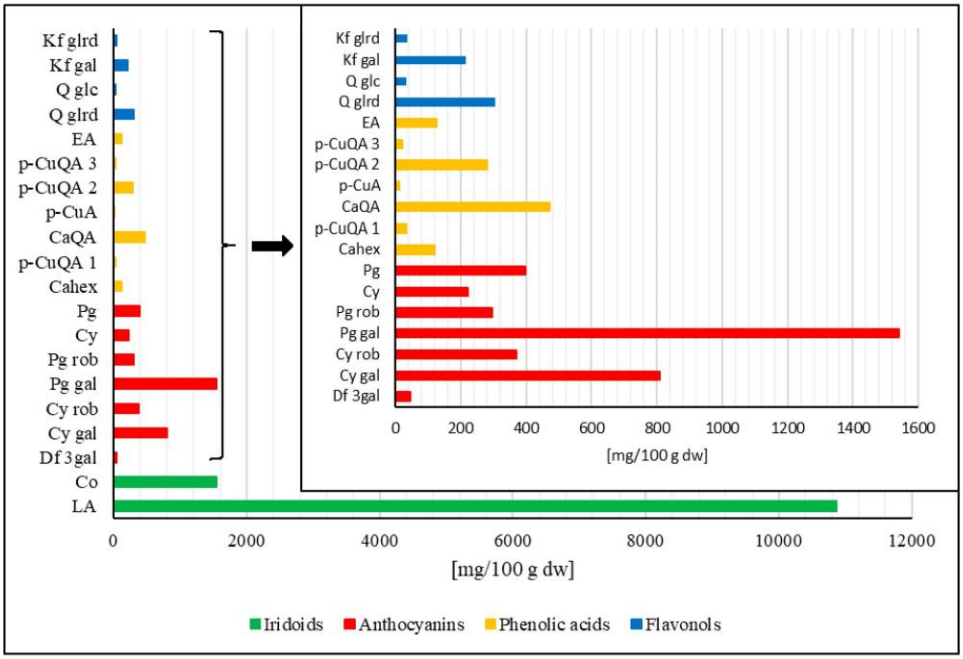


Figure 1. Iridoids and polyphenols (anthocyanins, phenolic acids, and flavonols) content in the purified cornelian cherry extract. Abbreviations: LA–loganic acid, Co–cornuside; Df 3 gal–delphinidin 3-*O*-galactoside; Cy gal–cyanidin 3-*O*-galactoside; Cy rob–cyanidin 3-*O*-robinobioside; Pg gal–pelargonidin 3-*O*-galactoside; Pg rob–pelargonidin 3-*O*-robinobioside; Cy–cyanidin; Pg–pelargonidin; Cahex–caffeoylhexoside; *p*-CuQA 1–*p*-coumaroilquinic acid 1; CaQA–caffeoylquinic acid; *p*-CuA–*p*-coumaric acid; *p*-CuQA 2–*p*-coumaroilquinic acid 2; *p*-CuQA 3–*p*-coumaroilquinic acid 3; EA–ellagic acid; Q glrd–quercetin 3-*O*-glucuronide; Q glc–quercetin 3-*O*-glucoside; Kf gal–kaempferol 3-*O*-galactoside; Kf glrd–kaempferol 3-*O*-glucuronide.

2.4. Quantification of Compounds by HPLC-PDA

The assay of iridoids and anthocyanins was carried out according to the method described by Kucharska et al. [26], with a Dionex HPLC system (Germering, Germany) equipped with the Ultimate 3000 model diode array detector. The Cadenza Imtakt column CD-C18 (75 × 4.6 mm, 5 µm) was used. The mobile phase was a mixture of 4.5% aq. formic acid, *v*/*v* (A) and 100% acetonitrile (B), flowing at 1.0 mL min − 1 under gradient elution conditions: 0–1 min 5% B in A, 1–20 min 25% B in A, 20–26 min 100% B, 26–30 min 5% B in A. Iridoids were monitored at wavelengths of 245 nm, ellagic acid at 254 nm, phenolic acids at 320 nm, flavonols at 360 nm, and anthocyanins at 520 nm. Iridoids were quantified as loganic acid, anthocyanins as cyanidin 3-*O*-glucoside, phenolic acids as *p*-coumaric acid, caffeic acid, and ellagic acid, flavonols as quercetin 3-*O*-glucoside and kaempferol 3-*O*-glucoside.

2.5. Measurement of Physical and Biochemical Parameters

The bodyweight of the rabbits was determined using a precision scale (Radwag, Radom, Poland). The serum levels of total cholesterol (TC) and triglycerides (TGs) were estimated based on calorimetric, enzymatic methods. High-density lipoprotein (HDL-C) and low-density lipoprotein cholesterols (LDL-C) were measured using calorimetric, direct methods (ABX Pentra 400, Horiba Ltd., Kyoto, Japan). Glucose levels were determined using the Trinder glucose activity test (ABX Pentra 400, Horiba Ltd., Kyoto, Japan).

2.6. Quantification of Adiponectin, Leptin, Resistin, Apolipoproteins (A1, B100, E), and Insulin by Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA method was used in the determination of adiponectin (ELISA kit for Rabbit ADP/Acrp30, ERB0002, Fine Test, Wuhan Fine Biotech Corp., Wuhan, China), leptin (Rabbit Leptin ELISA Kit, CSB-E06971Rb, Cusabio Technology LLC, Houston, TX, USA), resistin (Rabbit Resistin ELISA kit, ERB0105, Fine Test, Wuhan Fine Biotech Corp., Wuhan, China), apolipoprotein A1 (ELISA kit for Rabbit Apolipoprotein A1, ERB0009, Fine Test, Wuhan Fine Biotech Corp., Wuhan, China), apolipoprotein B100 (ELISA kit for Rabbit Apolipoprotein B100, ERB0010, Fine Test, Wuhan Fine Biotech Corp., Wuhan, China), apolipoprotein E (ELISA kit for Rabbit Apolipoprotein E, ERB0014, Fine Test, Wuhan Fine Biotech Corp., Wuhan, China), and insulin (Rabbit Insulin ELISA kit, 90186, Crystal Chem Inc., Elk Grove Village, IL, USA) levels, according to manufacturer's instructions. All concentrations were expressed as ng/mL or mmoL/L.

2.7. Histopathological Assessment of the Thoracic and Abdominal Aorta

The material fixed in buffered 7% formalin was embedded in paraffin and cut into 4 μm sections which were stained by the routine hematoxylin-eosin method. The preparations were viewed without knowing the division into the control group and experimental groups. Microscopic analysis was performed using the Olympus BX53 light microscope coupled with Olympus UC90 camera. The intima and media thickness measurements were made using a cellSens standard V1 software. Photos were made at an enlargement of 100× or 200×, the scale shown in the photo is 50 μm or 100 μm.

2.8. RNA Isolation, Reverse Transcription and Real-Time PCR

Total RNA was isolated from studied tissue samples with RNeasy Fibrous Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. To eliminate genomic DNA contamination, on-column DNase digestion was performed using RNase-Free DNase Set (Qiagen, Hilden, Germany). Quantity and purity of RNA samples were assessed by measuring the absorbance at 260 and 280 nm with NanoDrop1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). First-strand cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA) as described in the protocol. The mRNA expression of PPAR-α and PPAR-γ was determined by quantitative real-time PCR with 7500 Real-Time PCR System and Power SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference gene. The reactions were performed with RT^2 qPCR Primer Assay for Rabbit PPAR-α (PPN13311A, Qiagen, Hilden, Germany), PPAR-γ (PPN05175A, Qiagen, Hilden, Germany), and GAPDH (PPN00377A, Qiagen, Hilden, Germany). All reactions were performed in triplicates under the following conditions: activation of the polymerase at 50 °C for 2 min, initial denaturation at 94 °C for 10 min and 40 cycles of denaturation at 94 °C for 15 s followed by annealing and elongation at 60 °C for 1 min. The specificity of the PCR was determined by melt curve analysis for each reaction. The relative mRNA expression of the examined factors was calculated with the ΔΔCt method.

2.9. Western Blotting of LXR-α Expression

Rabbit livers were homogenized in buffer containing 25 mM Tris pH 7.5, 50 mM NaCl, 1% NP-40 and protease inhibitors set. After centrifugation, the clear supernatant was mixed with the SDS sample buffer, boiled at 95 °C for 5 min and subjected to SDS-PAGE on 12% gel. The resolved proteins were transferred to the PVDF membrane (Thermo Fisher Scientific, Waltham, MA, USA) using semi-dry transfer. After the transfer, the membrane was blocked with 1% casein in TBS at 4 °C, overnight, and then incubated with 1 μg/mL of antibody anti-LXR-α (NR1H3/LXR Alpha Antibody LS-B3526-50, LifeSpan Biosciences Inc., Seattle, WA, USA) and beta-actin C-04 (Santa Cruz Biotechnology Inc., Dallas, TX, USA) at room temperature for 1 h, followed by secondary horseradish peroxidase-labeled antibody

(Dako, Agilent, Santa Clara, CA, USA). The bounded antibodies were visualized using the West-Pico blotting detection system (Thermo Fisher Scientific, Waltham, MA, USA). The blots were scanned, and the optical density of bands was analysed with Image J software.

2.10. Statistical Analysis

Parametric data were expressed as mean ± standard deviation (mean ± SD). The statistical analysis was conducted using the Statistica v. 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). The normality of all continuous variables was verified with the Shapiro–Wilk test. One-way analysis of variance (ANOVA) with least significant difference (LSD) Fisher's post hoc test was performed for a comparison involving 3 or more groups. The p-values < 0.05 were considered statistically significant. Graphical representations of the statistical data were created using the Statistica v. 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results

We have studied the effects of the oral administration of resin-purified cornelian cherry extract on mRNA expression of PPAR-α and PPAR-γ in the aorta and LXR-α expression in hepatocytes. We have indicated the levels of various parameters in the serum, and we conducted also a histopathological analysis of the intima-media ratio in the thoracic and abdominal aorta.

3.1. Body Weight and Serum Lipid Levels

Firstly, we assessed the change in body weight of the rabbits. On day 60 (the last day of the study), we observed an increase in average body weight among all study groups. The highest increase in average body weight was noted in the SIMV 5 group and the CHOL group. The smallest weight gain compared to the control group was recorded in the EXT 50 group. Summary data are presented in Table 2.

Table 2. Rabbit average weight on days 0 and 60 and weight change during the study.

Group	Average Weight (kg) [Mean ± SD]		Weight Change (kg)	Weight Change (%)	Change in Weight Gain in Comparison to P Group (%)	Change in Weight Gain in Comparison to CHOL Group (%)
	Day 0	Day 60				
P	3.203 ± 0.338	3.380 ± 0.305	0.177	5.53	0	−57.14
CHOL	3.117 ± 0.453	3.530 ± 0.377	0.413	13.25	133.33	0
EXT 10	3.315 ± 0.320	3.696 ± 0.367	0.381	11.49	115.25	−7.75
EXT 50	3.161 ± 0.434	3.475 ± 0.422	0.314	9.93	77.40	−23.97
SIMV 5	2.975 ± 0.249	3.395 ± 0.274	0.420	14.12	137.29	1.69

In quantification of serum lipid levels, a significant increase in the total cholesterol level was observed in the CHOL group compared to the P group. In both groups fed with the cholesterol diet with the cornelian cherry extract, a slightly smaller increase in TC levels was observed (compared to the P group), but only in the simvastatin group, the change was statistically significant ($p < 0.001$). In the case of the LDL-C fraction, an identical trend was observed, while in the HDL-C level evaluation the alterations were negligible. On the contrary, the measurement of triglycerides showed significant decreases in their levels in all three research groups (EXT 10 $p = 0.036$; EXT 50 $p = 0.002$; SIMV 5 $p < 0.001$) (Figure 2).

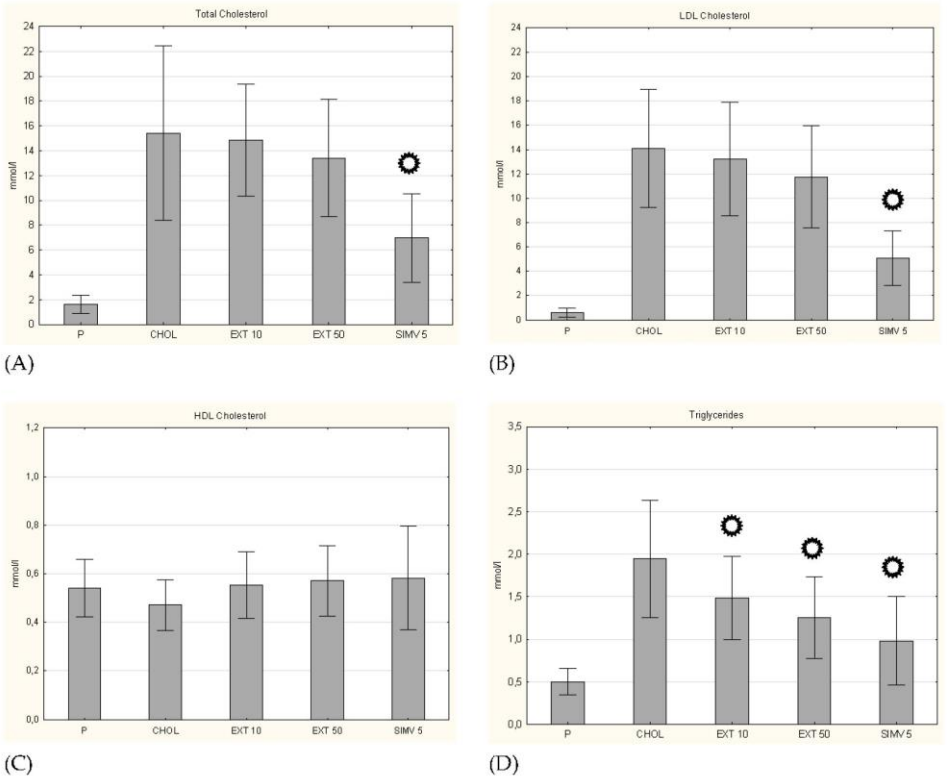


Figure 2. Serum lipid levels. (**A**) total cholesterol, (**B**) LDL cholesterol, (**C**) HDL cholesterol, and (**D**) triglycerides levels on day 60. of the study. P–standard chow; CHOL–standard chow + 1% cholesterol; EXT 10–standard chow + 1% cholesterol + cornelian cherry extract 10 mg/kg b.w.; EXT 50–standard chow + 1% cholesterol + cornelian cherry extract 50 mg/kg b.w.; SIMV 5–standard chow + 1% cholesterol + simvastatin 5 mg/kg b.w. Values are presented as mean ± SD. ✪ $p < 0.05$ vs. CHOL.

3.2. Expression of PPAR-α and PPAR-γ in the Aorta and LXR-α in the Liver

Feeding a cholesterol-rich diet compared to the baseline caused a decrease in the mRNA expression of PPAR-α and PPAR-γ in the aorta, while uptake of cornelian cherry extract led to an increase in the expression of both transcription factors, wherein receiving of 10 mg/kg b.w. extract changed significantly the expression of PPAR-α ($p = 0.035$) and receiving of 50 mg/kg b.w. extract changed significantly the expression of PPAR-γ ($p = 0.031$).

Using the Western blot method with bands optical density assessment, we determined the expression of another transcription factor–liver X receptor alpha–LXR-α. The cholesterol diet slightly decreased, while the consumption of cornelian cherry extract significantly induced the LXR-α expression, with a greater effect observed in the group receiving 10 mg/kg b.w ($p = 0.001$, in comparison to $p = 0.007$ in EXT 50 group), while the greatest augmentation was noted in the SIMV 5 group ($p < 0.001$) (Figure 3).

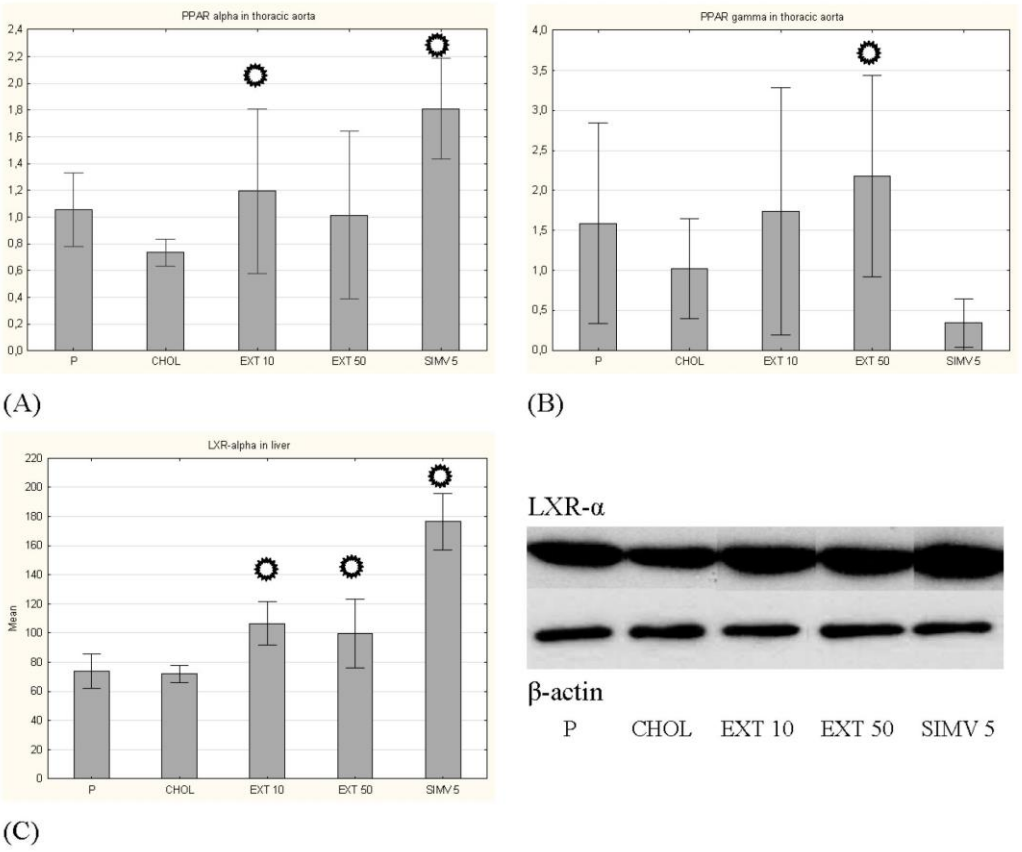


Figure 3. Expression of transcription factors. (**A**) PPAR-α in aorta, (**B**) PPAR-γ in aorta, (**C**) LXR-α in liver. P–standard chow; CHOL–standard chow + 1% cholesterol; EXT 10–standard chow + 1% cholesterol + cornelian cherry extract 10 mg/kg b.w.; EXT 50–standard chow + 1% cholesterol + cornelian cherry extract 50 mg/kg b.w.; SIMV 5–standard chow + 1% cholesterol + simvastatin 5 mg/kg b.w. Values are presented as mean ± SD. ✪ $p < 0.05$ vs. CHOL.

3.3. Adipokines Serum Levels

In the case of leptin serum level, a beneficial effect of cornelian cherry extract was observed in both dosing regimens—in both cases, changes were statistically significant (respectively $p = 0.011$ and $p = 0.003$). On the other hand, in the measurement of adiponectin, the beneficial effect of the extract was also observed for both dosing regimens, but only for the amount of 50 mg/kg b.w. the changes were statistically significant ($p = 0.027$). The positive effect of cornelian cherry extract on the serum level of resistin was also confirmed. Administration in the amount of 10 mg/kg b.w. caused a significant change ($p = 0.032$) in the level of resistin compared to the CHOL group (Figure 4).

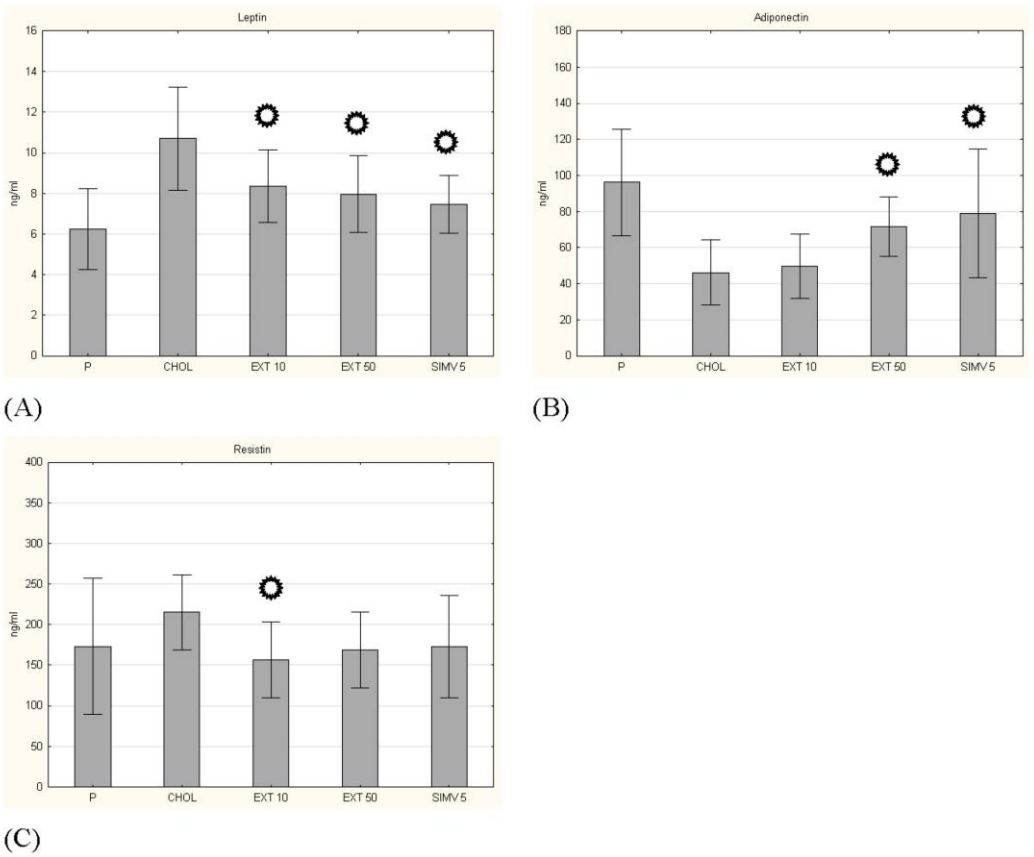


Figure 4. Concentrations of adipokines. (**A**) leptin, (**B**) adiponectin and (**C**) resistin. P–standard chow; CHOL–standard chow + 1% cholesterol; EXT 10–standard chow + 1% cholesterol + cornelian cherry extract 10 mg/kg b.w.; EXT 50–standard chow + 1% cholesterol + cornelian cherry extract 50 mg/kg b.w.; SIMV 5–standard chow + 1% cholesterol + simvastatin 5 mg/kg b.w. Values are presented as mean ± SD. ○ $p < 0.05$ vs. CHOL.

3.4. Apolipoproteins, Glucose and Insulin Levels

Other investigated parameters were the levels of selected apolipoproteins, i.e., protein fragments of lipoproteins that are responsible for lipid binding. The results for apolipoprotein A1, B100 and E were determined. In the case of Apo A1 and B100, we observed a certain favorable tendency caused by the oral intake of cornelian cherry extract, but the obtained changes did not turn out to be statistically significant (Figure 5). In turn, measurement of Apo E showed opposite effects of two studied doses, a slight decrease in Apo E level by 10 mg/kg b.w. dosage and a slight increase in Apo E level by 50 mg/kg b.w. dosage. Examination of glucose and insulin levels also did not show a significant result (Figure 6), similarly to the measurement of the insulin resistance index (HOMA-IR) (Table 3).

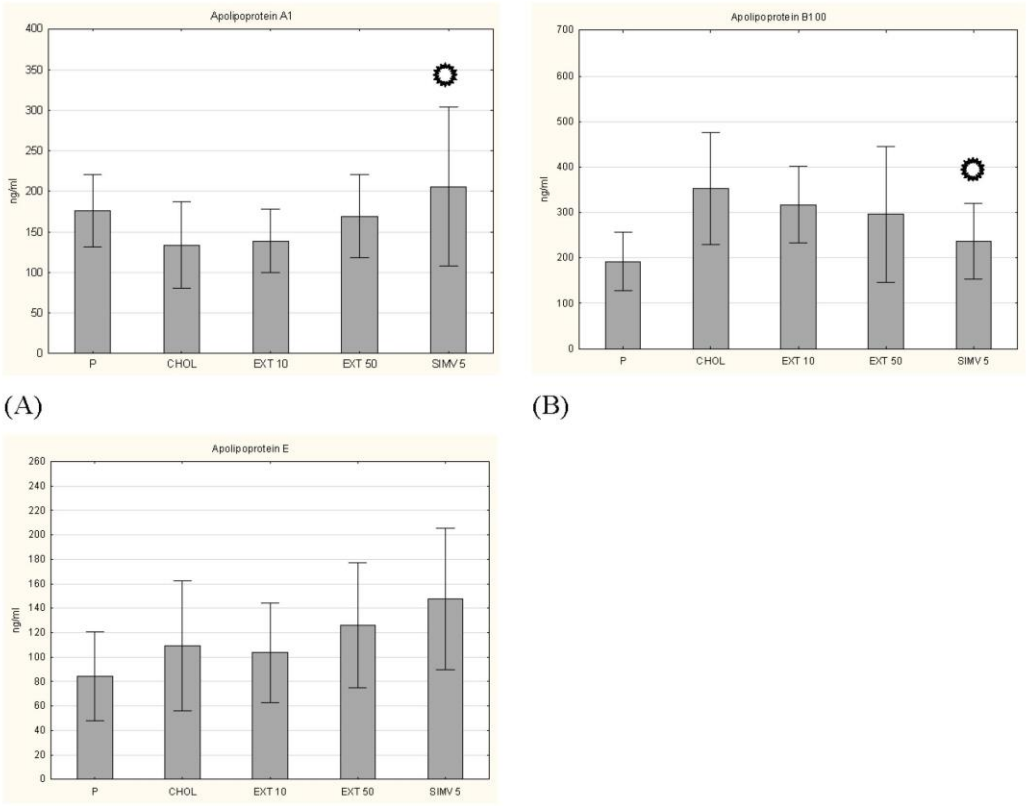
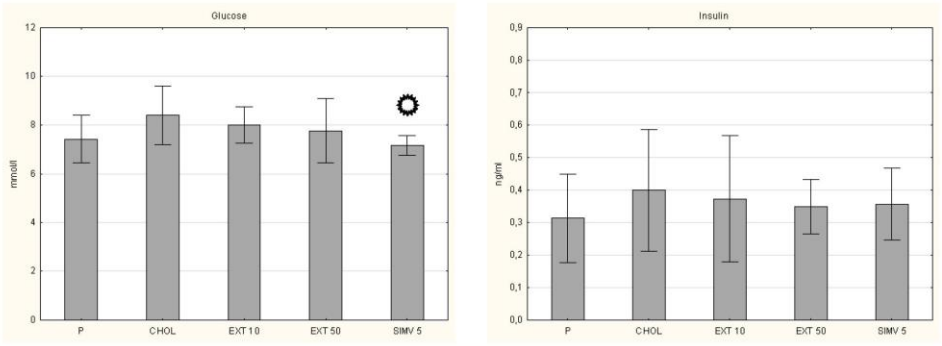


Figure 5. Concentrations of apolipoproteins. (**A**) apolipoprotein A1, (**B**) apolipoprotein B100 and (**C**) apolipoprotein E. P–standard chow; CHOL–standard chow + 1% cholesterol; EXT 10–standard chow + 1% cholesterol + cornelian cherry extract 10 mg/kg b.w.; EXT 50–standard chow + 1% cholesterol + cornelian cherry extract 50 mg/kg b.w.; SIMV 5–standard chow + 1% cholesterol + simvastatin 5 mg/kg b.w. Values are presented as mean ± SD. ✪ $p < 0.05$ vs. CHOL.

3.5. Intima, Media and I/M Ratio in the Thoracic and the Abdominal Aorta

In the histopathological examination of the thoracic aorta, favorable changes were observed in all groups fed with the assessed compounds, compared to the CHOL group, in which a considerable increase in the diameter of the artery wall was noted. In a complex approach (intima + media), a meaningful reduction in thickness was observed in all three research groups (respectively: $p = 0.001$, $p = 0.009$, and $p < 0.001$). This change particularly pertained to the intima (a significant decrease in all three groups), and to a lesser extent the media (a valuable alteration only in the EXT 10 group). In turn, the measurement of the I/M ratio indicated the EXT 50 ($p = 0.025$) and SIMV 5 ($p = 0.008$) groups as those with the most favorable changes (Figure 7). Moreover, in the histopathological examination of the abdominal aorta, in the collective approach (intima + media), an increase in the wall thickness in the CHOL group and a downward trend in the research groups (the most vivid in the SIMV 5 group–$p < 0.001$) were also observed. The alterations again were particularly visible in the intima assessment (all three groups $p < 0.001$), while in the case of the media, the changes were not statistically meaningful, and in the groups fed with the cornelian cherry extract even an increase in thickness was noted. However, the I/M ratio

in both the extract and simvastatin groups showed a significantly positive reduction in value ($p < 0.001$) (Figure 8).

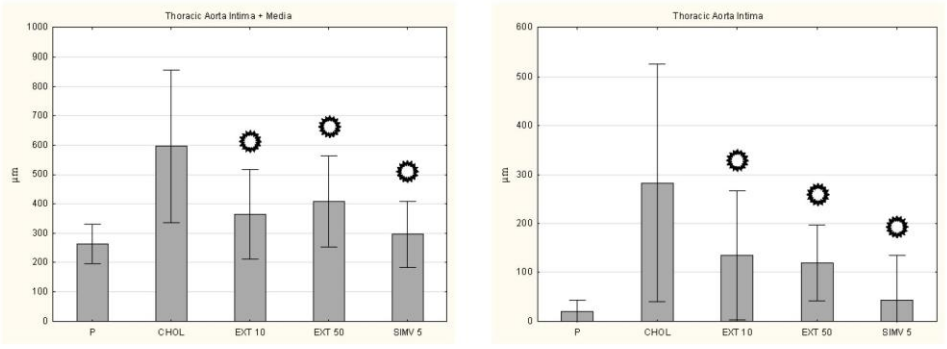


(A) (B)

Figure 6. Concentrations of (**A**) glucose and (**B**) insulin. P–standard chow; CHOL–standard chow + 1% cholesterol; EXT 10–standard chow + 1% cholesterol + cornelian cherry extract 10 mg/kg b.w.; EXT 50–standard chow + 1% cholesterol + cornelian cherry extract 50 mg/kg b.w.; SIMV 5–standard chow + 1% cholesterol + simvastatin 5 mg/kg b.w. Values are presented as mean ± SD. ✪ $p < 0.05$ vs. CHOL.

Table 3. Mean insulin resistance (HOMA-IR) index on day 60.

Group	P	CHOL	EXT 10	EXT 50	SIMV 5
HOMA-IR	2.48	3.58	3.19	2.90	2.73



(A) (B)

Figure 7. *Cont.*

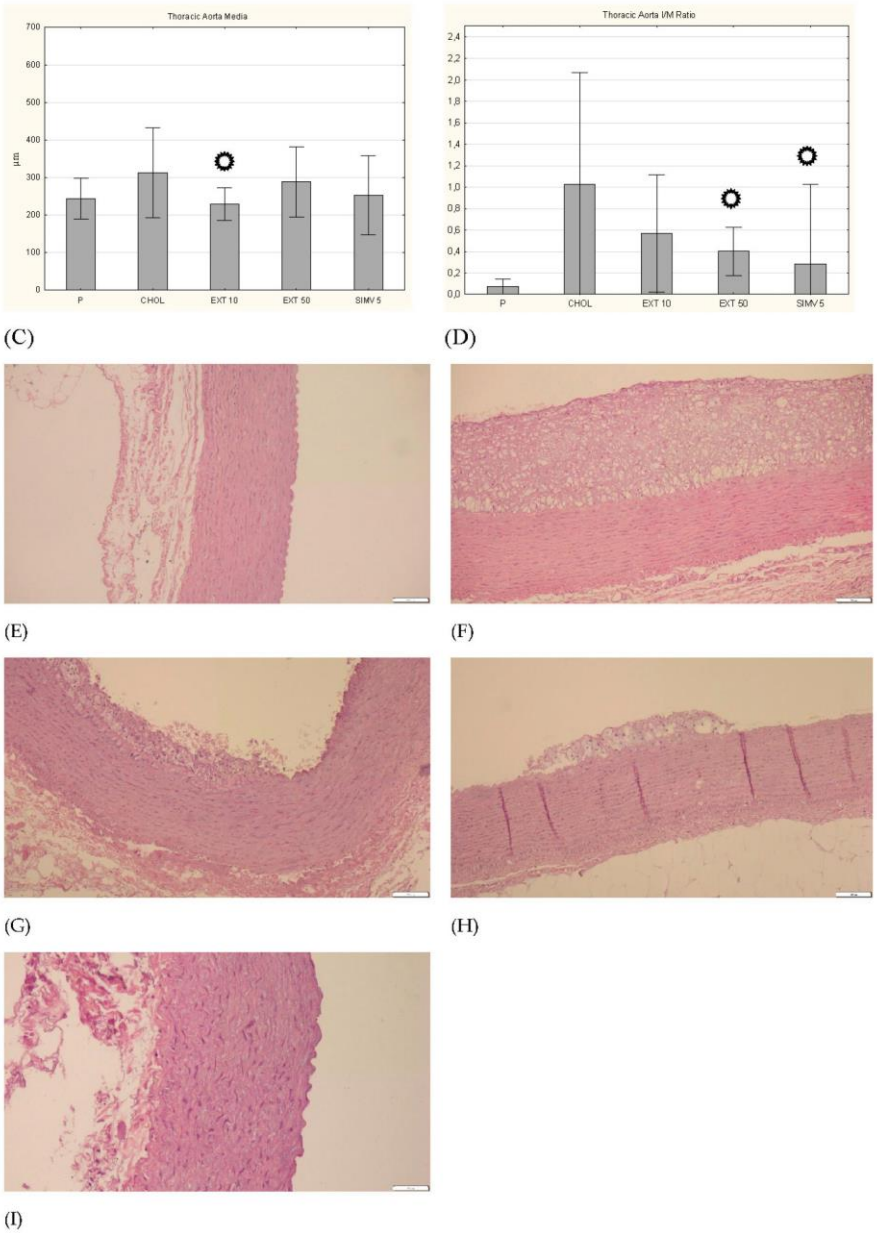


Figure 7. Intima and media thickness, and the intima/media ratio in the thoracic aorta. (**A**) intima + media thickness, (**B**) intima thickness, (**C**) media thickness, and (**D**) I/M ratio. P–standard chow; CHOL–standard chow + 1% cholesterol; EXT 10–standard chow + 1% cholesterol + cornelian cherry extract 10 mg/kg b.w.; EXT 50–standard chow + 1% cholesterol + cornelian cherry extract 50 mg/kg b.w.; SIMV 5–standard chow + 1% cholesterol + simvastatin 5 mg/kg b.w. Values are presented as mean ± SD. ○ $p < 0.05$ vs. CHOL. Representative cross-sections of the thoracic aorta segments, stained with hematoxylin-eosin in the (**E**) P group, (**F**) CHOL group, (**G**) EXT 10 group, (**H**) EXT 50 group and (**I**) SIMV 5 group.

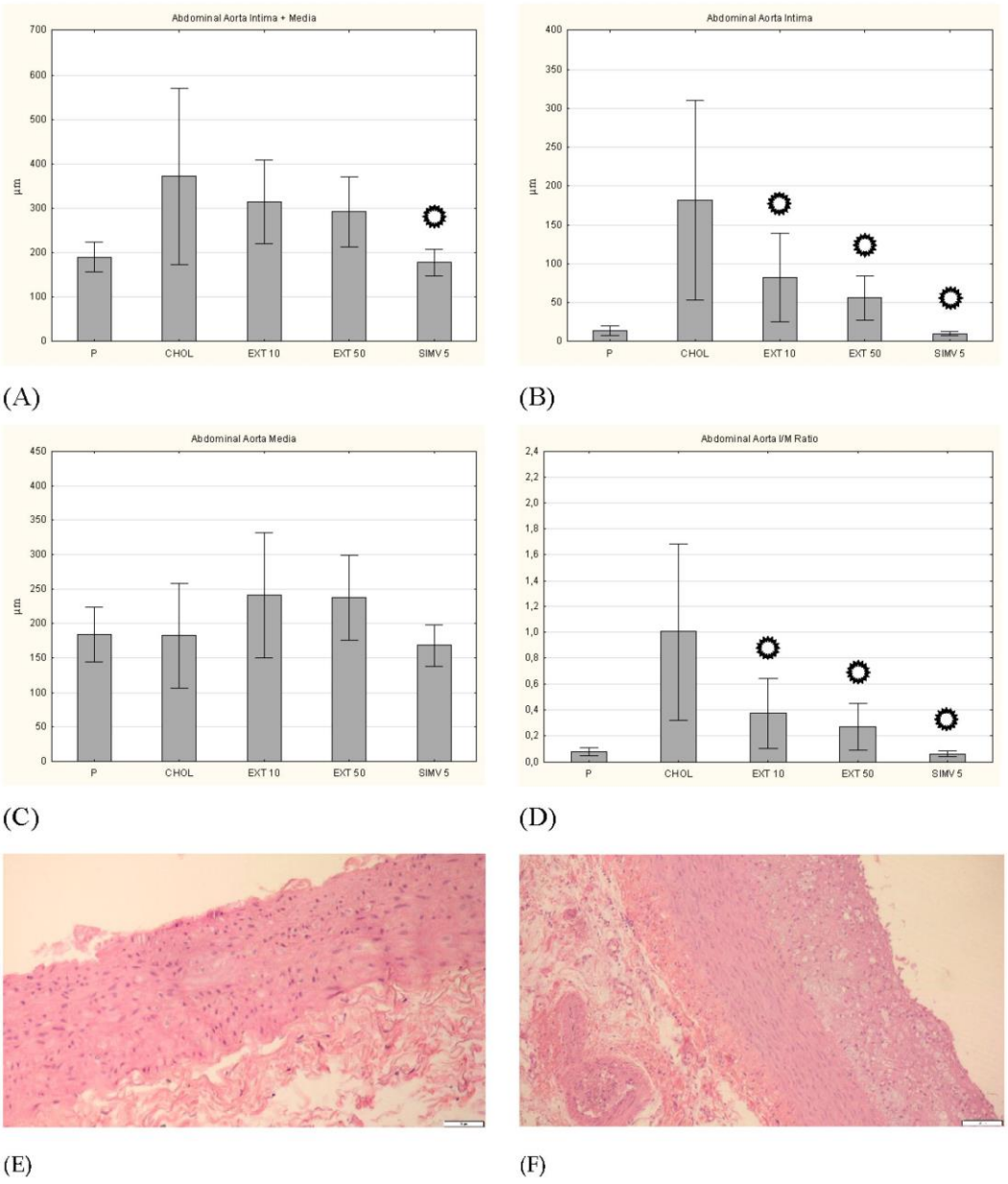


Figure 8. *Cont.*

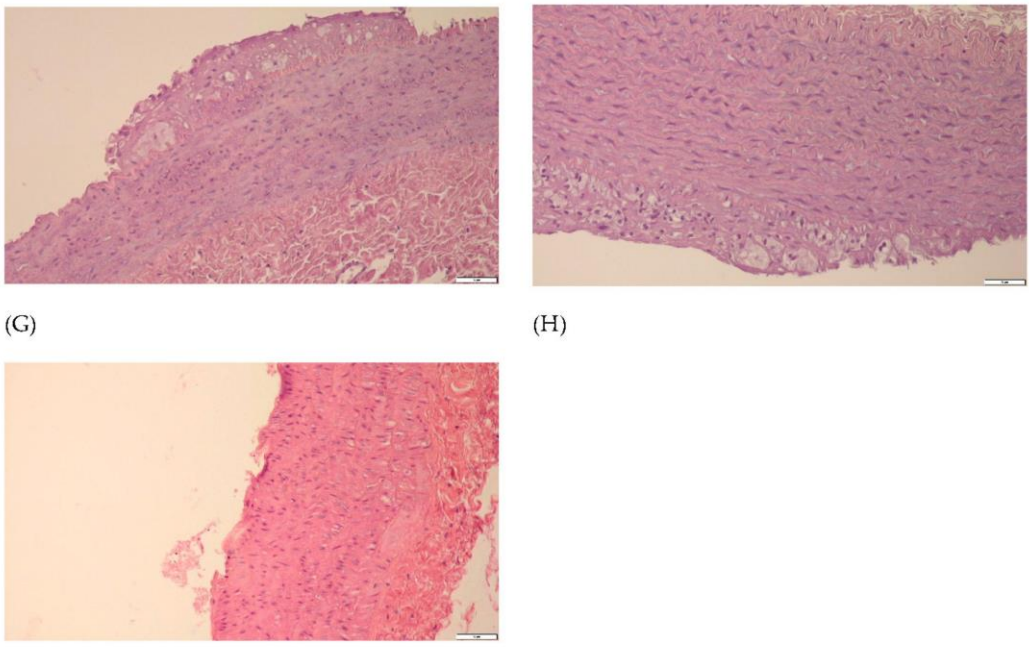


Figure 8. Intima and media thickness, and the intima/media ratio in the abdominal aorta. (**A**) intima + media thickness, (**B**) intima thickness, (**C**) media thickness, and (**D**) I/M ratio. P–standard chow; CHOL–standard chow + 1% cholesterol; EXT 10–standard chow + 1% cholesterol + cornelian cherry extract 10 mg/kg b.w.; EXT 50–standard chow + 1% cholesterol + cornelian cherry extract 50 mg/kg b.w.; SIMV 5–standard chow + 1% cholesterol + simvastatin 5 mg/kg b.w. Values are presented as mean ± SD. ○ $p < 0.05$ vs. CHOL. Representative cross-sections of the abdominal aorta segments, stained with hematoxylin-eosin in the (**E**) P group, (**F**) CHOL group, (**G**) EXT 10 group, (**H**) EXT 50 group and (**I**) SIMV 5 group.

4. Discussion

The main conclusion of our study is that oral administration of resin-purified cornelian cherry extract has a positive effect on factors that may contribute to the development of atherosclerosis-related cardiovascular disorders and these effects are at least partially evoked by changes in the expression of transcription factors in the aorta and liver. The key findings are that cornelian cherry extract increases expression of PPAR-α and PPAR-γ in the aorta, and LXR-α expression in hepatocytes. It also decreases the serum levels of leptin, resistin and triglycerides, concomitantly enhancing the level of adiponectin. Moreover, the extract has a positive impact on histopathological changes in the thoracic and abdominal aorta, reducing the I/M ratio. All the observed effects contribute to the limitation of the occurrence of functional and metabolic disorders, which may result in cardiovascular diseases such as atherosclerosis or metabolic syndrome.

As the greatest limitation of our work, we consider the lack of assessment of the extract's influence on the activity of particularly important target genes for the tested transcription factors. In addition, an increased incidence of cardiovascular diseases and metabolic syndrome is often associated with the Western diet, which apart from being rich in saturated fat and cholesterol, is also characterized by excess carbohydrates and low amounts of fiber. Therefore, the model of animal feeding adopted in our study may not have an unequivocal transfer to the conditions of an actual human diet. Nevertheless, the high-cholesterol feeding allowed us to study the effect of one isolated harmful factor and

the possible protective effect of a diet rich in anthocyanins and iridoids as well as provide a basis for further investigations.

Obesity is a condition conducive to the development of diseases of the cardiovascular system. Furthermore, obesity is also associated with an increased risk of all-cause and CVD mortality. Weight reduction is one of the first recommendations and the most important steps to reduce that risk [27,28]. The anthocyanins and iridoids contained in cornelian cherry extract may limit weight gain in a cholesterol-rich diet. Although during the study we observed an increase in body weight in each of the research groups, the gain was to some extent smaller in the EXT 10 and EXT 50 groups. This effect was especially noticeable in the group receiving the higher dose of the extract, where the weight increase was lower by almost 24% compared to the weight increase in the group fed with a cholesterol diet. Weight loss or reduction of weight gain as an effect of iridoids [29–32] and anthocyanins [33–36] were also observed in other research models. This also applied to models in which compounds from active extract groups were tested, e.g., loganic acid [37], cyanidin [38] or pelargonidin [39]. What distinguishes our research model from quoted studies, is that it was conducted not on commonly tested rats or mice, but on rabbits–nonrodents that share much more similar lipid metabolic pathways with humans. This may confirm that the positive effect of a diet rich in iridoids and anthocyanins may limit weight gain regardless of species, thereby demonstrating potential efficacy in humans.

Determination of serum lipid levels showed particularly favorable changes in the case of triglycerides. Both in the EXT 10 and EXT 50 groups, there was a statistically significant reduction in the level of TGs compared to the CHOL group. This is highly likely related to the enhanced expression of PPAR-α and PPAR-γ–also demonstrated in our previous studies in the liver [4,22], which activate genes responsible, inter alia, for lipolysis of triglycerides to free fatty acids [40,41]. The elevated concentration of adiponectin in the extract groups may also contribute to this. In turn, in the case of cholesterol levels, both in TC, HDL-C and LDL-C fractions, a certain positive effect of the extract was observed (lowering TC and LDL-C, increasing HDL-C levels), but these changes were relatively small, lesser than observed in the positive control group receiving simvastatin. Other publications reported a more significant effect of iridoids and anthocyanins on plasma/serum cholesterol levels [42–47]. However, the qualitative composition of iridoids and anthocyanins in mentioned research models, as well as laboratory animal selection, differed from the composition of the cornelian cherry extract and our animal model.

There are five known transcription factors, that play a crucial role in lipid and cholesterol metabolism: liver X receptor-α, peroxisome proliferator-activated receptor-α, peroxisome proliferator-activated receptor-γ, CCAAT/enhancer-binding protein α (C/EBPα), and sterol regulatory element-binding protein 1c (SREBP-1c) [48]. We assessed the expression levels of PPAR-α and PPAR-γ mRNA in the aorta and LXR-α in hepatocytes.

In both groups fed with the extract, we observed an enhancement in the expression of PPAR-α and PPAR-γ. Interestingly, the increase in expression differed from the dose of the extract used–a dose of 10 mg/kg b.w. caused a greater increase in PPAR-α expression, while the dose of 50 mg/kg b.w. in PPAR-γ. While the increase in PPAR-α expression can rather be evidently associated with a positive effect on cholesterol and lipid metabolism, the effect of PPAR-γ is not so unequivocal. PPAR-α is responsible for the catabolism and intracellular transformations of lipids, through the regulation of the transcription of multiplicitous genes, including acyl-CoA-oxidase, carnitine palmitoyl transferase (CPT) and several CYP4As. PPAR-α is closely related with fatty acid β-oxidation in the peroxisomes and mitochondria, and ω-oxidation in the microsomes. It was also proven that it modulates both acute and chronic inflammation [49–53]. In turn, PPAR-γ plays an important role in the differentiation and maturation of adipocytes, promotes fat storage in white adipose tissue and increases the tissues' sensitivity to insulin, which leads to a decrease in extracellular glucose levels [49,52,54]. PPAR-γ lowers the transcription of, among others, adipocyte fatty acid-binding protein (aP2), which is responsible for the intracellular binding of fatty acids [55] and lipoprotein lipase (LPL), which regulates the hydrolysis of

triglycerides [56,57]. It also enhances the transcription of i.a. fatty acid transporter (FATP), which adjusts transport of fatty acids inside the cells [58], fatty acid-binding protein (L-FABP), which participates in adipogenesis, transport and storage of fatty acids [59]. At the same time, PPAR-γ increases transcription of Acyl-CoA synthetase (lipogenesis and/or catabolism of lipids) [60], ATP-binding cassette G1 (ABCG1), which regulates the transport of cholesterol and phospholipids to macrophages [61], ATP-binding cassette transporter A1 (ABCA1) [62], and also adipocyte-related complement protein 30 (Acrp30), which decrease the concentration of glucose, triclycerides and free fatty acids [40]. Therefore, an enhancement of PPAR-γ expression can cause both positive and negative changes—it all depends on the expression of which of the target genes is activated. In our study, the increase in PPAR-γ expression was accompanied by a mitigation of the intensity of intima/media adverse changes, so it can be associated with a positive vascular effect. We observed a significant reduction in intima diameter in both the thoracic and abdominal aorta. This also translated into favorable I/M ratio results. We can thus hypothesize that the increase in both investigated PPARs expression in the aorta may contribute to limited arterial wall thickening observed in the course of atherosclerosis.

For LXR-α expression, it was the 10 mg/kg b.w. dose that caused a bigger augmentation of expression. This may suggest that a proper dose selection is particularly crucial in achieving the potential full therapeutic effect of the cornelian cherry extract, also in humans. Liver X receptor-α reverses transportation of cholesterol to peripheral tissues and transports the excess cholesterol into the liver, reduces cholesterol absorption by intestinal epithelial cells and directly induces the expression of the essential transcription factor for lipid and cholesterol synthesis in the liver, SREBP-1c [48]. One of the most pivotal target LXR-α genes is ATP-binding cassette transporter A1. LXR-α expression enhance leads to a robust upregulation of ABCA1 in macrophages, intestine, and in the liver and the efflux of cholesterol to apolipoprotein A1 and apolipoprotein E, which results in the formation of high-density lipoproteins (HDL-C) [22,63]. Noteworthy, in the CHOL group there was no increase in LXR-α expression, and even a slight decrease was noted. It seems that the most presumable hypothesis explaining the lack of change in LXR-α expression is that the study period was too brief to observe important alterations. However, since significant changes in LXR-α expression were noted in the test groups, it can be hypothesized that the active compounds contained in cornelian cherry extract activate LXR-α expression in the liver, which has a positive effect on the metabolism of cholesterol.

There are previous reports describing the impact of iridoids and anthocyanins on the expression of transcription factors crucial for cholesterol and lipid homeostasis [48,64]. However, none of these studies concerned the *Cornus mas* L. extract. Moreover, none of the iridoid or anthocyanin surveys have evaluated the changes of PPARs expression in the aorta and their consequences. Considering the results we have obtained, it seems that potentially greater efficacy of the lower dose is a positive result, as it would be more practical for technological development in the form of a drug, e.g., a tablet, which, due to a lesser dose of the active substance, could be smaller and easier for administering. For example: using the formula for dose translation based on BSA [65], calculated dose for adult humans weighing 70 kgs would amount to 227 mg (3.24 mg/kg b.w.), which could be easily compressed in the form of one tablet. As the results of our research are promising, further studies, both determining the dose-effect dependency and conducted in the human model, are necessary.

The adipose tissue secretes several mediators, called adipokines (e.g., adiponectin, leptin, and resistin). Adipokines play an important regulatory function in various metabolic processes and are involved in the development of metabolic diseases, such as type 2 diabetes and cardiovascular diseases. High levels of leptin and resistin occurring in obese individuals, lead to the emergence of insulin resistance, whereas adiponectin may prevent it [66,67]. Leptin is produced and secreted by the adipose tissue in direct relation to the amount of body fat [68]. Circulating leptin positively reflects adipose tissue size, and communicates energy storage status to the brain [69]. We observed a significant reduction

of leptin levels in all research groups. The lowering effect of anthocyanins on leptin concentrations was confirmed in many studies [70–74]. Fewer data are available on iridoids. Ishaq et al. hypothesize that asperuloside–iridoid glycoside found in many traditional Chinese medicinal plants–downregulates the gene expression of appetite regulators, i.a. leptin, in the hypothalamus. It overstimulates taste buds signaling in response to high-fat diet consumption leading to suppression of orexigenic signaling [32]. Contrarily, in the case of loganin, an iridoid glycoside isolated from *Cornus officinalis*, an elevation of leptin serum level in type 2 diabetic db/db mice was observed [75].

We noted a significant diminution in resistin level in EXT 10 group, in EXT 50 group the effect was lesser. This is in line with our previous findings that a lower dose of the extract could potentially prove to be more effective. There are not many sources describing the effect of the tested compounds on the level of resistin. The reduction of its level in the case of an anthocyanin-rich diet was observed in the rat [67] and mouse model [72]. However, we have not found a study evaluating this effect for iridoids. Resistin was shown to play a pivotal role in various metabolic, inflammatory, and autoimmune diseases [76]. Increased resistin levels are associated with the pathogenesis of obesity-associated insulin resistance and exert pro-inflammatory effects [77,78]. Resistin also appears to mediate the pathogenesis of atherosclerosis by promoting endothelial dysfunction, vascular smooth muscle cell proliferation, arterial inflammation, and the formation of foam cells [79]. Therefore, we can hypothesize that a decrease in the level of resistin together with an increase in PPAR-α and -γ expression translates into the positive effects we observed, limiting adverse changes in the aorta.

Measurement of adiponectin concentration showed the opposite dose–response result. Although the smaller dose augmented the adiponectin level compared to the CHOL group, a significantly beneficial effect of the 50 mg/kg b.w. dose was observed. An enhancement in adiponectin levels was also noted in other studies, evaluating among others asperuloside [80], cyanidin-3-*O*-β-glucoside [81], an anthocyanin-rich tart cherry extract comprised mainly of cyanidin glycosides [72] and cornelian cherry extract administered to humans, but only with total anthocyanin-base standardization [82]. An increase in adiponectin quantity is a desirable outcome since adiponectin is important homeostatic factor regulating glucose levels, lipid metabolism, and insulin sensitivity through its anti-inflammatory, antifibrotic, and antioxidant effects via two adiponectin receptors, AdipoR1 and AdipoR2 [83]. Circulating adiponectin levels are reduced in obese individuals, and it was proposed to have a crucial role in the pathogenesis of atherosclerosis and cardiovascular diseases associated with obesity and metabolic syndrome. Moreover, adiponectin concentration was found to be correlated with lipoprotein metabolism, especially the high-density lipoproteins and triglycerides. Adiponectin appears to increase HDL-C and decrease TGs levels, among others through activating ATP-binding cassette transporter A1 and lipoprotein lipase and decreasing hepatic lipase [84].

Despite our promising results for adipokines and transcription factors expression, we did not obtain statistically significant outcomes for both glucose and insulin levels. Similarly, the insulin resistance index (HOMA-IR) showed an observable positive trend, but the changes were still in the over normative range. The above findings may hail from species differences resulting from the applicated rabbit model and feeding pattern. However, since the decrease in the value of the HOMA-IR index was, at least in two tested doses, dose-dependent and increasing with its enhancement, it can be hypothesized that the use of a higher dose of the extract may have a more beneficial effect. Although a noticeable increase in HOMA-IR was observed in the CHOL compared to the P group, the feeding schema used (diet enriched with 1% cholesterol) probably also slightly limited the observed effects, because it did not take into account the high level of carbohydrates characteristic for Western diet. The same tendency (dose-dependency) was observed in the case of apolipoproteins, and determination of their levels also requires further research, with various diet models as well as the higher dose or prolonged application of the extract.

The overall positive effect of cornelian cherry extract on detrimental changes induced by a cholesterol-rich diet was confirmed by a decrease in intima and media thickness and the intima/media ratio in the thoracic and abdominal aorta observed in both groups (higher efficacy in the EXT 50 group). Thickening of the arterial wall, especially of the intima layer, is regarded as a risk-marker of atherosclerosis and is described as one of the earliest vascular pathologies observed in microscopic assessment during atherogenesis [85]. Comparable outcomes were obtained by Sozański et al. in another *Cornus mas* fruits study [4,86].

In the end, it is worth noting that in the course of our study we did not observe any adverse effects of cornelian cherry extract administration, regardless of the dose. Therefore, future determinations, including elongated administration or, feasibly, higher doses of the extract, promise a valuable application also in terms of its safety.

5. Conclusions

Oral administration of resin-purified cornelian cherry extract showed a diversified contribution to the regression of pathological changes induced by a cholesterol-rich diet in the rabbit model. The positive effects were mainly caused by the increased expression of PPAR-α and PPAR-γ in the aorta and LXR-α expression in hepatocytes, as well as a significant impact on the concentration of adipokines—a decrease in the level of leptin and resistin, and an increase in the level of adiponectin. Additionally, a reduction in the thickness of the thoracic and abdominal aorta walls was observed, and a diminution of the I/M ratio. Our study, to our knowledge, for the first time tested the influence of iridoids and anthocyanins extract on PPARs expression in the aorta, and the rabbit model stood out among the popularly used rats and mice models. The results we have obtained confirm the potential contribution of cornelian cherry extract to mitigate the risk of developing and the intensity of symptoms of obesity-related cardiovascular diseases and metabolic disorders such as atherosclerosis or metabolic syndrome. Thus, cornelian cherry extract may become a valuable resource for reducing the adverse effects caused by the Western diet and constitute a promising pharmaceutical agent. However, further research, especially in humans, is required to fully determine the properties of the extract.

Author Contributions: Conceptualization, M.D. and T.S.; methodology, M.D. and T.S.; investigation, M.D., A.Z.K., A.R., A.G., S.D. and T.S.; resources, A.Z.K. and N.P.; statistical analysis, M.D.; visualization, M.D., A.Z.K., A.R. and S.D.; supervision, M.D. and T.S.; writing—original draft preparation, M.D. and T.S.; writing—review and editing, M.D., A.Z.K., A.M., A.R., A.G., S.D., P.D., B.N., M.T., J.M., N.P., A.S. and T.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data underlying this article will be shared upon request to the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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VIII. Podsumowanie i wnioski

W niniejszej rozprawie przeanalizowano dane literaturowe dotyczące wpływu irydoidów i antocyjanów na funkcje układu sercowo-naczyniowego i wątroby oraz na ekspresję czynników transkrypcyjnych istotnych dla metabolizmu lipidów i cholesterolu, a także oceniono wpływ dwóch dawek ekstraktu z owoców derenia jadalnego na wybrane czynniki wpływające na ryzyko powikłań sercowo-naczyniowych w modelu króliczym. W części doświadczalnej, zwierzęta podzielono w sposób randomizowany na 5 grup badawczych: grupę P – otrzymującą standardową karmę; grupę CHOL – otrzymującą standardową karmę + 1% cholesterol; grupę EXT 10 – otrzymującą standardową karmę + 1% cholesterol + ekstrakt z owoców derenia jadalnego 10 mg/kg m.c.; grupę EXT 50 – otrzymującą standardową karmę + 1% cholesterol + ekstrakt z owoców derenia jadalnego 50 mg/kg m.c. oraz grupę SIMV 5 – otrzymującą standardową karmę + 1% cholesterol + simwastatynę 5 mg/kg m.c. Ocenie poddano wpływ ekstraktu z owoców derenia jadalnego na ekspresję PPAR-α i PPAR-γ w aorcie i LXR-α w wątrobie, stężenia cholesterolu (całkowitego, HDL, LDL), trójglicerydów, adipokin (adiponektyny, leptyny, rezystyny), apolipoprotein (A1, B100, E), glukozy i insuliny, a także na zmiany histologiczne w ścianach aorty piersiowej i brzusznej.

Ekstrakt z owoców derenia został wybrany jako materiał badawczy, bowiem owoce derenia jadalnego zawierają stosunkowo duże ilości związków czynnych z grup irydoidów i antocyjanów. Dokonany przegląd dostępnej w tym temacie literatury potwierdził, że związki z grup irydoidów i antocyjanów posiadają bardzo szeroki terapeutyczny wpływ na funkcje układu sercowo-naczyniowego i wątroby, i mogą być efektywnie wykorzystane w leczeniu ich stanów patologicznych. W tym kontekście opisano dotychczas m.in. działanie hipotensyjne, hipoglikemiczne, hipolipemiczne, przeciwzapalne i hepatoprotekcyjne irydoidów i antocyjanów. Prozdrowotne działanie tych związków wynika głównie z ich właściwości antyoksydacyjnych. Działanie to jest zarówno bezpośrednie, m.in. poprzez eliminację reaktywnych form tlenu, jak i pośrednie, poprzez stymulację systemów antyoksydacyjnych organizmu.

Wielokierunkowy, pozytywny wpływ irydoidów na czynniki sprzyjające zaburzeniom funkcji wątroby i układu krążenia, m.in. na uwalnianie prozapalnych cytokin, stężenia i aktywność niektórych enzymów i białek (np. ALT, AST, AMPK – *AMP-activated protein kinase*, ACC – *Acetyl-CoA carboxylase*, VCAM-1 – *vascular cell adhesion molecule-1*, ICAM-1 – *intercellular adhesion molecule-1*) czy też metabolizm kwasów żółciowych, wolnych kwasów tłuszczowych i glukozy, pozwala na rozpatrywanie ich jako wartościowej opcji terapeutycznej w leczeniu m.in. alkoholowego i niealkoholowego stłuszczenia wątroby, cholestazy, zwłóknienia

wątroby, miażdżycy czy choroby wieńcowej. Podobnie jest w przypadku antocyjanów, które m.in. zmniejszają aktywność reduktazy 3-hydroksy-3-metyloglutarylokoenzymu A (reduktazy HMG-CoA, *HMG-CoA reductase),* hamują białko transportujące estry cholesterolu (CETP, *cholesteryl ester transfer protein*) oraz wpływają na stężenia niektórych apolipoprotein i cytokin.

Związki czynne z grup irydoidów i antocyjanów wpływają także w sposób istotny na ekspresję czynników transkrypcyjnych odgrywających kluczową rolę w metabolizmie lipidów i cholesterolu, tj. LXR, PPAR-α i -γ, SREBP i C/EBP. Potwierdzono, że mogą być one bezpośrednimi ligandami tych receptorów, w większości przypadków hamując główne adipogeniczne i lipogenne czynniki transkrypcyjne (SREBP i C/EBP) oraz aktywując te o przeciwnym działaniu (LXR i PPAR-α). W przypadku PPAR-γ wpływ irydoidów i antocyjanów w różnych modelach jest bardzo zróżnicowany, a jednoznaczne określenie roli tego czynnika w patogenezie chorób układu sercowo-naczyniowego i wątroby nie jest proste, bowiem pobudzenie tego receptora z jednej strony może przyczyniać się do ograniczenia stanu zapalnego, ale z drugiej strony stymulować lipogenezę.

W przeprowadzonym doświadczeniu stwierdzono pozytywny wpływ podawania ekstraktu z owoców derenia jadalnego na ekspresję badanych czynników transkrypcyjnych, stężenia wybranych markerów surowicy krwi oraz zmiany histologiczne w ścianach aorty piersiowej i brzusznej. Chociaż w przebiegu doświadczenia zaobserwowano wzrost masy ciała w każdej z badanych grup, to jednak wzrost ten był do pewnego stopnia mniejszy w obu grupach przyjmujących wyciąg, a w szczególności w grupie EXT 50, gdzie zaobserwowano redukcję przyrostu masy ciała o prawie 24% w porównaniu do grupy CHOL.

W obu grupach otrzymujących ekstrakt zaobserwowano wzrost ekspresji PPAR-α i PPAR-γ. Co ciekawe, zwiększenie ekspresji różniło się w zależności od użytej dawki ekstraktu, bowiem dawka 10 mg/kg m.c. spowodowała większy wzrost w przypadku PPAR-α, natomiast dawka 50 mg/kg m.c. w przypadku PPAR-γ. Z kolei, w kontekście receptora LXR-α, to niższa dawka wywołała bardziej znaczący wzrost jego ekspresji. Warto odnotować, że w grupie CHOL nie tyle nie zaobserwowano zwiększenia ekspresji LXR-α, choć liczba receptorów LXR-α jest zależna od poziomu cholesterolu, co nawet lekkie jej zmniejszenie. Prawdopodobną przyczyną takiego rezultatu mógł być krótki okres eksperymentu. Ponieważ jednak odnotowano istotne zmiany w ekspresji LXR-α w grupach badawczych, można hipotetyzować, że irydoidy i antocyjany zawarte w ekstrakcie aktywowały ekspresję LXR-α w wątrobie, co przełożyło się na pozytywne efekty przyjmowania ekstraktu.

Oddziaływanie związków czynnych z grup irydoidów i antocyjanów na ekspresję powyższych czynników transkrypcyjnych było opisane w innych publikacjach naukowych,

jednak żadne z wcześniej przeprowadzonych doświadczeń nie dotyczyło ekstraktu z owoców derenia jadalnego. Co więcej, żadne z opisanych badań nie oceniało zmian ekspresji PPAR w aorcie i efektów, jakie te zmiany wywołują.

W ocenie stężenia lipidów w surowicy, szczególnie korzystną zmianę odnotowano w przypadku poziomu trójglicerydów. Zarówno w grupie EXT 10, jak i EXT 50, zaobserwowano statystycznie istotną redukcję poziomu TG. Zmiana ta wynikała najprawdopodobniej ze zwiększenia ekspresji PPAR-α i PPAR-γ oraz stężenia adiponektyny, które zaobserwowano w grupach otrzymujących ekstrakt. Natomiast w przypadku cholesterolu modyfikacje stężenia okazały się relatywnie niewielkie, ale dało się zauważyć pozytywny trend, np. obniżenie stężenia frakcji LDL i podwyższenie HDL.

Tkanka tłuszczowa wydziela mediatory zwane adipokinami (np. adiponektynę, leptynę i rezystynę), które odgrywają ważną rolę regulatorową wielu przemian metabolicznych lipidów i węglowodanów, w tym pełnią istotną rolę w rozwoju insulinooporności. Zaburzenia poziomu adipokin są obserwowane w przebiegu wielu chorób związanych z nieprawidłową dietą, np. otyłości i cukrzycy typu II. Doustne przyjmowanie ekstraktu z owoców derenia spowodowało znaczące, korzystne zmiany stężeń trzech oznaczanych adipokin, tj. redukcję stężenia leptyny (porównywalny efekt w obu grupach EXT) i rezystyny (większy w EXT 10) oraz wzrost stężenia adiponektyny (większy w EXT 50). Warto zaznaczyć również, że o ile istnieje co najmniej kilka publikacji opisujących wpływ antocyjanów na poziom adipokin, to źródeł opisujących wpływ irydoidów jest niewiele, a autorowi nie udało się wyszukać żadnej publikacji odnoszącej się do oddziaływania irydoidów na stężenie rezystyny.

Nie odnotowano natomiast istotnych statystycznie różnic w kontekście wyników badanych apolipoprotein (A1, B100, E), a także stężeń glukozy i insuliny. Tym samym, także wyniki wskaźnika insulinooporności (HOMA-IR) pozwoliły jedynie na zobrazowanie zauważalnej pozytywnej tendencji, nie mniej jednak wszystkie zmiany utrzymywały się ciągle w zakresie wartości ponadnormatywnych. Na powyższe wyniki wpływ mógł mieć szereg różnorakich czynników, np. wybór zwierzęcia laboratoryjnego, długość doświadczenia czy przede wszystkim schemat karmienia, czyli zastosowanie diety wzbogaconej o 1% cholesterol, co nie odzwierciedla charakterystycznego dla zachodniej diety wysokiego poziomu węglowodanów.

W niniejszym badaniu, zwiększeniu ekspresji receptorów PPAR-α i PPAR-γ w aorcie towarzyszyło zmniejszenie intensywności niekorzystnych zmian warstw intima i media. Zaobserwowano znaczącą redukcję średnicy intima zarówno w przypadku aorty piersiowej, jak i aorty brzusznej. Przełożyło się to także na zmniejszenie wskaźnika I/M, przy czym zmiana

ta była większa w przypadku grupy EXT 50. Zgrubienie ściany aorty, szczególnie warstwy intima, jest uważane za jeden z ważniejszych markerów ryzyka miażdżycy i jest opisywane jako jedno z najwcześniejszych zaburzeń obserwowanych w ocenie mikroskopowej zmian arteriosklerotycznych. Tym samym wyciąg z owoców derenia może okazać się potencjalnie skutecznym środkiem farmakologicznym, który można wykorzystać do zapobiegania zmianom miażdżycowym.

Uwzględniając przedstawione powyżej wyniki, można wyciągnąć następujące wnioski:

Doustne przyjmowanie ekstraktu z owoców derenia jadalnego:

1. Ze względu na zawartość związków czynnych z grup irydoidów i antocyjanów, ma pozytywny wpływ na czynniki, które mogą przyczyniać się do rozwoju związanych z nieprawidłową dietą i otyłością chorób układu sercowo-naczyniowego i wątroby.
2. Sprzyja ograniczeniu przyrostu masy ciała spowodowanego dietą obfitującą w cholesterol.
3. Powoduje zwiększenie ekspresji czynników transkrypcyjnych PPAR-α i PPAR-γ w aorcie i LXR-α w wątrobie.
4. Przyczynia się do korzystnych zmian stężenia niektórych parametrów surowicy krwi, przede wszystkim adipokin i trójglicerydów.
5. Prowadzi do ograniczenia przyrostu grubości ścian aorty piersiowej i aorty brzusznej, zmniejszając jednocześnie wskaźnik I/M.
6. Nie wywołuje istotnych zmian w stężeniach apolipoprotein, cholesterolu, glukozy i insuliny, przynajmniej w zastosowanych dawkach.
7. W obu zastosowanych dawkach nie wywołuje żadnych działań niepożądanych, przynajmniej w 60-dniowym okresie stosowania.
8. Po przeliczeniu dawek na właściwe dla dorosłych ludzi (korzystając ze wzoru na przeliczenie dawek opartego na BSA), pozwala na proste technologicznie przygotowanie odpowiedniej dawki ekstraktu w formie gotowego leku, np. jednej tabletki.
9. Może stanowić skuteczną i bezpieczną opcję farmakoterapeutyczną w profilaktyce i leczeniu niektórych chorób układu sercowo-naczyniowego i wątroby, np. miażdżycy czy zespołu metabolicznego.
10. Wymaga przeprowadzenia badań klinicznych, bowiem choć model króliczy jest bliższy ludzkiemu niż popularnie stosowane modele szczurze czy mysie, daje jedynie obiecujące przesłanki, a nie miarodajne wyniki reakcji organizmu ludzkiego na doustne przyjmowanie ekstraktu z owoców derenia.

IX. Piśmiennictwo

1. Narodowe Centrum Edukacji Żywieniowej. Jarosz M. Piramida Zdrowego Żywienia i Aktywności Fizycznej dla osób dorosłych. https://ncez.pzh.gov.pl/abc-zywienia/zasady-zdrowego-zywienia/piramida-zdrowego-zywienia-i-aktywnosci-fizycznej-dla-osob-doroslych-2/. Dostęp: 20 kwietnia 2022.
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Oświadczam, że w pracy *„Danielewski M, Matuszewska A, Nowak B, Kucharska AZ, Sozański T. The Effects of Natural Iridoids and Anthocyanins on Selected Parameters of Liver and Cardiovascular System Functions. Oxid Med Cell Longev. 2020; 2020: 2735790”* mój udział polegał na krytycznej ocenie manuskryptu.

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OŚWIADCZENIE

Oświadczam, że w pracy *„Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (Cornus mas L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621"* mój udział polegał na przygotowaniu oraz analizie składu ekstraktu z owoców derenia jadalnego metodą HPLC-PDA, a także przygotowaniu tekstu manuskryptu w podrozdziałach 2.2, 2.3 i 2.4, w tym ryciny 1.

Alicja Kucharska

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Oświadczam, że w pracy *„Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (Cornus mas L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621"* mój udział polegał na nadzorze nad wykonaniem analiz techniką Western Blot oraz przygotowaniu tekstu manuskryptu w podrozdziale 2.9.

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OŚWIADCZENIE

Oświadczam, że w pracy „*Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (Cornus mas L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621*" mój udział polegał na wykonaniu analiz techniką RT-PCR, a także przygotowaniu tekstu manuskryptu w podrozdziale 2.8.

Agnieszka Gomułkiewicz

Wrocław, 07.03.2022r.

dr hab. Stanisław Dzimira, prof. uczelni
Zakład Patomorfologii i Weterynarii Sądowej
Uniwersytet Przyrodniczy we Wrocławiu
ul. Cypriana Kamila Norwida 31
50-375 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy „*Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (Cornus mas L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621*" mój udział polegał na wykonaniu analizy histopatologicznej pobranego materiału i jej zobrazowaniu oraz przygotowaniu tekstu manuskryptu w podrozdziale 2.7.

Wrocław, 07.03.2022r.

prof. dr hab. Piotr Dzięgiel
Katedra Morfologii i Embriologii Człowieka
Uniwersytet Medyczny we Wrocławiu
ul. Tytusa Chałubińskiego 6a
50-368 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy *„Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (Cornus mas L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621”* mój udział polegał na rewizji finalnej wersji manuskryptu.

Wrocław, 07.03.2022r.

dr hab. Beata Nowak
Katedra i Zakład Farmakologii
Uniwersytet Medyczny we Wrocławiu
ul. Jana Mikulicza-Radeckiego 2
50-345 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy „*Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (Cornus mas L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621*" mój udział polegał na rewizji finalnej wersji manuskryptu.

Beata Nowak

Wrocław, 07.03.2022r.

dr hab. Małgorzata Trocha, prof. UMW
Katedra i Zakład Farmakologii
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ul. Jana Mikulicza-Radeckiego 2
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OŚWIADCZENIE

Oświadczam, że w pracy „*Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (Cornus mas L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621*" mój udział polegał na rewizji finalnej wersji manuskryptu.

M Trocha

Wrocław, 07.03.2022r.

dr hab. Jan Magdalan
Katedra i Zakład Farmakologii
Uniwersytet Medyczny we Wrocławiu
ul. Jana Mikulicza-Radeckiego 2
50-345 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy „*Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (Cornus mas L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621*" mój udział polegał na rewizji finalnej wersji manuskryptu.

Jan Magdalan

Rzeszów, 14.03.2022r.

dr hab. Narcyz Piórecki
Instytut Nauk o Kulturze Fizycznej
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ul. Cicha 2A
35-326 Rzeszów

OŚWIADCZENIE

Oświadczam, że w pracy *„Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (Cornus mas L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621"* mój udział polegał na dostarczeniu owoców derenia jadalnego do doświadczenia oraz na przygotowaniu surowca do zielnika.

Narcyz Piórecki

Wrocław, 07.03.2022r.

prof. dr hab. Adam Szeląg
Katedra i Zakład Farmakologii
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ul. Jana Mikulicza-Radeckiego 2
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OŚWIADCZENIE

Oświadczam, że w pracy „*Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (Cornus mas L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621*" mój udział polegał na rewizji finalnej wersji manuskryptu.

Wrocław, 07.03.2022r.

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ul. Jana Mikulicza-Radeckiego 2
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OŚWIADCZENIE

Oświadczam, że w pracy „*Danielewski M, Kucharska AZ, Matuszewska A, Rapak A, Gomułkiewicz A, Dzimira S, Dzięgiel P, Nowak B, Trocha M, Magdalan J, Piórecki N, Szeląg A, Sozański T. Cornelian Cherry (Cornus mas L.) Iridoid and Anthocyanin Extract Enhances PPAR-α, PPAR-γ Expression and Reduces I/M Ratio in Aorta, Increases LXR-α Expression and Alters Adipokines and Triglycerides Levels in Cholesterol-Rich Diet Rabbit Model. Nutrients. 2021; 13(10): 3621*" mój udział polegał na kierowaniu projektem naukowym obejmującym badania opisane w tej pracy oraz rewizji finalnej wersji manuskryptu.

Uniwersytet Medyczny we Wrocławiu
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