Review

Hesperidin - A bioflavonoid's antidiabetic action: A Therapeutic and Epigenetic approach

Vijayakumar Sakthivel[1], Ponnulakshmi Rajagopal[1]*, Selvaraj Jayaraman[2], Krithika Chandrasekaran[3], Jaideep Mahendra[1]

[1]Department of Central Research Laboratory, Meenakshi Ammal Dental College and Hospitals, Meenakshi Academy of Higher Education and Research (MAHER), Chennai-600 095, India

[2]Department of Biochemistry, Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai India

[3]Meenakshi Academy of Higher Education and Research (MAHER), West K.K Nagar, Chennai-600 078, India

Correspondence Author*

Dr. Ponnulakshmi Rajagopal

Department of Central Research Laboratory

Meenakshi Ammal Dental College and Hospitals

Meenakshi Academy of Higher Education and Research (MAHER)

Chennai-600 095, India

drponnulakshmi.researchscientist@madch.edu.in

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Abstract: Anomalous epigenetic modifications are described in different pathological conditions, which includes Type 2 Diabetes, cardiovascular disease, neurodegenerative disease, obesity and cancer. The recent progress in Epigenetic modification studies and ability to reverse of epigenesist makes them an encouraging target. e.g., treatment of Diabetes. Hence, several epigenetically active compounds studied for their role in treatment of several diseases including diabetes. Many Flavonoids which are phytochemicals present in plants have ability to alter epigenetic cellular mechanisms. In this review, to facilitate the compilation of the sources, we studied biosynthesis, characterization, pharmacokinetics, antioxidant and anti-diabetic activities of Hesperidin conventional and online-literature that comprises the electronic search (Sci Finder, Pubmed, Google Scholar, Scopus, and Web of Science etc) and books on Diabetics /Epigenetics were studied. These Flavonoids that are natural phenol compounds has been found in plants and can be sub-characterized into subclasses which affect the two top characterized epigenetic mechanisms viz., DNA methylation and Histone Modification. High intake of Dietary flavonoid intake helps to reduce the threat of cardiovascular disease which includes Diabetes Mellitus.

Keywords: Flavonoids; Therapy; Type 2 Diabetes; Hesperidin; Orange Peel, epigenetics

1. Introduction

Hesperidin is a bioflavonoid flovanone glycoside (biophenolic compound) available in citrus fruits which performs the following functions, antioxidant, neuroprotective and anti-inflammatory activity [1]. They are abundant in oranges and lemons. Among various biophenol, Hesperidin plays a major as a naturally occurring therapeutic drug [2]. Hesperidin is a strong micronutrient and prevalent in most of the citrus plants that are ingested by animals through diet [3]. European Foods Safety Authority (EFSA) recommended that intake of Hesperidin with diosmin, troxerutin and hesperidin, is adequately characterised. The demanded effect, upkeep of standard venous-capillary permeability, is a valuable physiological outcome [4]. Hesperidin can reduce intestinal glucose and cholesterol absorption, suppress hepatic glucose production and along with peripheral glucose uptake rise insulin sensitivity [5, 6] Diabetes mellitus is a universal metabolic disorder and are swiftly increasing prevalence.

In the estimation made by the International Diabetes Federation (IDF), in 2015 it was 415 million and the current number of patients with diabetes would shoot up from 537 million to 783 million by 2045 [1]. There are some limitations in the currently used therapeutic option which is used for diabetic management. Thus, there is an urgent need for safe and efficient substitute anti-diabetic agents [7]. Several scientific reports documented that it holds antinociceptive [8], anxiolytic & sedative effects [9], analgesic [10], immunomodulatory [11], antimicrobial [12], anticancer [13], anti-inflammatory [14], oxidative stress [15], defensive effect against NAFLD [16], lipid reducing effect [18], hepato-protective [19, 22], shielding effect on respiratory diseases [24], wound healing effect [17], antioxidant [20], anti-diabetic activity [25], result on epigenetic modification.

2. Sources

Hesperidin is a main bio-flavonoid present in citrus fruits, lemons and sweet oranges (Fig.1) and similarly in other vegetables and several poly-herbal formulations. The metabolite of Hesperidin is Hesperetin is available biologlically. Hesperidin shows many pharmacological actions such as anti-hyperlipidemic, cardioprotective, anti-hypertensive, antidiabetic activities which are mainly recognized to an antioxidant defence mechanism and suppression of pro-inflammatory cytokine creation.

Figure 1 Source of Hesperidin from Citrus fruits (adapted from dw.com)

Table 1. The Physical and Chemical Characteristics of Hesperidin

S.no.	Facts	Properties of Hesperidin	Reference
1	Molecular Formula	$C_{28}H_{34}O_{15}$	[Binkowska 2020, Ref.27), 28
2	Molecular Weight	610.6 g/mol	[28, 29] [NCBI 2022, Ref. 28] [Agrawal PK, Agrawal C, 2021, Ref. 29]
	Colour	Yellowish Brown	36, Chaudhri VK 2016
3	Melting Point	262.0 °C	[NCBI 2022, 28]
4	IPUAC	(2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one	[NCBI 2022, 28]
5	UV absorption	283 nm	[33, Kuntić, V., Pejić, N 2012]
6	Solubility and stability	Hesperidin established, pH independent, aqueous solubility. Solubility enhanced intensely under the presence of 2-hydroxypropyl-beta-cyclodextrin (HP-β-CD) and the outcomes found supported 1:1 complex formation	[32, Majumdar S, Srirangam R. 2009]

3. Characterization

Hesperidin is an significant structural element of plant cell and it belongs to the family of Flavonoids (Fig. 2) and it plays important part in the membrane fluidity regulation and permeability [23, 34]. The Table 2 depicts the Physical and chemical properties of Hesperidin. It is known as (2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one. It is yellowish brown in colour. It has chemical structure (figure 3) similar to other flavanones.

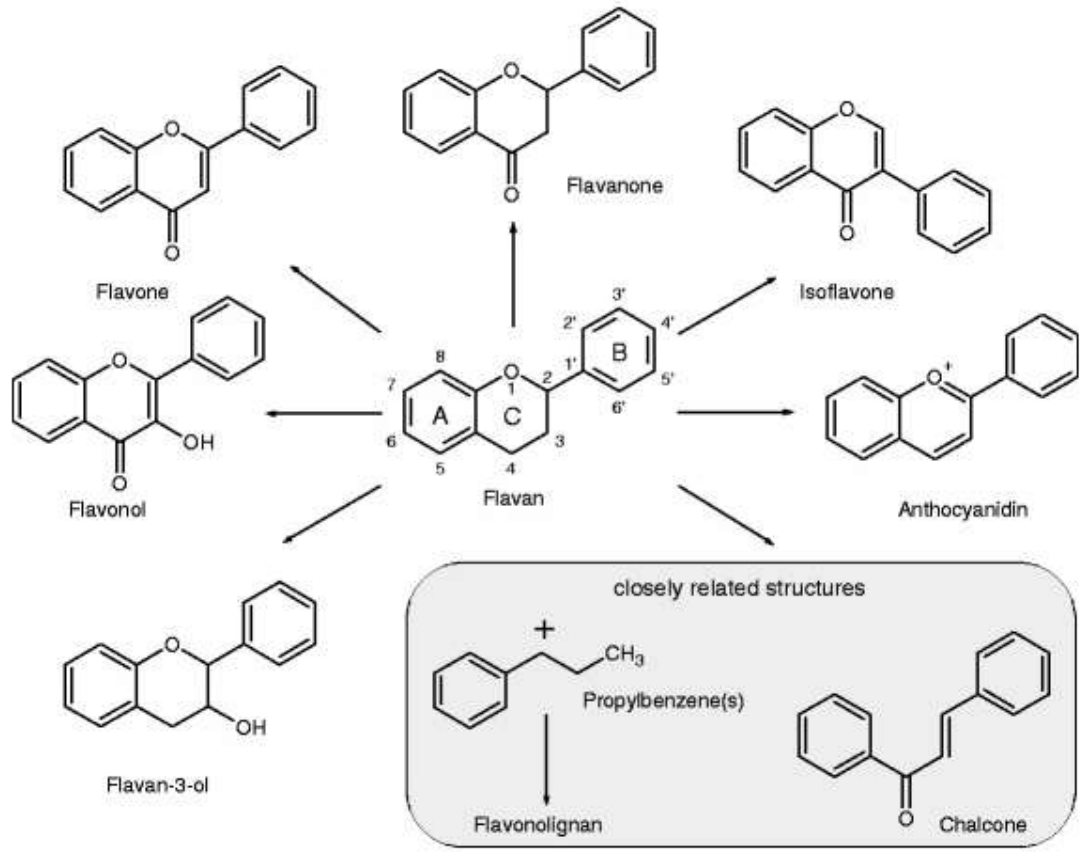


Figure 2: The Chemical structures of flavonoid subclasses

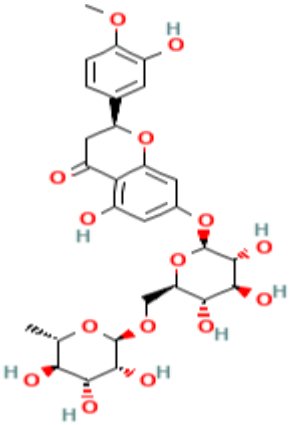


Figure 3:

Chemical structure of Hesperidin (C28H34O15)

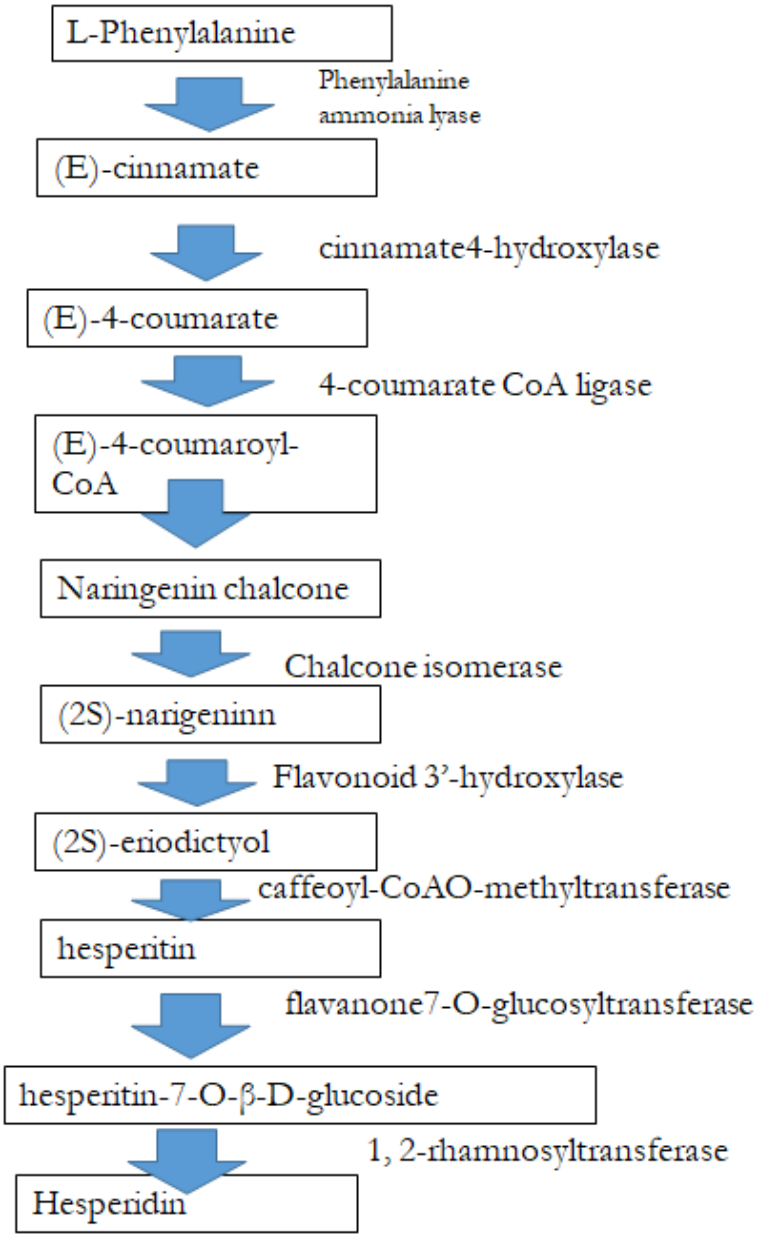
4. Biosysnthesis of Hesperidin

Hesperidin can be extracted from orange peel and the hesperidin extraction was explored on a laboratory scale by changing the solvent composition and the solid-to-solvent ratio, and then scaling this process (volume: 20 L). [37], The biosynthesis of hesperidin creates stems from the phenylpropanoid pathway (Figure 4), where the natural amino acid L- phenylalanine undertakes a deamination by phenylalanine ammonia lyase to release (E)-cinnamate

Later monocarboxylate go through an oxidation by making cinnamate 4-hydroxylase to discharge (E)-4-coumarate, [39] that is converted into (E)-4-coumaroyl-CoA by 4-coumarate-CoA ligase. Isolation of a cDNA for a cytochrome P450, cinnamate 4-hydroxylase (C4H), of Arabidopsis thaliana using a C4H cDNA from mung been as a hybridization probe [39] (E)-

4-coumaroyl-CoA is then exposed to the type III polyketide synthase naringenin chalcone synthase, which undergoes consecutive condensation reactions and eventually a ring-closing Claisen condensation to pay for naringenin chalcone. The corresponding chalcone go through an isomerization by chalcone isomerase to afford (2S)-naringenin, that is oxidized to form (2S)-eriodictyol by flavonoid 3'-hydroxylase. After O-methylation by caffeoyl-CoA O-methyltransferase,[18] the hesperitin product undertakes a glycosylation by flavanone 7-O-glucosyltransferase to afford hesperitin-7-O-β-D-glucoside.[19] Lastly, a rhamnosyl moiety is introduced to the monoglycosylated product by 1,2-rhamnosyltransferase, forming hesperidin

Figure 4: Biosynthesis of Hesperidin (Phenylpropanoid pathway)



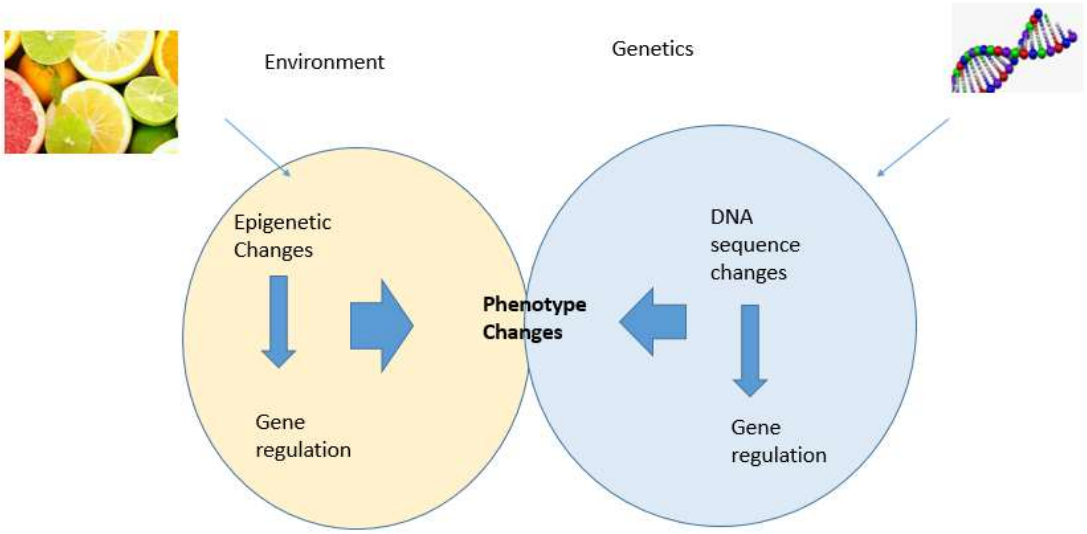


Figure 5: Interaction of epigenetic and Genetic mechanisms

Table 1. Biological activity of Hesperdin

S.No	Biological activity of Hesperidin	Type of study	Model	Dose/Source	Inference	Reference
1	Antinociceptive	In Vivo	Rat	It produced a dose-dependent and important response with an ED25 = 1666.72 mg/kg while comparing to an ED25 = 302.90 mg/kg for the extract or an ED25 = 0.47 mg/kg for the reference ketorolac in PIFIR model. (ethanol extract of the Rosmarinus officinalis)	study observed the anti-nociceptive properties; additive and supra-additive response after it is combined with the NSAID ketorolac.	**[Martínez, A. L 2010** [8]
2	anxiolytic & sedative effects	In vivo	Adult Male Swis Mice	i.p. (10 mg/kg) in volume 0.15 ml/30 g of body weight, 30 min before completing the behavioural	pERKs reduction relates with the depressant efficacy of flavonoids, suggests a step	[**Martínez, M. C** 2008, [9]

				tests in mice.	that involves ERKs to get inactivated may account for sleep-inducing & sedative actions.	
3	Analgesic effects	In Vivo	Male wistar rats	Solid filtrate of orange peel after an acidic pre-treatment	showed anti inflammatory and analgesic effects	[**Galati, E. M**, 1994], [10]
4	Immunomodulatory effects	In vivo	Male wistar rats	hesperetin (C16H14O6, purity >95%) Sigma Aldrich (Canada Ltd).	moderates immune cell functions in physiological and pathological conditions	[**Sassi, A., , 2017,** 11]
5	Antimicrobial effects	In vitro	*Staphylococcus aureus* ATCC (American Type Culture Collection) 25923, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231.	Hesperidin (HES) bought from Sigma Aldrich (USA),	High glucose levels and liver and kidney damage markers decreased by administering hesperedin	[**Corciova, A., 2015** 12]
6	Anticancer effects	In Vivo	Diethylnitrosamine /CCl4-induced rats	11 mg/kg	Hesperdin controls oxidative stress,inflammation, and cancer cell death increase oxidative stress, inflammation, cell proliferation, TGF-b1/Smad3 signalling, along with collagen deposition by activating Nrf2/	[**Aggarwal, V., 2020,** 13], Mahmoud AM, 2017, 53]

					ARE/HO-1 and PPARc pathways	
7	Anti-inflammatory effects	In vivo	Human Mesechymal Stem cells (bone marrow cells collected from bone pieces of patients (Second Hospital, Shandong University)	hesperidin (0, 1, 5 and 10 μM)	Enhance chondrogenesis of human MSCs - cartilage tissue repair.	[**Xiao, S., , 2018** 14]
8	Oxidative stress	In vivo	Male wistar rats	100 mg/kg b.w. oral dose - Sigma	High glucose levels and liver and kidney damage markers decreased /Oxidative stress and NF-kB levels increased	[**Iskender, H. 2017** 15]
9	Protective effect against NAFLD	In vivo	Human with NAFLD	1 g Hsp supplementation (12 weeks)	improved glucose and lipid metabolism/ reduced inflammation and hepatic steatosis (meticulous attenuation parameter - NAFLD patients)	[**Yari, Z., 2021, Ref.**16]
10	Lipid-Lowering effect	In vivo	male Sprague –Dawley rats	Hesperetin - 0.02%, 0.066 mml/100 g diet]	Plasma lipid-lowering actions cholesterol biosynthesis and esterification - reduced	[**Kim, H. K., 2003, Ref.**18]

11	Hepatoprotective	In vivo	Adult male wistar rats	Hesperidin –(H-Isoniazid – oral dose (27 mg/kg, p.o)	liver damage protected / oxidative stress-mediated natural & chemical toxins. antitubercular drug induced oxidative liver injury and necrosis	[**Tabeshpour J** 2020 Ref.19], [**Shanmugam, Nathiya&Rajaram** , 2015, 22]
12	protective effect on respiratory diseases	In vivo	Female BALB/c mice	5 and 1 mg kg−1, Sigma-Aldrich Korea - Oral	inhibitory effects on airway inflammation (asthma)	[**Kim SH, 2011, Ref.**24]
13	Wound Healing effect	In vivo	Adult Sprague Dawley rats	25, 50 and 100 mg/kg, p.o. - Hesperidin and Streptozotocin (Sigma)	Ihronic diabetic foot ulcers it speeds up angiogenesis and vasculogenesis via up-regulation of VEGF-c, Ang-1/Tie-2, TGF-β and Smad-2/3 mRNA expression to improve wound healing	[**Li W, Kandhare AD** 2018 Ref. 17]
14	Anti diabetic effect	In vivo	24 male Sprague Dawley rats	3, 10, 30 or 100 µg/ml hesperidin at 37˚C Sigma-Aldrich	Lessened hyperglycemia by activating the IR/PDK1 signaling pathway	[**Peng, P., 2021, Ref.** 25]
15	Anti oxidant	In vivo	Yeast cells/*Saccharomyces cerevisiae*	Hesperidin and the stressing agents hydrogen peroxide	antimutagenic effect in rats against N-methyl-N-amylnitrosamine hesperidin treatment ameliorated HG-induced insulin	[**Wilmsen** , 2005, Ref.20], **Tian, M., 2021, Ref.**26]

					resistance - by reducing oxidative stress and mitochondrial dysfunction moderately by suppressing DNMT1-mediated miR-149 silencing	
16	Anti-Covid-19	Review	Prophylactic agents- blocks viral infection and replication		hesperidin and hesperetin avert the SARS-CoV-2 virus from binding to the ACE2 enzyme of the host cell and prevent virus replication	[Agrawal PK 2021, Ref.29]

5. Anti-diabetic activity of Hesperidin and its epigenetic and therapeutic effects

We study one of the Pharmacological properties of Hesperidin mainly on its Anti-Diabetic activity in detail along with epigenetic changes in Table 2. The basic epigenetic and genetic modification example is given in figure 5. In most of the study the diabetes is induced by STZ. In the study by Akiyama et al (2010), we noted that Hypoglycemic and hypolipdemic effects while a dose of 10 g/kg diet was given to the three-week-old Wistar male rats. We noted that in the study done by Swapna et al (2019) when 100 mg/kg of Hesperidin was given to Male albino wistar rats aged 3 months, the resulted in decrease of total lipid profiles and plasma insulin concentration which was supplemented by anti-hyperglycemic, hypo-lipidemic activity. In the study done by Akiyama et al (42), we noted that altering gene expression encoding PPARs, HMG-CoA reductase, and LDL receptor when 1% of Hesperdin and 4.6% CD-hespertin was adjusted by diet with corn starch fed to Goto-Kakizaki rats which results in epigenetic changes.

Table 2 Anti-Diabetic activity of Hesperidin along with therapeutic activity through epigenetic changes

S.No	Hesperidin	Dose used/Study	Model/Study (Animal/Cell line/Clinical/Review)	Observation	References
1	Hesperidin from Toyo Sugar Refining	10 g/kg diet	18 3 wk old -Wistar male rats	Hypoglycemic and hypolipdemic effects The initial and final body weights (54 g and 242–247 g, respectively)	Satoko Akiyama, Katsumata et al 2010 J Clin Biochem Nutr. 2010 Jan; 46(1): 87–92 [Ref. 41]

2	Hesperidin	100 mg/kg bw of Hesperidin	Male albino Wistar rats, aged 3 months old	decrease in total blood lipid profiles and plasma insulin concentrations supplemented by the anti-hyperglycemic, hypo-lipidemic activity in DMI rats	Somesula Swapna et al, 2019, [Ref. 43]
3	Hesperidin from Toyo Sugar Refining	1% hesperidin and of 4.6% CD-hesperetin was adjusted by corn starch in diet	Goto-Kakizaki rats	altering the gene expression encoding PPARs, HMG-CoA reductase, & the LDL receptor.	Satoko AKIYAMA et al, (2009) 73:12, 2779-2782, [42]
4	Hesperidin and Naringin	50 mg/kg b. w for 30 days	While male albino rats	Antihyperglycemic and anti dyslipidemic efficacies as well as cardiac function improving action in HFD and STZ-induced type 2 diabetic rats	Ahmed, Osama (2012) 41. 53-67 [Ref. 40]
5	Hesperidin and Naringin	0.2 g/kg diet	Male Mice	Increase hepatic glycolysis along with glycogen concentration; reducing hepatic gluconeogenesis.	Jung, Un & Lee, 2004. 10.1093/jn/134.10.2499 [Ref.44]
6	Hesperidin	200 mg/kg bw - oral	Male albino rats	Hesperidin hepato-protective effects against CIS would be mediated by anti-inflammatory, antioxidant and anti-apoptotic properties	Aboraya DM et al 2022 [Ref.46]
7	Hesperidin from *C. reticulata* fruit peel hydroethanolic extract	100 mg/kg b.w./day for 4 wks	male rats (adult) Wistar (bw 130-150 g, 10-12 wks old	Exerted anti-hyperglycemic and anti-hyperlipidemic	Alaa M. Ali,, 2020. https://doi.org/10.1155/2020/1730492 [43]
8	Flavonoids	NA	Review	Improvement of pathogenesis of diabetes/regulation of glucose metabolism	AL-Ishaq RK, et al,. 2019; 9(9):430. [47]

9	Hesperidin (H5254) from Orange and diethylnitrosamine (DEN) (N0756) as hepatocarcinogen inductor (Sigma)	Hesp concentration (from 0.78 to 25 mM) to evaluate the cytotoxic doses; inhibitory concentration 50 (IC50)	HL60 human leukaemia cancer cell line (American Type Culture Collection)	Hesperidin exerts a hypomethylating effect on the LINE-1 sequence (up to 47% hypomethylation at 12.5 mM) and on the ALU-M2 repetitive sequences (up to 32% at 6 mM) in HL60 tumor cells. hesperidin suggested as a nominee molecule in chemoprevention in epigenetic therapies.	Fernández-Bedmar, Z. et al., 2017, *56*(6), 1653–1662. https://doi.org/10.1002/mc.22621 [48]
10	Epigenetic abnormalities - abnormal methylation of CpG islands, are inherited over cell divisions (Cancer cells)	NA	Review	DNA demethylating agents - effective for hematological malignancies, and tested in solid tumor	Kazuaki Miyamoto and Toshikazu 2005 35(6), 293–301 [49]
11	Wound Healing for Diabetics – Epigenetic changes	NA	Review	Gene expression profiling - instantly after injury to normal skin -> alteration in gene expression. 3% of 4000 genes studied -> upregulated within 30 min. of injury. Histone and DNA methylation - wound healing process	Rafehi et al. 2011 Feb;8(1):12-21 [50]
12	Epigenetic factors interplay between genes and environment	NA	Review	Epigenetic factors adjust complex interaction between genes/environment - affect human diseases., Diabetes	Villeneuve LM, et al. 2011;38(7):451-459. doi:10.1111/j.1440-1681.2011.05497.x [51

6. CONCLUSION.

We noted from different studies that hesperidin standardizes blood glucose levels and also alters the function of glucose-regulating enzymes, where lowering serum lipid levels in STZ-induced diabetic MI rats were noted no change in body-weight loss owing to the modulatory effect in bio-transformation enzymes. Hesperidin studies shows that it is a phytocompound

and has bio-therapeutic properties which works on by lessening the making of additional cholesterol by liver and also have anti-diabetic activity. With the advent of Epigenetics as one the major form of therapy, we observe that several studies would arrive at a conclusion that antidiabetic activity can well be obtained from Hesperidin with notable changes through epigenetics. The researchers also found that hesperidin has antioxidant property which paves way for this bioactive compound to be treated as biomedicine in STZ induced diabetic rats on hypoglycemic and hypolipidemic actions.

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