REVIEW ARTICLE

Effect of Plant Extracts on Protein Changes During Adipogenesis: A Scoping Review

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ABSTRACT

Plant extracts are gaining popularity among researchers as alternatives from natural sources for the treatment of obesity and inhibition of adipogenic differentiation is one of the mechanisms targeted by these extracts. The main focus of this scoping review is to specifically identify the phytochemicals within the extracts, and the protein changes that occurred during adipogenesis when subjected to the various plant extracts as well as to identify the gaps in the previous studies. A systematic search was conducted using predetermined keywords on three online databases (SCOPUS, PubMed, and ScienceDirect). Overall, a total of 988 articles were retrieved, leaving only 43 articles after applying the exclusion criteria. The selected studies looked at the effects of phytochemicals found in plant extracts on the alterations in adipogenesis-related proteins that results in adipocyte differentiation inhibition mainly in 3T3-L1 cells and mice. Despite plant extracts being the basis of numerous hyperlipidemic treatments, not much is focused on the changes in adipogenic proteins such as PPARs, CEBPs, or SREBPs. Thus, in this review, we discuss how the plant extracts aid in obesity prevention, and possible further research required to fully utilize the natural sources for the betterment of public health.
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INTRODUCTION

Obesity is a chronic metabolic disorder associated with insulin resistance, hyperplasia, and/or hypertrophy of fat cells. This condition sometimes resulted from genetic predisposition (1) and has significantly increased with current dietary changes, leading to lifestyle-related diseases such as hypertension, heart disease, fatty liver disease, and diabetes (2,3) posing a serious threat to public health. This ever-growing disorder has become a global health issue that affects both genders, every ethnicity, and all ages (4). The widespread concern has led to a surge of interest in studies involving understanding the framework of adipogenesis in search of possible treatment and prevention steps against obesity. Basally, obesity is caused by a persistent imbalance between energy source intake and expenditure. This is due to the increased differentiation of preadipocytes to mature adipocytes which are regulated by several transcription factors (5).

An elevation in adipose tissue mass is directly linked to the dysregulation of multiple transcription factors, namely peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα), which are vital in adipogenesis for sustaining the size and number of fat cells produced (6). Both PPARγ and C/EBPα are considered the transcription factors responsible for the induction of adipogenesis. According to Moseti et al. (7), the factors that are positive regulators of PPARγ expression and adipogenesis are the Kruppel-like factors (KLFs), the sterol regulatory element-binding protein 1 (SREBP-1), the cyclic AMP response element-binding protein (CREB), and the zinc finger protein 423 (ZFP423). Previous research also reported that when the main regulators of adipogenesis such as PPARγ, C/EBPα, and SREBP-1 are altered, the process of lipid accumulation is inhibited (8). This shows that the complete differentiation of adipocytes requires the participation of the respective proteins as they are responsible for the second differentiation process of preadipocytes to mature adipocytes which is the terminal differentiation (9). Thus, understanding the adipogenesis cascade may be promising in terms of controlling adipose tissue growth and altogether curbing the ever-rising obesity-related disorders in society.

Despite multiple studies (10–14) demonstrating the effectiveness of a healthy lifestyle and promotion of

physical activities in daily life, reports of obesity cases continue to rise. This might be due to the fact that people are not willing to step out of their comfort zones and try to change their sedentary lifestyle for a better one. Eventually, all the attempts for the treatment of obesity are focused on drugs and medication-based solutions. Statins, fibrates, niacin, and bile acid-binding sequestrants are the common drugs for lowering LDL cholesterol. However, there are side effects that come with the usage of these drugs such as constipation, nausea, increased risk of myopathy, rhabdomyolysis, myotoxicity, central nervous system complaints, and hepatotoxicity among others (15–17). In addition, to prevent adipose tissue development and expansion, several anti-obesity medications namely orlistat, fenfluramine, rimonabant, and sibutramine have been created but these treatments are accompanied by a variety of negative effects, resulting in most of them being withdrawn from the market (3,18). This calls for alternatives from natural remedies to provide consumers with a safer option to combat obesity without detrimental side effects (8).

Plants have always been one of the main resources for treatments against human illnesses. Even though traditional herbs are frequently overlooked and neglected (19), the utilization of medicines originating from various parts of various plants has been going on for centuries and has brought excellent results. Current modern medicine is also thought to be a branch of the ancient medicinal practice and improvements are constantly explored so that the previous knowledge is still applied today (20). Among all phytochemicals, there is an increasing number of reports on polyphenols having beneficial effects in fighting or preventing obesity (21). Blueberries, seaweed, wormwood, barley sprout, sweet potatoes, and chokeberries are examples of plants reported to have anti-obesity properties. These plants and fruits possess compounds responsible for the inhibition of adipogenesis and some even induce lipolytic action (1,3,8,17,22,23). Phenolic acids and flavonoids are the phytochemicals most commonly found to exhibit these health benefits. Therefore, this scoping review will summarise the studies conducted focusing on the changes in adipogenesis-related proteins by various types of plants. This paper will also identify the gaps which will provide a guide for future research in the field.

METHODOLOGY

The review was conducted in accordance with the framework outlined by Arksey & O'Malley (24). There are five stages of processes involved in producing this review and they are outlined below.

Stage 1: Identification of Research Question

This review aimed to explore the effects of plant extracts on the protein changes during adipogenesis and the phytochemicals involved. This research question was constructed following the Arksey & O'Malley (24) suggestion to begin with a broad review area to determine the data available before narrowing down the search. The current authors are presently researching the protein expressions during lipolysis and are collecting additional information from previous related articles and published literature. Identification of research questions was crucial for setting the direction of the review and deciding the process of relevant studies identification and selection. Thus, the research question for this review is: what are the protein changes induced by phytochemicals from plant extract during adipogenesis?

Stage 2: Identification of Relevant Studies

Search Terms

The key terms were chosen to identify previous studies that are related to the research questions. A search string was then constructed according to the search terms obtained. The search was initiated on 15th December 2021 with the string; ("Plant Extract*" OR Phytochemical*) AND (Lipolysis OR "Anti-Adipogen*" OR "Anti-Obesity" OR Inhibit*) AND ("Protein Change*" OR "Protein Expression*" OR "Gene* Expression*").

Databases

Three databases were chosen for this review based on the topic area: SCOPUS (life and health sciences), ScienceDirect (science and medicine), and PubMed (life sciences and biomedical topics). These databases are supposed to provide all relevant publications in the field of interest. A total of 5467 articles were identified from Scopus, 4804 articles from ScienceDirect, and 1173 articles from PubMed. Overall, after limiting the search to publications from five recent years and only the English language, 998 articles were found in the chosen databases.

Stage 3: Selection of relevant and reliable studies

From the three databases, an overall total of 11444 articles were obtained from the initial string search. The search was then limited to only include peer-review publications, the English language, and studies published between 2017 and 2021. Though there was research done before the year 2017, the goal of this scoping review was to collect information from the most recent and updated articles, thus exclusion of older publications was conducted. The duplicated articles retrieved from the three databases were also removed (n = 10). This leaves 988 articles for further screening, where 897 articles were excluded for irrelevant topics in the title or abstract. The eligibility criteria include the discussion of the plants tested for their antiadipogenic properties and the changes in the protein expression of adipogenesis-related genes. The proteins of interest are PPARγ, and C/EBPs since these transcription factors are the major regulators of adipogenesis that are responsible for the induction of adipose cell differentiation (7). The

sterol regulatory element-binding protein 1 (SREBP-1) is also commonly studied in articles involving adipogenic studies and it is one of the positive regulators of adipogenesis. Articles with either in vivo and/or in vitro experimental designs were included as long as the data collected on protein changes are via protein expression. Therefore, papers presenting results by way of gene expressions were excluded. Additionally, all secondary sources such as review articles or commentary articles were excluded from the study to ensure assessment only involved primary data. Sorting and organization of articles were done on Mendeley. The following step was to screen and read the entire article, focusing primarily on the results section, leaving only 43 articles to be included in the review. The flowchart for article selections is shown in Figure 1.

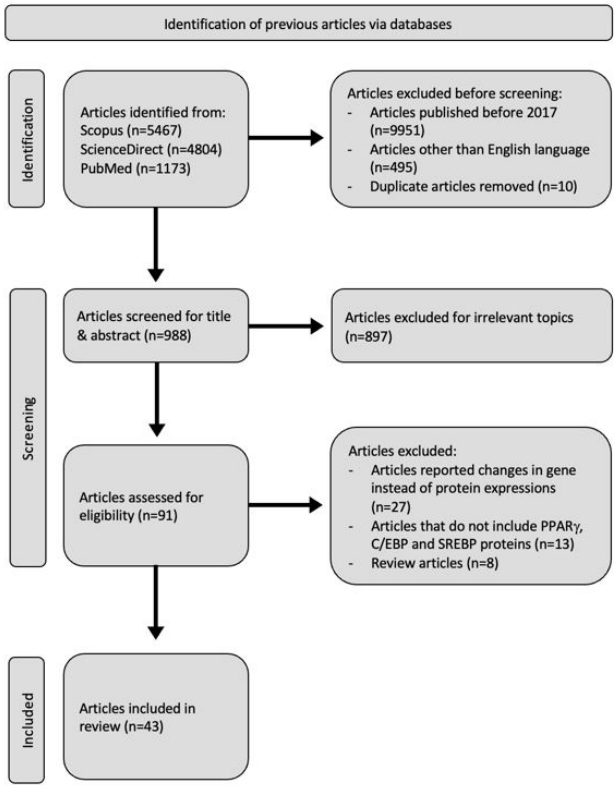

Figure 1: Study selection for the scoping review. Articles identified from Scopus (n = 5467); ScienceDirect (n = 4804); PubMed (n = 1173). 43 articles were selected after screening and application of the exclusion criteria.

Stage 4: Data Charting

Data collected from the articles included in this review was tabulated into six columns which were plant extracts, solvents, phytochemicals, experimental designs, protein changes, and references. The plant chosen should include its species name and the part utilized in the study. Solvents used for the extraction of the plant material should also be stated. However, papers that do not use solvents were also included. Phytochemicals separated and identified from the extracts, whether they were directly responsible for the protein changes or not, were also stated. Studies that do not mention the compounds involved were not excluded. The experimental designs for the articles were divided into in vivo and in vitro in which the in vivo experiments involved the species of the animal model utilized while in vitro tests majority used the 3T3-L1 preadipocytes. The protein changes charted include the increase or decrease of protein expression of PPARγ, C/EBPs, SREBPs, and a few more adipogenesis-related proteins.

Stage 5: Result Collating, Summarizing, and Reporting

According to the framework by Arksey & O'Malley (24), the final stage was to organize the relevant findings into several groups and prioritize the data according to the relevance of the research questions and focus more on the results that answer the main research question decided in the earlier stage. Significant data, including the samples, methods, intervention, and outcomes were all included. The details of all the data obtained from the reviewed articles are provided in Table I.

RESULTS

Plant Extracts

Different parts of plants usually produce different bioactive compounds (25) which necessitates the complete analysis of the plant of interest to prove their maximum potential in human medicine. The majority of the articles (30%) focused on the leaves of the plants chosen. Six articles opted to analyze the stem of their selected plant species while another five chose the fruits. Hwang et al., Joo et al., and Shen et al. (26–28) decided on the flowers of Castanea crenata, Magnolia denudata, and Citrus aurantium as their potential natural source of the anti-adipogenesis agent. Additionally, Geum et al. (64) reported on the anti-obesity effect of Hibiscus manihot flowers. Also, the roots of several plants such as Eurycoma longifolia (29), Polygonum multiflorum (30), turmeric rhizome (31), Lithospermum erythrorhizon, and Rheum palmatum L. from the Gangjihwan polyherbal composition (32) and Zingiber officinale (33) were tested for their antiobesity properties. Four studies reported on the anti-adipogenic capabilities of the seeds and grains of several plants (34–37) while Caulerpa okamurae (8) and Grateloupia elliptica (38) were two seaweed species selected by researchers in their respective studies. Aside from certain plant parts, two articles made use of the whole plant of Sargassum muticum (39) and Acalypha autralis L. (40) in their analysis. Other plant segments selected were peanut kernels and sprout (41), barley grass (42), barley sprouts (22), corn silk (43), and cacao mass (44).

Extraction Solvents and Methods

Fifteen studies (35%) chose water as the extraction solvent for the plant materials used. After extraction with water, one article synthesized gold nanoparticles from Dendropanax morbifera Lйveille leaves extract to be used in further testing (45). Eight studies opted for methanol while 14 opted for ethanol. For methanol

Table I: Summary of included studies sorted according to the plant extracts and adipogenic proteins changes

Plant extracts (Plant part)	Solvents	Phytochemicals	Experimental design	Protein changes	References
Acalypha australis L. (plant)	Distilled water	Flavonoids	*In vitro* (3T3-L1)	Decrease in PPARγ and C/EBPα expressions	(40)
Acer okamotoanum Nakai (leaves)	Methanol	-	*In vitro* (3T3-L1)	Dose-dependent down-regulation of PPARγ and C/EBPα Dramatic reduction in levels of SREBP-1 Decrease in FAS and adiponectin	(60)
Acer tegmentosum Maxim (stem)	70% Ethanol	-	*In vitro* (3T3-L1)	C/EBPβ, C/EBPα, and PPARγ protein expression levels decreased in a dose-dependent manner Significant dose-dependent downregulation of FAS, LPL, and aP2 expression levels	(61)
Arachis hypogaea L. (kernels & sprout)	80% ethanol	-	*In vitro* (3T3-L1)	Significant decrease in PPARγ protein expression	(41)
Aster yomena (Kitam.) (leaves)	Methanol (Ethyl acetate fraction)	-	*In vitro* (3T3-L1)	Significant reduction of PPARγ, C/EBPβ, and C/EBPα protein expressions	(47)
Barley sprout	Water	Saponarin	*In vitro* (3T3-L1)	Decreased levels of C/EBPα and PPARγ protein expression	(22)
Carica papaya (fruit flesh)	(juice)	β-carotene and vitamin C extraction	*In vivo* (Male Sprague Dawley rats)	Significant decrease in PPARγ expression	(50)
Castanea crenata var. dulcis (flower)	n-hexane	Cinnamyl alcohol	*In vitro* (3T3-L1)	Inhibition of PPARγ, C/EBPα, SREBP-1c, and FAS	(26)
Catharanthus roseus (leaves)	Distilled water	1α, 25-dihydroxy Vitamin D3	*In vitro* (3T3-L1)	Reduction of PPARγ1, PPARγ2, and PLN1 protein level	(57)
Caulerpa okamurae (seaweed)	Ethanol	-	*In vitro* (3T3-L1)	Strong reduction of PPARγ, SREBP-1C, and C/EBPα expression	(8)
Corn silk	Water	β-sitosterol	*In vitro* (3T3-L1)	Significant suppression of PPARγ, C/EBPα, and C/EBPβ expressions	(43)
Cerasus humilis (fruit flesh)	70% Acidic ethanol	Procyanidin B2, cyanidin-3- glucoside, and pelargonidin-3-glucoside	*In vivo* (Male C57BL/6 mice)	Downregulation of PPARγ and C/EBPα protein expression	(65)
Cissus quadrangularis (stems and leaves)	Aqueous water	-	*In vitro* (3T3-L1)	Significant decrease in PPARγ protein Dose-dependent downregulation of C/EBPα protein expression	(53)
Citrus aurantium (blossoms)	85% Ethanol	Hesperidin, naringin, neohesperidin	*In vitro* (3T3-L1)	Dose-dependent reduction of C/EBPα protein expression	(27)
Dendropanax morbifera Léveille (leaves)	Distilled water (Gold nanoparticles)	-	*In vitro* (3T3-L1, HepG2 cells)	Significant inhibition of PPARγ, C/EBPα, SREBP-1 and FAS	(45)
Dendropanax morbifera (leaves)	Water	-	*In vitro* (3T3-L1)	Reduction of PPARγ, SREBP-1, C/EBPα, and FAS	(56)
Fagopyrum esculentum (*Monascus ruber*- fermented buckwheat)	Deionized distilled water	-	*In vitro* (3T3-L1)	Decreased PPARγ and C/EBPα protein expressions	(36)
Ephedra intermedia stem, *Lithospermum erythrorhizon* root, and *Rheum palmatum* root (Gangjihwan polyherbal)	Water, 30% Ethanol, 70% Ethanol	Ephedrine, pseudoephedrine, aloe-emodin, chrysophanol, shikonin	*In vitro* (3T3-L1)	Down-regulation of SREBP-1C, PPARγ, and C/EBPα	(32)
Eurycoma longifolia (root)	95% Methanol (HP 20 resin fraction)	Eurycomanone	*In vitro* (3T3-L1)	Reduction of PPARγ1 Significant reduction of PPARγ2, C/EBPα, and FAS	(29)
Euterpe oleracea (Aзai seed)	-	-	*In vitro* (3T3-L1)	Decreased expression of PPARγ receptor, SREBP-1, FAS	(34)
Grateloupia elliptica (Red Seaweed)	60% Ethanol	-	*In vivo* (Male C57 BL/6J mice), *In vitro* (3T3-L1)	Significant reduction of PPARγ, SREBP-1, and FABP4 protein	(38)

Table I: Summary of included studies sorted according to the plant extracts and adipogenic proteins changes (continued)

Plant extracts (Plant part)	Solvents	Phytochemicals	Experimental design	Protein changes	References
Ginkgo vinegar (ginkgo seed coat)	-	Quercetin	*In vitro* (3T3-L1)	Significant inhibition of C/EBPδ and PPARγ protein levels	(35)
Hibiscus manihot (leaves)	Distilled water	-	*In vitro* (3T3-L1)	Suppression of C/EBPα, PPARγ, perilipin-1, FABP4, and adiponectin levels	(52)
Hordeum vulgare L. (barley grass)	(Squeezed juice)	Flavonoids, saponins, and terpenoids	*In vivo* (Male albino Wistar rats)	Downregulation of PPARγ expression	(42)
Korean Chungtae-jeon tea (lyophilized tea)	Distilled water	-	*In vitro* (3T3-L1)	Significant decrease of PPARγ, C/EBPα, and aP2 proteins	(55)
Liupao tea (leaves)	70% Ethanol	Rutin, catechin, taxifolin, astragalin.	*In vivo* (C57BL/6J mice)	Suppression of PPARγ and C/EBPα expressions	(64)
Magnolia denudata (browned and fresh flowers)	Deionized water	Rutin, acteoside, isoacteoside, caffeyolquinic acids	*In vivo* (Male C57BL/6J mice)	Suppression of PPARγ and C/EBPα	(28)
Methylxanthine derivative-rich Cacao Extract	50% Ethanol	Theobromine, caffeine, epicatechin, catechin, procyanidin B2, procyanidin C1, cinnamtannin A2	*In vivo* (Male ICR mice), *In vitro* (3T3-L1)	Dose-dependent decrease of PPARγ, C/EBPs, SREBP-1, and FAS protein expression levels	(44)
Myrica nagi Thunb. (fruit)	Methanol	Alkaloids, glycosides, flavonoids, steroids, amino acids, carbohydrates, tannins, and phenols	*In vitro* (3T3-L1)	Reduction of PPARγ	(70)
Oenocarpus bacaba (fruit)	80% aqueous acetone	Total phenolics	*In vitro* (3T3-L1)	Reduction of PPARγ, C/EBPα, FABP4, IR-β, and adiponectin	(49)
Oryza sativa L. (rice hull)	Water	-	*In vitro* (3T3-L1)	Significantly lowered PPARγ, SREBP-1, and C/EBPα protein expressions	(37)
Panax ginseng (green & dried leaves extracts)	Distilled water	-	*In vivo* (Male Sprague-Dawley rats)	Dried leaves- Significant decrease of C/EBPα, PPARγ, and adiponectin protein expression levels Green leaves- Significant increase of PPARγ protein expression.	(54)
Polygonum multiflorum Thunb. (root)	70% Ethanol	Polyphenol, flavonoids	*In vitro* (3T3-L1)	Significant decrease in C/EBPα and PPARγ protein expressions	(30)
Pterocarpus santalinus (heartwood)	Methanol (Chloroform fraction)	Pyrans, benzoates, lignans, chalcones, santalin, savinin, pterolinus, vanillic acid	*In vitro* (3T3-L1)	Dose-dependent down-regulation of PPARγ, SREBP-1c, TNF-α, and IL-6 levels	(46)
Ramulus mori (twigs of *Morus alba*)	80% Ethanol	Mulberroside A, quercetin, quercetin-3-β-glucoside, resveratrol, oxyresveratrol	*In vitro* (3T3-L1)	Dose-dependent reduction of PPARγ, C/EBPα, and SREBP-1	(62)
Rhus verniciflua (leaves)	Methanol (Butanol fraction)	Sulfuretin	*In vitro* (3T3-L1)	Suppression of PPARγ	(48)
Sargassum muticum (whole plant)	Ethanol	Saringosterol	*In vitro* (3T3-L1)	Expressions of PPARγ and C/EBPα at the protein level were suppressed	(39)
Taiwan Tea Experiment Station No.12 (TTES) (leaves)	Water	Epigallocatechin gallate (EGCG), gallic acid, caffeine, catechin	*In vivo* (Male Wistar albino rats)	Downregulation of PPARγ, C/EBPα, C/EBPβ and C/EBPδ protein expression	(76)
Tectona grandis (bark)	Chloroform & methanol	Betulinic acid	*In vitro* (3T3-L1)	Dose-dependent reduction of PPARγ2	(58)
Turmeric rhizome (BCM-95)	Dissolved in ethanol	Curcuminoids	*In vitro* (3T3-L1)	Decreased PPARγ and C/EBPα	(31)
Viburnum opulus L. (fruit)	Fresh juice undergoes methanol elution, dissolved in water to pure juice	Chlorogenic acid, procyanidins, and catechins,	*In vitro* (3T3-L1)	Reduction of PPAR and SREBP-1C protein expression Significant downregulation of FAS expression	(51)

Table I: Summary of included studies sorted according to the plant extracts and adipogenic proteins changes (continued)

Plant extracts (Plant part)	Solvents	Phytochemicals	Experimental design	Protein changes	References
Zingiber officinale (fresh ginger)	Ethanol	-	*In vivo* (Male C57BL/6), *In vitro* (3T3-L1)	Considerable decrease of PPARγ, C/EBP1α, SREBP-1c and FAS protein expressions	(33)
Ziziphus jujuba (leaves)	50% methanol	Apigenin	*In vitro* (Human Simpson-Golabi-Behmel syndrome (SGBS) preadipocyte cell)	Strongly decreased PPARγ and C/EBPα protein level	(59)

extraction, four studies proceeded with fractionation using chloroform (46), HP 20 resin (29), ethyl acetate (47), and butanol (48). Other than that, Lauvai et al. (49) chose aqueous acetone as the extraction solvent for Oenocarpus bacaba fruits while Hwang et al. (26) used n-hexane for Castanea crenata flower extraction. A study done by Thatiparthi et al. (42) analyzed the properties of Hordeum vulgare L. but did not use any extraction solvents as the juice of the barley grass was directly used in the next steps of the study. This is similar to Od-Ek et al. (50) and Zaklos-Szyda et al. (51) that used the fruit juices of Carica papaya and Viburnum opulus L. in their studies.

Phytochemicals

Due to the different species of plants used in the chosen articles, the phytochemicals focused on in each study were also different. Phenolic acids and flavonoid compounds are usually the main components analyzed in the studies. Out of all the included articles, nine did not analyze the bioactive compounds present in the extracts obtained (22,36,37,45,52–56). Several papers quantified the total phenols and flavonoids available in their plant extracts, however, no phytochemical profiling was done to identify the specific compounds that were responsible for the protein changes in the test subjects (30,40,42,49). Eleven studies (26%) analyzed the antiadipogenic properties of the selected plants focusing on a specifically selected compound. They were 1α, 25-dihydroxy Vitamin D3 (57), eurycomanone (29), saringosterol (39), saponarin (22), cinnamyl alcohol (26), neohesperidin (27), sulfuretin (48), β-carotene and vitamin C (50), curcumonoids (31), betulinic acid (58) and apigenin, betulinic and maslinic acid (59).

Previous Study Designs

There were two types of study designs applied in the studies collected, which were in vivo studies and in vitro studies. From a total of 43 articles, 35 used an in vitro approach by utilizing the 3T3-L1 preadipocyte differentiation to generate the condition of the adipocyte-like cells for the monitoring of protein changes. Another article also applied the in vitro study setting but decided to use Human Simpson-Golabi-Behmel syndrome (SGBS) preadipocyte cells to provide the environment for testing against adipogenesis-related proteins (59). The remaining 10 articles conducted the experiments with in vivo study settings where two articles opted for male albino Wistar rats, two opted for male Sprague Dawley rats, five opted for C57BL/6 mice and one opted for ICR mice. Only three articles utilized both in vivo and in vitro settings to demonstrate the changes in adipogenic genes at the protein level (33,38,44).

Protein Changes

The changes in adipogenesis proteins were quantitated via Western blotting and the proteins of interest included PPARγ, C/EBPα, and SREBP-1 which are three of the most common proteins studied. The changes in PPARγ were reported in 42 out of 43 articles. Five papers specifically showed a dose-dependent reduction of PPARγ protein expression (44,46,60–62). The majority of the articles focused on the changes in C/EBPα as it is one of the main regulators of adipogenesis, and 13 also included the SREBP-1 changes in their investigations. In papers that include C/EBPα and SREBP-1 proteins, it is observed that the proteins decreased as the reduction of PPARγ level occurred. Some other protein changes such as fatty acid synthase (FAS), lipoprotein lipase (LPL), adipocyte P2 (aP2), also known as Fatty Acid-Binding Protein 4 (FABP4), and several adipokines were also noted in the studies.

DISCUSSION

This review features the promising potential of various plant extracts as novel remedies with prospective applications in treating obesity. Simultaneously, it provides clarity on the significant processes that need to be undertaken in battling the disease. This scoping review examined 43 peer-reviewed research articles that include the potential natural alternatives with the ability to hinder the occurrence of adipogenesis. Most of the plants of interest are already traditionally consumed for their known health benefits (55,62), however, the full potential of these natural resources is yet to be discovered. The variety of plant material sources in the selected articles is an example of how researchers are currently looking into every possibility to maximize the potential of different vegetation available in the efforts of expanding the range of medicinal encyclopedias from natural sources. Numerous parts of plants from numerous species have proven to exhibit different bioactive compounds with different capabilities in the same direction of impeding the process of adipogenesis. This also shows that there are health benefits that can be obtained from every part of every plant. Among all the included articles, the various plant extracts seem to

be able to reduce the occurrence of fat accumulation via similar pathways which are by tackling the main regulators of adipogenesis. Therefore, focusing on the changes that occur to the associated proteins will provide insight into the extracts' capabilities to aid in obesity prevention.

Solvents and Extracts

Fifteen studies used water for the extraction process as water is the most polar solvent and is able to draw out a wide range of polar compounds and maximize the retrieval of compound yield (63). Twelve studies used diluted alcohols ranging from 30% to 95% methanol or ethanol as their extraction solvents (27,29,30,32,38,41,59,61,62,64–66). Similarly, the presence of water molecules in diluted alcohol increases the polarity compared to absolute alcohol, and this also allows the extraction of bioactive compounds with a broad range of polarity (67,68). This was demonstrated in a study done by Jang et al. (32) where Gangjihwan polyherbal composition was extracted in water, 30% and 70% ethanol, and it was reported that the most effective extract in down-regulating lipogenic transcription factors was the 70% ethanol extract. The result obtained might be owed to the greater amount of bioactive components extracted using 70% ethanol in contrast to water and 30% ethanol. Too high or too low polarities of extraction solvents might not be able to bring out the compounds of interest, thus choosing the right concentration of aqueous solvents is important to ensure the maximum retrieval of compounds from the plant materials. The majority of the studies utilized either ethanol or methanol as their extraction solvent and according to Sultana et al. (69), these alcohols, with or without mixtures with other solvents can recover the most amount of phenolic compounds from the plant material. Prashar and Patel (70) reported that methanol produced the highest percentage yield of Myrica nagi fruit extract at 8.83% and it also showed the presence of the maximum number of compounds compared to hexane and chloroform.

Fractionations were also done in some studies to narrow down and focus on the targeted compounds among the numerous components available in the extracts. This process separates the extracts into portions consisting of only several compounds where they segregate according to their polarities when selected solvents of increasing polarity are introduced (63). Borah et al. (57) showed that one out of 37 Catharantus roseus leaf extract fractions that contains a higher percentage of 1α, 25-dihydroxy vitamin D3 inhibited the adipocyte differentiation more effectively compared to the other fractions. Apart from that, a study done by Lamichhane et al. (48) demonstrated that fractionation of the Rhus verniciflua leaves extracts to obtain a pure compound which was sulfuretin, that suppressed the protein expression of PPARγ in 3T3-L1 cells. Similarly, Kim et al. (47) selected the ethyl acetate fraction of the methanolic extract of Aster yomena leaves which was previously proven to exert a higher antioxidant activity compared to other extracts and fractions due to the presence of high phenolic contents. The results obtained from the study showed a significant reduction in PPARγ, C/EBPβ, and C/EBPα levels.

Phytochemicals

Phenolic compounds are the most common components reported as antidiabetic and are usually effective in suppressing adipogenesis (49,51,70). Several other studies have also reported the presence of plant phenolic contents with the inhibition of adipogenesis in 3T3-L1 cells (49,51,62,65). Phenolic compounds have been extensively studied in the last few years due to their antioxidant and anti-obesogenic properties. Multiple compounds namely quercetin, curcumin, catechin, and apigenin are among the few examples that have been tested in vitro and in vivo assays to establish their effectivity in adipogenesis inhibition. As mentioned by Aranaz et al. (71), different compounds affect the differentiation process at different stages. Apigenin and myricetin are active during the early and late stages of adipogenesis, hesperidin is active during the intermediate stage while quercetin and resveratrol are involved in adipogenesis inhibition during the whole process. Even so, any hiccups in the differentiation process led to an incomplete transformation of preadipocytes to mature adipocytes which causes an impairment in the function of the fat cells. Thus, any protein changes brought upon by the compounds are worth to be noted.

Flavonoids are one of the many bioactive compounds quantified in plant extracts. When O. bacaba fruit extract was analyzed using HPLC/MSn, quercetin was detected, and it was the compound responsible to inhibit adipogenesis by down-regulating PPARγ and C/EBPα (49). On top of that, Park et al. (62) also reported the presence of quercetin among mulberroside A, quercetin-3-β-glucoside, resveratrol, and oxyresveratrol in Ramulus mori which reduced the protein expression levels of PPARγ, C/EBPα, and SREBP-1 in a dose-dependent manner. Quercetin is one of the most prevalent flavonoid compounds that has been shown to lower the expression of adipogenic transcription factors in multiple studies (72–74). Even so, 17 out of 43 of the articles did not analyze the phytochemical composition of their plant extract and mostly rely on the previous studies for information on the antiadipogenic properties of the plants tested. For instance, Hosoda et al. (35) reported that ginkgo vinegar obtained from ginkgo seed coat significantly inhibited the expression levels of C/EBPδ and PPARγ protein due to the presence of quercetin. Previously, ginkgo leaf extract has shown inhibitory effects on adipogenesis, and it was assumed that a similar bioactive compound, quercetin is also present in ginkgo vinegar.

Other than quercetin, neohesperidin which is the most

abundant flavonoid compound found in C. aurantium blossoms, alongside hesperidin and naringin has also been reported to hinder the process of adipogenesis as studied in 3T3-L1 cells (27). The dose-dependent reduction of C/EBPα protein expression in the cells treated with C. aurantium extract was observed to be also accompanied by the reduction of FAS. Not only that, taxifolin, astragalin, catechin, and rutin found in liupao tea extract have also exhibited properties of anti-obesity effects (64). Rutin was also found in M. denudata flowers (28), and it was shown to have the greatest suppressive effect on adipocyte differentiation in 3T3-L1 cells (75). Several other studies reported the presence of procyanidins (44,51,65) and catechin (44,51,76) where both compounds have been shown to take part in the downregulation of adipogenesis-related factors.

Among the various compounds extracted from cacao mass, theobromine which is an alkaloid was suggested as a strong candidate for an effective compound in the reduction of adipogenesis (44). Theobromine and caffeine are present in the minimal concentration of cacao extract required for lipid accumulation inhibition. This shows the great potential of each compound in adipogenesis inhibition. Resveratrol, a phenolic compound detected in R. mori extract (62) has also been reported to have multiple pharmacological effects on adipocytes and is capable of modulating lipid metabolism (77). 3T3-L1 cells treated with resveratrol showed a significant decrease in lipid accumulation and depression of various adipogenic markers including C/EBPα, LPL, FAS, and SREBP-1c. Chlorogenic acid, which is an ester of caffeic acid is also found in V. opulus extract which showed a reduced amount of PPARγ and SREBP-1c and significant downregulation of FAS protein expression (51). Other than that, triterpenoids namely betulinic acid and maslinic acid extracted from Tectona grandis bark and Ziziphus jujuba leaves showed their antiadipogenic potentials by the reduction of PPARγ2 level (58) and the significant decrease of PPARγ and C/EBPα (59) respectively.

Previous Study Designs

Only 10 articles studied the changes of adipogenesis protein using in vivo subjects, while another 33 were in vitro. Three out of all the articles applied both in vivo and in vitro experimental designs (33,38,44). The liver tissue, mesenteric adipose tissue, and epididymal adipose tissue in mice were used to examine the protein changes that had occurred when they are induced with each selected plant extract. As stated by Loncar et al. (78), epididymal tissue in rats is thought to be "pure" white adipose tissue and the mesenteric adipose tissue is one of the adipose tissue depots that have been linked to the risk of developing obesity-related diseases (79). Thus, it is only appropriate that this tissue is chosen as a vessel to study any changes that occur during adipogenesis. The white adipose tissue is important for energy storage where an increase in tissue deposition will lead to obesity (7). This also explains the choice of samples acquired from the test subjects as five studies (28,33,50,54,76) collected the epididymal adipose tissue and another one (44) collected the mesenteric adipose tissue for the examination of adipogenesis-related protein changes.

For in vitro studies, one of the articles used the human Simpson-Golabi-Behmel syndrome (SGBS) preadipocyte cells as they are not converted nor immortalized, and their ability to maintain the adipogenic differentiation capability while being able to multiply for up to 50 generations makes them an almost limitless supply (80). Furthermore, after growing them in adipogenic media, SGBS cells behave extremely similarly to human primary preadipocytes, and developed white SGBS adipocytes cannot be distinguished from human primary adipocytes in terms of shape or function (81). The rest of the articles preferred the cell line derived from mouse 3T3 cells as the 3T3-L1 adipocytes are also considered to be a well-documented model system for in vitro adipogenesis studies (82).

Protein Changes

The changes in adipogenic protein expression after treatment with plant extracts are observed via the Western Blotting technique as demonstrated in Figure 2. A reduction in the intensity of the bands produced indicates a decrease in the protein of interest. The main protein changes that are observed in the articles are PPARγ and C/EBPs, which are the main regulators of adipogenesis (7). The downregulation of protein expressions in the adipogenesis cascade by the plant extracts is simplified in Figure 3. All plant extracts studied had shown downregulation of PPARγ in the test subjects. The PPARs consist of 3 isoforms which are (α, β, γ). PPARγ plays a major role in adipogenic differentiation, glucose metabolism, inflammation, and other physiological processes (7). Additionally, the two primary isoforms of PPARγ are PPARγ1 and PPARγ2. It is reported that PPARγ2 is the isoform responsible for the differentiation of preadipocytes (83). This is shown by the failure of 3T3-L1 cells to differentiate with only the presence of PPARγ1 but successfully undergo adipogenic differentiation when PPARγ2 is restored. The structures of the two isoforms are similar except the N-terminus of PPARγ2 contains an additional 30 amino acids. A reduction in PPARγ2 expression has led to the failure of 3T3-L1 cells to undergo adipogenic differentiation but an exogenous restoration of the protein reinstated the adipogenic differentiating ability. On the contrary, PPARγ1 reactivation showed no impact on adipogenic differentiation, indicating that PPARγ2 rather than PPARγ1 is important in adipogenesis (83).

As mentioned, the C/EBPs, specifically C/EBPα is also the master regulator of adipogenesis alongside PPARγ. C/EBPα is an isoform of C/EBPs which is responsible for the terminal differentiation of adipocytes, and it

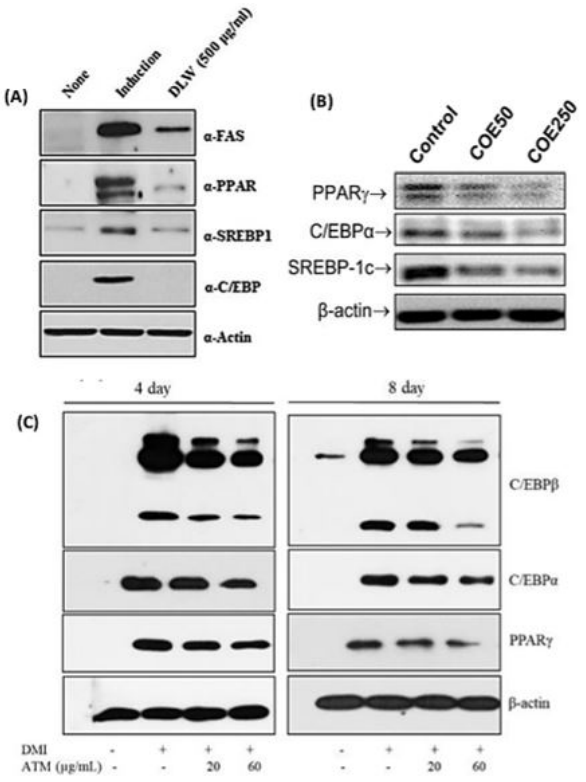

Figure 2: Effects of plant extracts on protein expression levels of adipogenesis-related proteins in 3T3-L1 cells. (A) 500μg/mL D. morbifera leaves water extract caused significant reductions of FAS, PPARγ, SREBP-1, and C/EBPα protein expression. (B) 250μg/mL C. okamurae ethanolic extract showed down regulations of PPARγ, C/EBPα, and SREBP-1 in 3T3-L1 cells. (C) The decrease in protein expression levels of C/EBPβ, C/EBPα, and PPARγ in 8 days was observed when treated with 60μg/mL A. tegmentosum ethanolic extract. Image adapted and modified from (56), (8), and (61).

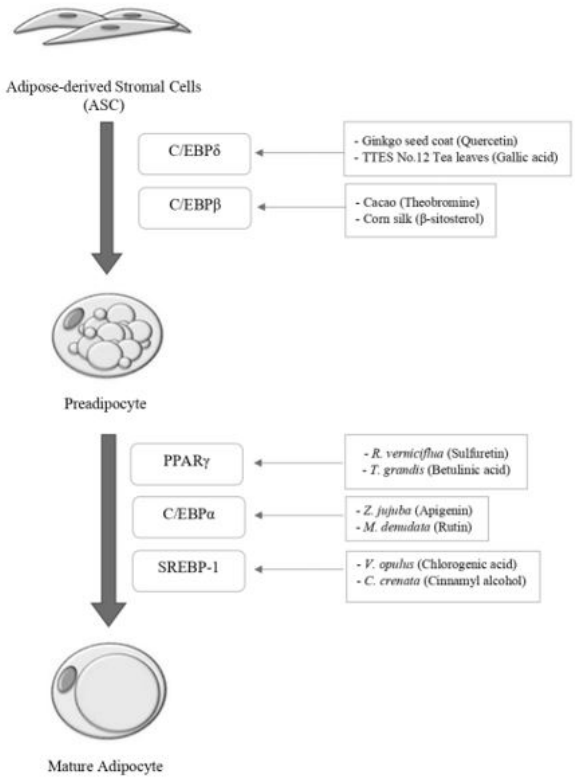

Figure 3: The simplified adipogenesis cascade and the examples of respective plant extracts with their phytochemicals that act on specific proteins that inhibit the complete differentiation process. Image adapted and modified from (84).

is generally expressed in adipose tissue, liver, lung, adrenal gland, and placenta (85). C/EBPβ and C/EBPδ are not less important as they are the first transcription factors induced during adipogenesis. Cells with the absence of these two factors were not able to proceed with differentiation and thus fail to express the other major adipogenic markers such as C/EBPα, PPARγ, or aP2. Even so, in vivo studies exhibited the expression of C/EBPα and PPARγ even without the presence of C/EBPβ and C/EBPδ but the adipogenesis is severely impaired, indicating that co-expression of C/EBPα and PPARγ is insufficient for complete adipocyte differentiation in the absence of the other two isoforms of C/EBPs (7).

In response to hormonal induction, C/EBPδ, and C/EBPβ are highly expressed during the early stages of adipocyte differentiation. They are responsible for the catalytic functions in the differentiation pathway, then diminish to be replaced by PPARγ and C/EBPα in the later stages of differentiation (85). Once activated, PPARγ promotes the production of other adipogenesis-related genes. C/EBPα, which is also induced by PPARγbinds to the promoter region of PPARγ, establishing a self-regulatory loop. This shows the dependency of both proteins for the differentiation cascade where the expression of either protein leads to the expression of the other. The continuous expression of C/EBPα, which may trans-activate multiple adipocyte genes, helps to maintain terminal differentiation. C/EBPα also has a C/EBP binding site in its proximal promoter, which enables it to auto-activate its expression, which is crucial for maintaining the marker's expression (7).

Apart from the two master regulators of adipogenesis, several other proteins positively regulate the PPARγ expression, at the same time enhancing the process of adipogenesis. They are the Sterol Regulatory Element-Binding Protein 1 (SREBP-1), Kruppel-Like Factors (KLFs), Cyclic AMP Response Element-Binding Protein (CREBP), and Zinc Finger Protein 423 (7). Among all, changes in SREBP-1 are most commonly monitored when protein changes in adipogenesis are studied. SREBP-1 enhances the activity of PPARγ via the production of endogenous ligands. Moreover, it was reported that the ectopic expression of SREBP-1 in 3T3-L1 induces endogenous PPARγ mRNA levels, indicating that SREBP-1 amplifies the differentiation process by means of PPARγ expression induction (86). The two key genes involved in fatty acid metabolism, FAS and LPL are also regulated by SREBP-1 (87). FAS is the enzyme responsible for the synthesis of long-chain fatty acids (88) and is expressed by the adipose tissues. Therefore, the levels of FAS measured can also indicate the adipocyte count in the test subjects.

In this review, the reduction in all adipogenesis-related proteins is expected as it marks the success of the plant extracts in impeding the differentiation of preadipocytes to mature adipocytes. Dose-dependent reductions of proteins showed that the concentration of extract plays an important role in the extent of protein inhibition. The higher the concentration of extract, the greater the suppression of proteins. This causes the preadipocytes to maintain their immature form and not differentiate into fully functional mature adipocytes.

What is more interesting is that as the main regulators of adipogenesis are downregulated, the downstream proteins that are subsequently induced are therefore also decreased. This is shown in several studies where the reduction of PPARγ leads to the reduction of SREBP-1 and causes a significant decline in FAS, LPL, and aP2 protein levels (26,34,56,62). All this eventually leads to a decrease in lipid accumulation in the cells and a reduction in fat cell counts. Among all the results showing the positive impact of plant extracts on adipogenesis inhibition, a study on ginseng leaf extract which utilized the dried leaves and green leaves extracts showed otherwise (54). The treatment with dried leaf extract significantly decreased the protein expression levels of C/EBPα, PPARγ , and adiponectin, which were commonly seen in the results of other studies. However, the analysis of the tissues treated with green ginseng leaf extract showed a significant increase in PPARγ . It was hypothesized that this event occurred as the PPAR expression was considerably elevated, which improved glucose homeostasis and energy expenditure. Though, proving this concept requires further experiments which include investigations into glucose homeostatic changes following the green leaf extract treatment.

With the data collected from these studies, this review should be able to provide a basis for the idea of a safer antihyperlipidemic agent from natural sources. As mentioned, the harmful side effects caused by readily available pharmaceuticals are of major concern and there is a pressing need for a solution to this issue. The evidence provided by these previous studies shows that multiple plant extracts are capable of interfering with the differentiation of adipogenesis thus impeding the process of excessive lipid accumulation. The information gathered also provides a deeper understanding of the mechanism of phytochemicals that caused changes in the proteins involved. The knowledge at hand can facilitate the effort in the production of safer drugs for consumers. Indirectly, this provides an alternate path for the development of a treatment plan for obese patients. This also increases the possibility of the inclusion of natural products in the synthesis of remedies for obesity.

Limitations and suggestions

One of the gaps identified in the articles analyzed is that some reports did not specify the phytochemicals responsible for the changes in adipogenesis proteins. This limits the knowledge of the species of the plant extracts as the pure active compound involved remains unknown whilst this specific information can aid in the efforts of developing new biopharmaceuticals. However, the absence of this information allows for further investigations on the extract focusing on isolation and identification of bioactive components exerting effective inhibition of adipogenesis. Further studies on the conversion of plant extracts into fully functioning therapeutic agents for obesity are required to ultimately commercialize them for public use. More high-quality clinical research and trials are necessary to assess the efficacy and safety of these extracts. Also, the establishment of quality assurance systems to guarantee that the extracts are procured and made to a high grade is an issue that should not be disregarded in future studies.

CONCLUSION

In summary, this scoping review provides insight into the effects of phytochemicals on the protein changes that occur during adipogenesis. From the literature analyzed, the majority of the phytochemicals extracted from plant parts are able to halt the differentiation process of preadipocytes by diminishing the levels of master regulators of adipogenesis. This review summarizes the pertinent literature on plant extracts and protein alterations in adipogenesis, as well as future research possibilities. It has become evident that numerous other plant extracts are viable options to aid in obesity prevention as natural alternatives to the current pharmaceuticals. Further, more studies on this topic are beneficial to help society as a whole and bring about the solution that is already right under our noses.

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