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VITEXIN AS A CHRONOTHERAPEUTIC AGENT FOR CIRCADIAN-ASSOCIATED COLONIC COMPLICATIONS IN DIABETES: A REVIEW

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ABSTRACT

Diabetes mellitus is increasingly recognized as a disorder that extends beyond glycemic dysregulation to encompass significant colonic pathology, characterized by epithelial injury, barrier dysfunction, and microbial dysbiosis mediated by hyperglycemia-induced oxidative and inflammatory stress. Concurrently, circadian clock genes—most notably *Bmal1*—have emerged as critical regulators of colonic epithelial renewal, tight-junction integrity, and mucosal immune homeostasis, with circadian disruption shown to independently exacerbate colonic injury. Vitexin, a naturally occurring C-glycosylated flavone, exhibits well-documented antidiabetic, anti-inflammatory, anti-apoptotic, and gut-microbiota-modulating properties that converge mechanistically on several molecular nodes governed by BMAL1, raising the hypothesis of an unexplored circadian dimension to its pharmacological action.

Vitexin was found to inhibit intestinal α-glucosidase activity, attenuate NF-κB-mediated inflammatory signaling and adhesion-molecule expression, activate Nrf2-dependent antioxidant defenses, preserve mitochondrial dynamics, protect intestinal barrier integrity in experimental colitis models, and favorably modulate gut microbial composition. These effects operate through Nrf2, NF-κB, and AMPK/SIRT1 signaling axes, each independently established as reciprocal regulators of the CLOCK–BMAL1 transcription–translation feedback loop. Notably, a structurally analogous polyphenol has been demonstrated to restore BMAL1 rhythmicity in metabolically stressed hepatocytes, lending indirect support to the plausibility of a similar effect for vitexin. However, no published study to date has directly examined vitexin's influence on BMAL1, CLOCK, PER, or CRY expression, nor assessed whether its protective effects are time-of-administration dependent. Vitexin represents a mechanistically plausible, though experimentally unverified, chronotherapeutic candidate for circadian-associated diabetic colonic disease.

KEYWORDS: Circadian rhythm; BMAL1; diabetic colonic injury; vitexin; chronopharmacology; α-glucosidase inhibition.

1. INTRODUCTION

Diabetes mellitus belongs to the most impacting NCD epidemics of the current times. As of 2024, an estimated 589 million adults (about one in nine) in the world had diabetes, spending more than USD 1 trillion per year on health costs; by 2050 it is estimated that the number of adults with diabetes will approach 850-900 million [1,2]. This is very apparent in India where there are currently more than 100 million adults with diabetes or pre-diabetes; and more than 40% of all adults over 45 years of age have a high diabetes risk as per the Indian Diabetes Risk Score (IDRS) [3,4]. Gastrointestinal (GI) symptoms occur in up to a quarter of diabetic patients [5–7] in population surveys, with a high burden of diabetic gastroenteropathy (DGE) reported in regional populations, and a cross-sectional study in the Aseer region of Saudi Arabia during 2023–2024, suggesting a prevalence of diabetic gastroenteropathy that was underestimated [8].

The combination of converging neuropathic, structural and metabolic injury give rise to diabetic colopathy. Loss of enteric neurons and interstitial cells of Cajal disrupts colonic motility [9,10] and chronic hyperglycaemia leads to GLUT2 dependent transcriptional remodeling of the intestinal epithelium, which negatively regulates TJs, resulting in the ability to translocate bacterial components from the lumen, seen clinically by increased levels of circulating LPS, zonulin and intestinal FAP [11,12]. This barrier failure is not a passive bystander; experimentally re-establishing or breaking down gut barrier integrity in direct correlation to altering the activation status of islet-reactive T-cells, reflecting bi-directionality between the colon and systemic glycaemic disease [13]. Superimposed oxidative stress and NF-κB induced inflammation then form a vicious cycle of epithelial injury, which has been described histologically and biochemically in diabetic rat colon [14].Strong evidence exists in a distinctively different literature that a circadian clock intrinsic to the colon exists. The SCL entrains peripheral oscillators all over the body through a central transcription-translation feedback loop in which the heterodimer CLOCK-BMAL1 regulates rhythmic expression of activators CLOCK and BMAL1 themselves, while they regulate the expression of their repressors PER and CRY, respectively, in these peripheral oscillators [15-17]. In the colon specifically, this clock remains active in the same time frame even in constant conditions of darkness and fasting, reflecting a true and food-independent autonomous oscillator that regulates, amongst other things, motility, epithelial turnover, nutrient transport and mucosal permeability during the 24-hour cycle [18-20]. BMAL1 is an integral part of this

organization: it can participate in the Wnt pathway, and also in the RNA-binding protein MEX3A, maintaining the Lgr5-positive intestinal stem-cell compartment which favors intestinal epithelial renewal, and its genetic loss enhances the initiation of tumors and impairs the regenerative signalling [21-23].

There is bidirectional mechanologic coupling between circadian misalignment and diabetes. In a couple of days, controlled human shift-work simulations lower the insulin-sensitivity of skeletal-muscle [24,25] and mice lacking Clock or BMAL1 suffer from overt hyperglycaemia and overt hypoinsulinaemia similar to those of type 2 diabetes [26]. Glycated haemoglobin is also inversely correlated to the levels of BMAL1/CLOCK/PER3 cycling in skeletal-muscle and leucocytes of diabetic patients [27], and diabetic patients have an inverse correlation between CLOCK polymorphisms and insulin resistance [28]. It also affects the gut microbiome, which, similar to the brain, shows 24-hour rhythmicity, which is lost in obesity, diabetes and can precede and predict disease onset [29-31]. The deregulation of BMAL1 temporal control in intestinal tissues has been observed to promote experimental colitis, depending on the time of day, in a time-of-day-dependent way, by deregulating the expression pattern of tight-junctions and by deregulating epithelial apoptosis [32-34]; and in human inflammatory bowel disease cohorts, mucosal BMAL1 is decreased compared to healthy controls [32-34].

The naturally occurring vitxin (apigenin-8-C-β-D-glucopyranoside) is a C-glycosylated flavone that is found in mung bean, hawthorn, bamboo, passionflower and Vitex negundo [35,36]. It has well-documented antioxidant, anti-inflammatory, antidiabetic and anticancer activity, being an inhibitor of intestinal α-glucosidase which attenuates the postprandial hyperglycaemia, and inhibiting NF-kB-mediated expression of adhesion molecules in hyperglycaemic endothelial cells [37-39]. In a very specific manner, vitexin has pro-apoptotic, chemosensitising and macrophage-repolarising activity in colorectal carcinoma models [40-43] binding to molecular nodes, apoptosis, NF-κB, macrophage polarisation that are independently targeted by BMAL1, but also dysregulated in the colon of diabetes. But its main pharmacokinetic problem is the limited availability after oral administration attributable to extensive deglycosylation in the intestines (about 5% oral bioavailability; see also above), which is not a limitation for its application in the colon, since a large proportion of an oral dose reaches the colon in the unaltered form [44] and its preclinical safety profile is generally favourable [45,46].

Chronotherapy (timing the delivery of an existing drug to its circadian phase of maximum effect) is already used for several therapeutic classes, such as evening administration of some antihypertensives, and some cancer chemotherapeutics that have circadian-dependent toxicity [47-49]. In metabolic diseases, metformin has been demonstrated to enhance glycaemic control when administered in the evening as compared to morning; the drug also alters the expression of clock genes in the liver in a clock fashion by dégradation of PER2 via AMPK-dependent mechanisms, showing a true bidirectional correlation between drug and clock [50]. Direct pharmacological clock modulators, like synthetic REV-ERB agonists and the ROR agonist nobiletin have been shown to reduce hyperglycaemia in diet-induced obese mice, indicating that enhancing clock-gene function is a metabolically-validated biological strategy that can be extended to the consideration of clock-adjacent naturally-occurring phytochemicals like vitexin (51-54).

No study has ever investigated the action of vitexin on the colon, from the point of view of circadian and BMAL1-dependent regulation, nor studied it as a chronotherapeutic agent in order to treat the complications associated with diabetic colorectal cancer; there is therefore no previous study which linked the vitexin colorectal-cancer literature with the vitexin antidiabetic literature along this shared mechanistic axis. This review therefore seeks to: (i) summarise the evidence for the antidiabetic, anti-inflammatory and anti-apoptotic pharmacology of vitexin; (ii) examine the evidence for the relationship between circadian and BMAL1 dysregulation with diabetic colonic pathology; and (iii) present, and clearly define the scope of, an integrated hypothesis for the use of vitexin as a chronotherapeutic agent that engages BMAL1.

2. CIRCADIAN REGULATION OF COLONIC PHYSIOLOGY

The mammalian clock is a cell autonomous transcription-translation feedback loop. Together, the CLOCK and BMAL1 proteins heterodimerise and bind to E-box elements to promote rhythmic transcription of Per1/2 and Cry1/2, with protein product accumulation and inhibition of the CLOCK-BMAL1 complex providing a self-correcting ~24-hour oscillation [55,56]. This core loop is estimated to be binding to approximately 40% of the tissue specific protein coding transcriptome [57,58] and this is reinforced by another, interlocking loop, where REV-ERB and ROR nuclear receptors compete for binding to the same response element in Bmal1 promoter [59,60].

In the colon the site of this molecular clockwork, this translates into measurable time-of-day dependent colonic physiology. Tight-junction genes (Claudin-1, Occludin, ZO-1) are also rhythmically expressed in wild-type mice, and are seen to be constitutively increased and more severe in the colitis of Bmal1 deficient mice, as recovery is impaired [61,62]. Epithelial apoptosis and loss of barrier integrity act as markers of colonic Bmal1 mis-regulation by circadian disruption, and is mediated by autophagy pathway [33]. Colonic immune compartment is also endowed with its own clock signature: intestinal epithelial group 3 Innate lymphocytes (ILC3) exhibit circadian oscillation in their IL-22/IL-17 output, colonic epithelial Bmal1 knockout mice are more susceptible to colitis, lose their microbiota of short chain fatty-acid (SCFA)-producing bacteria, and have increased STAT3 signalling [63-65]. Intestinal stem-cell proliferation is also rhythmic, provided by crosstalk between BMAL1/Clock and the Wnt, Notch and Hippo pathways, and in Apc-mutant mice, loss of Bmal1 or simple photoperiod disruption enhances tumour initiation, suggesting a role for an intact colonic clock in the control of stem-cell self-renewal [22,23].

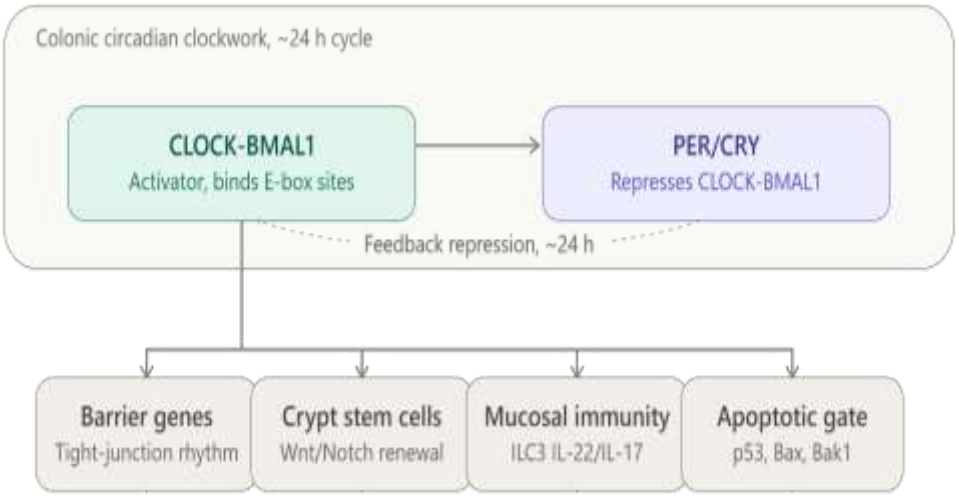

Figure 1: CLOCK–BMAL1 heterodimer drives its own repressors, PER and CRY, closing a self-correcting ~24-hour loop stabilised by competing REV-ERB/ROR binding at the *Bmal1* promoter [55–60]. That loop times four colonic functions the review returns to repeatedly: tight-junction rhythmicity, crypt stem-cell renewal, ILC3-driven mucosal immunity, and — critically — a *protective*, time-gated apoptotic programme via p53/Bax/Bak1 [21–23,61–65].

There is a two-way interaction between the clock and the gut microbiota. Roughly 25% of the bacterial taxa vibrate on a 24-hour rhythm aligned to feeding/ fasting cycles and this rhythmicity is quickly disrupted by inappropriate feeding and/or high fat diets [66,67]. The microbial metabolism in turn regulates the host clock via several mechanisms: antimicrobial peptides such as host-derived are essential for shaping microbial cycling; microbial metabolites also regulate the host clock, for example via short-chain fatty acids that promote the transcription of Per2 by inhibiting histone-deacetylase and by secondary bile acids that signal through FXR/TGR5 to modulate circadian glucose and lipid rhythms [68-71]. Circadian gene expression levels are found to differ in germ free mice, which were partially restored by microbial colonisation, demonstrating the causal, bidirectional axis [72]. However, in the colon, insufficient expression of Bmal1 leads to diminished populations of short-chain-fatty-acid-producing microbes, and to a more severe colitis via STAT3-mediated inflammation [73,65], pointing to an additional link between disruption of circadian rhythm function and microbial metabolite deficiency leading to colonic injury, which is hypothesized to intertwine with the diabetes-colon axis discussed below.

3. PATHOPHYSIOLOGY OF CIRCADIAN-ASSOCIATED COLONIC COMPLICATIONS IN DIABETES

The closest known mechanism for colonic oxidative damage is sustained hyperglycaemia. Polyols, advanced-glycation-end-products, protein-kinase C and hexosamine pathways, which all lead to excess generation of reactive oxygen species (ROS) and depletion of antioxidant defence, are all stimulated by excess intracellular glucose [74,75]. This results in a pro-oxidant cellular redox imbalance that retains this hyperglycaemic ‘memory’ following the subsequent improvement of glycaemic control [76] and it is measurable as malondialdehyde accumulation and damage to the epithelial structure in colonic mucosa, proportionate to the cells’ exposure to hyperglycaemia [14].

There is a mechanistic continuum between oxidative stress and inflammation: The release of ROS activates NF-κB, leading to concerted induction of TNF-α, IL-6, IL-1β and IFN-γ, which is exacerbated by the diabetic gut through activation of TLR4 by translocated LPS [77,78]. Both high-sensitivity C-reactive protein and mucosal histologic neutrophilia and inflammation have been reported to be positively associated with glycated haemoglobin in experimental diabetic rodents [14]. This inflammatory state is accompanied by a progressive breakdown of the barrier; first, increased levels of GLUT2 drive and lower levels of ZO-1, occludin and claudins (causing lower levels of tight junctions), and second, reduced expression of barrier-protective commensals like *Akkermansia muciniphila*, which is driven by dysbiosis [11,79].

The reduction of taxa that produce short-chain fatty acids (SCFA) – anti-inflammatory nutrients and signals for colonocytes (*Faecalibacterium prausnitzii*, *Roseburia, Akkermansia*) and increase of pro-inflammatory Proteobacteria appears to share a consistent trend in type 2 diabetes and is generally accepted as being functionally significant, as the lack of SCFA is a key loss of nutrients and anti-inflammatory signals for colonocytes [78,80]. This dysbiotic state also has an inherent circadian rhythm which is dampened in diabetes and can occur preceding disease. This state can directly connect to clock-gene disturbances and represent a direct link from a dysbiotic state to a breakdown in the clock [30,31].

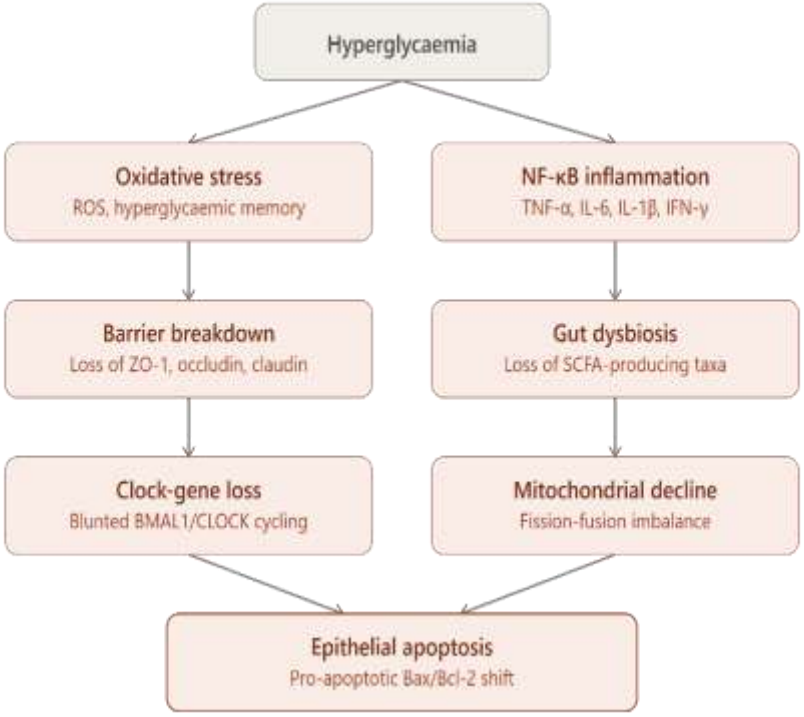

Figure 2: This is the review's core pathological argument, and the text presents it as a linear narrative even though it is really a converging network. Hyperglycaemia drives oxidative stress and NF-κB inflammation in parallel, these compound into barrier breakdown and dysbiosis, and the resulting clock-gene suppression and mitochondrial decline jointly culminate in uncontrolled epithelial apoptosis [11,14,27,32,33 , 74–83]. Seeing it as a converging cascade rather than a list makes clear why the authors argue a multitarget agent is needed rather than one that hits a single node.

It is now recognized that there is dys-regulation of clock genes in diabetic tissue. Skeletal-muscle and leukocyte studies indicate blunted cycling of BMAL1 expression is linked with an inverse trend for glycated haemoglobin; colonic study of genetically disrupted, environmentally stressed and metabolic studies shows decrease in BMAL1 and associated with dysregulated expression of tight-junctions or cerebral apoptosis; human inflammatory bowel disease mucosa shows decrease in BMAL1 relative to expression in healthy mucosa. The Clock-null mice have been found to have parallel insulin and glucose abnormalities (hyperglcaemia and hypoinsulinaemia) [26], and CLOCK polymorphisms have been associated with insulin resistance in human genetic studies [28]. Alterations in PER and CRY are also noted in diabetes, not always in the same direction as BMAL1/CLOCK, and suggesting that diabetes can affect progress in the relative role of the positive and negative arms of the clock [54].

Loss of BMAL1 alters mitochondrial dynamics in a tissue-specific manner: In pancreatic β-cells, upon loss of BMAL1, expression of mitofusin is decreased, the morphology of mitochondria is fragmented and their insulin secretion activity is impaired; the fission GTPase, DRP1, which is clock-controlled like BMAL1, is involved in a bidirectional cross-talk, rendering increased insulin secretion that leads to mitochondrial dysfunction, which in turn increases RNA circadian rhythmicity, thus reinforcing the clock's function [81-83]. These are the common pathways which converge, oxidative stress, inflammation, barrier failure, dysbiosis, loss of clock genes and mitochondrial loss, ending in an apoptotic pathway of epithelial cells. The tight regulation of Bax and Bak and Bcl2 is normal at the colonic crypt, and in diabetic tissue, the ratio is measurably altered with reports that diabetic tissue shifts toward a pro-apoptotic Bax/Bcl-2 ratio, which positively correlates with glycaemic control [76]. Since BMAL1 is a direct inducer of pro-apoptotic genes such as p53, Bax, and Bak1 in the homeostatic intestinal epithelium, the extent of BMAL1 regulation in colon tissue is directly associated with the extent of the apoptotic and structural injury burden, completing the mechanistic linkage between diabetic systemic disease and circadian-related colonic disease [32,33].

4. PHARMACOLOGICAL PROFILE OF VITEXIN

Vitexin (5,7,4'-trihydroxyflavone 8-glucoside, C21H20O10; molecular weight = 432.38 g/mol) is a C-glycosylated flavone which is common in edible and medicinal plants and employed in traditional Chinese medicine preparations [35,84]. Its main commercial source are the leaves and seeds of Hawthorn (Crataegus spp.) while also occurring in high quantities in mung bean, beetroot, bamboo, pearl millet, fenugreek, buckwheat, Passiflora species and Vitex negundo agriculture [84]. In contrast to the O-glycosylated flavonoids, vitexin contains a glycoside interface bound to C-8 that is unresponsive to glycosidase action, and show greater metabolic stability, explain their relatively high antioxidant and antidiabetic activity, compared to O-glycosides and aglycone apigenin [36,85].

Unfortunately, vitexin has poor oral pharmacokinetics that limits its clinical translation. It is extensively cleared by 94% intestinal, 30% gastric and less by hepatic metabolism to give it an absolute oral bioavailability of only 2.8-5% in rodent models, mostly via deglycosylation by intestinal β-glucosidases [44,86]. After receiving intravenously, vitexin was rapidly excreted, with a half-life of approximately 21-44 minutes [86]. The very limited absorption of this component(s) in the acidic gut is chemically favourable for a colon-specific application: only a small percentage of the compound(s) present is(are) absorbed in the upper gastrointestinal tract and the remainder, if any, is digested by resident gut bacteria (e.g., deglycosylation and C-ring opening) [87]. Because of this poor and variable systemic bioavailability, efforts in the area of

active formulation are ongoing with an injectable formulation in Phase I clinical testing in China and controlled release microspheres and microcapsules for improved gastrointestinal absorption in development [88,89]. Another pharmacokinetic concern is the phenomenon of hepatic enzyme interaction as vitro, vitexin inhibits CYP3A1 concentration- and time-dependently, and modulates CYP2C11 in a time- and concentration-dependent fashion, implying possible clinically relevant herb-drug interactions with co-administered substrates of these enzymes [90,91].

While limited, these available safety data are reassuring, but stem almost exclusively from preclinical studies. The considerable levels of vitro cytotoxicity of vitexin have yet to be noted at concentrations below approximately 100 μM, and repeated administration of high doses to rodents has resulted in no detectable liver or stomach mucosal damage or genotoxicity [45,46]. Toxicological data on vitexin-rich extracts of hawthorn (H. Oleate) as well as from Ficus deltoidea also support this, with an acute oral LD50 of > 5,000 mg/kg for both species and absence of mutagenic activity in the Ames assay [45,46]. However, it has been found that the majority of the toxicological evidence available comes from rodents or from the use of complex plant extracts, while in humans to date studies have been in the form of case reports and anecdotes, and the Phase I injectable-vitexin trial is currently being performed in China as a first step toward closing this gap [88].

Table 1: Vitexin's pharmacokinetic and safety profile.

Parameter	Finding	Citation
Molecular identity	Apigenin-8-C-β-D-glucopyranoside; C21H20O10; MW 432.38 g/mol	[35,84]
Principal sources	Hawthorn (Crataegus spp.), mung bean, bamboo, passionflower, Vitex negundo	[84]
Oral bioavailability	Approximately 2.8–5% in rodent models	[44,86]
First-pass metabolism	~94% intestinal, ~30% gastric, lesser hepatic loss	[44,86]
IV elimination half-life	~21–44 minutes	[86]
Colonic delivery	Substantial fraction of an oral dose reaches the colon intact	[44,87]
Hepatic enzyme interaction	Inhibits CYP3A1; modulates CYP2C11 bidirectionally	[90,91]
In vitro cytotoxicity	None appreciable below ~100 μM	[45,46]
Acute oral toxicity (vitexin-rich extracts)	LD50 >5000 mg/kg; no Ames mutagenicity	[45,46]
Clinical development stage	Injectable formulation in Phase I trial (China); controlled-release microsphere/microcapsule formulations in development	[88,89]

5. THERAPEUTIC PROPERTIES OF VITEXIN RELEVANT TO DIABETIC COLONIC INJURY

Vitexin is a direct free-radical scavenger with radical-scavenging capacity (RSC) increased by the C-glycosidic bond compared with O-glycosidic bonds due to the presence of the polyhydroxylated A-ring which increases electron density at the C-3 oxygen [36,92]. In high glucose-stressed endothelial cells, a model system pertinent to the vascular component of diabetic colonic injury, vitexin decreases ROS and malondialdehyde production and restores cell viability by activation of the Wnt/β-catenin and Nrf2 pathways [39,93]. In addition to scavenging, vitexin activates endogenous antioxidant means, including upregulation of glutathione, superoxide dismutase, catalase and glutathione peroxidase, primarily through Nrf2 activation and in streptozotocin-induced diabetic rats the inhibition of GPX1-dependent ferroptosis and the increase of antioxidant enzyme activities [92,94,95].

Vitexin directly inhibits NF-κB signalling, the main transcription system involved in hyperglycaemia-associated inflammation. It inhibits p38 MAPK and NF-κB activation in high glucose stimulated endothelial cells, in turn inhibiting expression of adhesion-molecules and the adhesion of monocytes [38,93] and in a DSS-induced colitis model it inhibits the phosphorylation of the NF-κB subunit p65, IκB, and STAT1 in colon tissue [96,97]. Similarly, consistent with this, vitexin modifies cytokine expression to favour an anti-inflammatory pattern, reducing the expression level of both tumor necrosis factor α and IL-1β, and increasing that of IL-4 and IL-10, and leading to a measurable decrease of IL-1β and tumour necrosis factor α in the colon, and an improvement in the diversity of colon microbiota in DSS colitis [98,99].

Vitexin protects the cell through cell-fate signalling in models of diabetic complications, in high glucose stressed endothelial cells, it reduces the rate of cell death (apoptosis) through the Wnt/β-catenin and Nrf2 signalling pathway, and in injury by bile-acids, it restores mitochondrial membrane potential by suppressing the JAK2/STAT3 signalling pathway [39,100]. Vitexin protects against mitochondria damage in myocardial ischaemia-reperfusion injury by down regulating mitochondrial-effects downstream of Epac1-Rap1 signalling, via inhibiting activation of the caspase enzyme system, and by balancing the ratio of fission (Drp1) and fusion (mitofusin-2) protein in mitochondria [101,102]. These dosage-dependent, protective properties should be separated from separately reported pro-apoptotic properties of vitexin in colorectal cancer cell lines, which involve “direct activation” of the caspase-3/9 axis and Bax/Bid axis to kill cancerous cells [40] (as would be consistent with broadly described polyphenol properties; see above).

Vitexin has direct, colon-specific evidence for barrier protection; in a DSS-induced colitis model, it was found to significantly improve colitis symptoms, with no interference of barrier integrity, and by down-regulating colonic IL-1β and TNF-α, an effect that was in addition to its suppression of NF-κB/STAT1 signalling [103,96]. Vitexin also changes gut microbiota significantly in a beneficial way: it was found that.Veillonella and Klebsiella – genera common in IBD – were depleted, whereas the presence of beneficial genera such as Parabacteroides, Flavonifractor, and Blautia increased after in vitro fermentation of faeces collected from IBD patients and after treatment of DSS-colitis mice [99]. This microbiota-modulating activity reaches to distant organs: In mice fed a high-fat diet, the combined antioxidant, anti-inflammatory and microbiota-modulating activity of vitexin decreased diet-induced brain oxidative stress and inflammation, highlighting the gut microbiome as one way in which vitexin’s local colonic activity leads to systemic activity [104].

Table 2: vitexin's pharmacological profile as it relates specifically to diabetic colonic injury.

Pathway / target	Experimental model	Key documented effect	Citation
Intestinal α-glucosidase inhibition	Enzyme assays	Blunts postprandial hyperglycaemia	[37–39]
Nrf2 antioxidant activation	High-glucose endothelial cells; STZ diabetic rats	↓ROS/MDA; ↑glutathione, SOD, catalase, GPx; ↓GPX4-mediated ferroptosis	[39,92,94,95]
NF-κB suppression	High-glucose endothelial cells; DSS colitis	Blocks p38 MAPK and p65/IκB/STAT1 phosphorylation; ↓adhesion-molecule expression	[38,93,96,97]
Cytokine rebalancing	DSS colitis mice	↓TNF-α, IL-1β; ↑IL-4, IL-10	[98,99]
Anti-apoptotic / mitochondrial protection	Endothelial cells; hepatocytes; myocardial ischaemia-reperfusion	↑Mitofusin-2, ↓Drp1 recruitment; restores membrane potential and ATP; inhibits caspase activation	[39,100–102]
Pro-apoptotic activity (tumour-selective)	Colorectal cancer cell lines	Activates caspase-3/9 and the Bax/Bid axis	[40]
Intestinal barrier protection	DSS-induced colitis mice	Maintains barrier integrity; ↓colonic IL-1β/TNF-α	[96,103]
Gut microbiota remodelling	IBD patient faecal fermentation; DSS colitis mice	↓*Veillonella, Klebsiella*; ↑*Parabacteroides, Flavonifractor, Blautia*	[99]
Vitamin D receptor agonism	Colitis-associated carcinogenesis model	VDR nuclear translocation; M1 macrophage polarisation	[43]
Macrophage repolarisation	AOM/DSS carcinogenesis model	Shifts macrophages away from pro-tumourigenic phenotype	[42]

6. VITEXIN AS A CHRONOTHERAPEUTIC AGENT: A BMAL1-CENTRED HYPOTHESIS

Chronopharmacology considers circadian variability in the absorption, distribution, metabolism and elimination of drugs as well as changes in the sensitivity of targets tissues, in regard to drug efficacy and toxicity [47,105]. Chronopharmacology considers the CLOCK-BMAL1 loop simply as a therapeutic target rather than as a confound, as well as a therapeutic variable, since many drug receptors, transporters and metabolising enzymes are regulated by this loop, and in turn its activity can be modulated to influence drug pharmacology [57,106]. Clinical "proof" of this is known: chronomodulated chemotherapy yields approximately twofold increases in efficacy with significantly less toxicity than constant rate delivery [48]; evening dosing of certain antihypertensiva measurably reduces the cardiovascular risk in diabetic patients [107]; and specifically for metabolic disease, insulin sensitivity in early type 2 diabetes exhibits an inverted diurnal cycle with a morning dip (the "dawn phenomenon") [108]. A randomised controlled trial, limiting feeding to a 10-hour window during the day, compared to the same level of restriction of calories but without circadian timing, induced changes in HbA1c, fasting glucose, and insulin sensitivity, and found circadian timing of an intervention to be as important as what it provides [109,110].

This study review does not obscure, but makes obvious, that no study to date has directly tested the effect of vitexin on the timing of drug administration or that its effect on the expression of BMAL1, CLOCK, PER and CRY in any tissue—a result of its molecular targets could not be said to indicate it. Nevertheless, this inference is a concrete one. Each of these pathways, Nrf2, NF-κB and the AMPK/SIRT-family signalling (see Section 5), is a regulator of the opposite direction of the CLOCK-BMAL1 loop that regulates DNA binding of the loop, with AMPK and SIRT1 regulating the turnover of CRY1 as well as its deacetylation of CLOCK, and NAD+ availability being rhythmical and regulating the DNA-binding activity of the CLOCK-BMAL1 loop [111-113]. The most comparable example is another dietary polyphenol that, in a different model, partially reverses the impaired daily fluctuation in expression of Bmal1/Clock, which is observed under conditions of metabolic stress, and that, in this model, is causally required for the protective effect observed, as silencing of Bmal1 blocks the benefit [114]. A similar isoflavone (isovitexin) has previously been shown to restore expression of Bmal1 and the architecture of intestinal cells challenged with low glucose, providing the nearest molecular model to what is expected for vitexin [115].

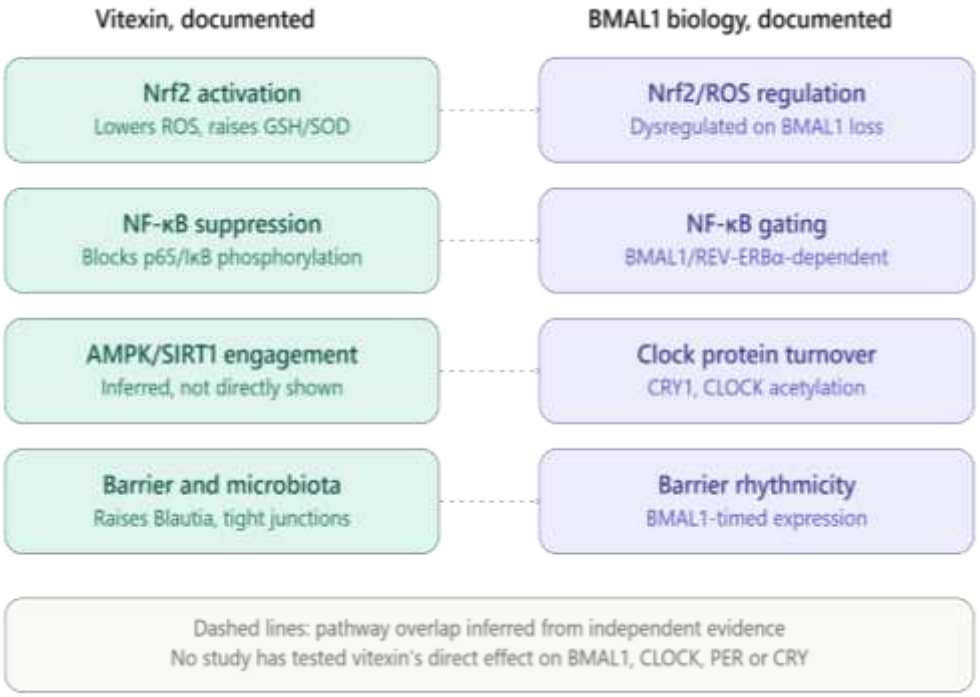

Figure 3: This is the paper's most important and most carefully hedged claim. Each dashed line links a pathway vitexin is *shown* to act on with the corresponding function BMAL1 is *shown* to regulate — Nrf2/ROS handling, NF-κB-driven inflammation, AMPK/SIRT1-gated clock protein turnover, and barrier/microbiome rhythmicity [92,96,111–117]. The precedent cited for a causal (not just parallel) link is resveratrol restoring hepatic *Bmal1* rhythmicity under metabolic stress, and isovitexin doing something similar in glucose-stressed intestinal cells [114,115]. But as the authors state explicitly, this is convergence on shared nodes, not proof that vitexin acts *through* BMAL1 — which is why the diagram keeps the two columns visually separate rather than merging them.

If both parts of a comparison are separately well supported, the resemblance is real, but only correlative. I first note that there is no indication in the data that vitexin acts on BMAL1; rather, there is evidence that vitexin and BMAL1 affect the same pathway—in this case, the pathway described as Nrf2 signalling.But first I note that there is no evidence in the data that vitexin acts on BMAL1, but only that, in this case, both photonics and BMAL1 targets the same pathway, namely the pathway described as Nrf2 signalling. This is also true for inflammation, where Vitexin downregulates NF-κB p65/IκB phosphorylation, but also loss of BMAL1 alone drives NF-κB-induced colonic inflammation [96,117,63]; and for barrier function, where Vitexin protects the integrity of the intestinal barrier in colitis and BMAL1 directly regulates t Barnier expression of the same tight-junction genes [103,62]. Mechanistically, vitexin's ability to enrich short-chain-fatty-acid-associated genera like Blautia and Parabacteroides is interesting in that these microbial metabolites feed back into the host and regulate Bmal1/Per2 gene expression, a point that should be tested to determine if vitexin can indirectly affect host circadian gene expression by a microbiota-to-clock-signalling pathway [99,68].

A physicochemical precedent to these clock genes comes from a treatment with a chitosan based gel formulation of vitexin, which resulted to very fast wound healing in excision wound experiments in vivo, where histological analysis showed enhanced re-epithelialisation, a skin treatment cause but also mechanistically relevant to the wound healing and regenerative capacity of epithelia [118]. This was reinforced by the observation that vork2 also has pro-apoptotic activity demonstrated in colorectal cancer cell lines [22,40] and that the turnover of THB is circadian under control of Wnt/Notch/ Hippo crosstalk with BMAL1/ CLOCK and that BMAL1 also imposes a time-gated apoptotic rhythm that helps maintain THB function.This was further supported by the induction of a separately documented pro-apoptotic activity of vork2 in colorectal cancer cell lines [22,40] and by the observation that the turnover of THB is circadian and under control of Wnt/Notch/ Hippo crosstalk with BMAL1/CLOCK, and that BMAL1 also imposes a time-gated apoptotic rhythm that helps maintain THB function.

Finally, because of its own hepatically mediated clearance, it is proposed that vitexin has a chronopharmacokinetic hypothesis of action. Besides its own ability to inhibit this enzyme, vitexin is also eliminated through CYP3A1-dependent pathways [90,91] and Cyp3A11 (mouse orthologue of human CYP3A4) itself is transcriptionally regulated by Bmal1 via the clock output genes Dbp and Hnf4α, so that in Bmal1 deficient mice there is a diminished circadian rhythm in the activity of Cyp3A11 [119]. These two independent discoveries suggest a testable and not yet proven prediction: That the systemic exposure of vitexin, and any potential for drug interaction, would be predictable by the time of dosing, with evidence in support of such prediction provided with circadian regulation of P-glycoprotein, shown to be a translator of vitexin [105]. If the above predictions are correct a circadian-timed vitexin regimen would have theoretical benefits, as it would be administered when colonic BMAL1 is at its most vulnerable point and could extend local exposure by giving the dose when the greatest proportion of the enzymes involved in the metabolism of colonic flavonoids are least active. However, the above advantages are theoretical and remain predictions based on the principles of chronopharmacology, since none of them have been obtained from an ad hoc trial optimized for vitexin delivery with the timings suggested by chronopharmacological principles.

7. PRECLINICAL AND CLINICAL EVIDENCE, AND RESEARCH GAPS

The evidence base for potential effects of vitexin is substantial, but arranged separately in different model systems which did not have a consistent focus on the diabetic colon. There are three lines of work most directly related to the question of the colon. Vitexin decreases the IL-1β and TNF-α levels in the colon, restores barrier function and alters gut microbiota composition so that it is more anti-inflammatory in an acute colitis model induced by DSS [103,99]. Vitexin helped to suppress the progression of tumours in the colon in an azoxymethane/DSS-induced model of colitis-associated carcinogenesis by altering the function of macrophages, repolarising them away from a pro-tumourigenic state and another example of its anti-inflammatory properties that attenuates chronic colon injury via NF-κB centred mechanisms [42] and a mechanistic study in 2024 identified that vitexin is a novel vitamin D receptor (VDR) agonist that blocks chronic inflammation to CRC by acting through VDR nuclear translocation and M1 macrophage polarisation which is a different mechanism to its NF-κB centred anti-inflammatory activity [43]. The ante ngăn of NF-κB suppression and activation of Nrf2 is maintained across tissues injured by hyperglycaemia, including diabetic nephropathy, where vitexin showed protective effects against injury due to GPX4-related ferroptosis [94] and diabetic rats, as well as in diet-induced brain injury, but it also occurs to other tissues damaged by disruption of circadian rhythms, where it protected against brain injury associated with microbiota remodelling [104] showing that a common mechanism is maintained across diverse organs, both in the context of an injury triggered by hyperglycaemia and to other organs suffering from an injury and caused by circadian disruption.

However, the animal model chosen has a very big impact on what can be inferred. The streptozotocin-induced diabetic rat model yields identical systematic hyperglycaemia, but weak colonic histopathology (increased expression of TNF-α/RAGE) and less representative colonic-associated phenotype (disorganised rhythm of tight-junctions and timing of colonic apoptosis) compared to circadian disruption models (jet-lag and Bmal1 knockout) which more closely reproduce the phenotype of colonic inflammation under study [120]. Two major arguments for this review appear to be lacking an adequate evidence base: protection against diabetic colonic injury by vitexin has been shown, but not circadian-mediated protection; conversely, circadian protection without vitexin was shown, but not protection from diabetic colonic injury.

There is limited evidence on the clinical uses of vitexin. In humans, at the preclinical stage there is only an injectable formulation in Phase I evaluation in China, because of the many known poor human oral bioavailability problems of vitexin [88]. There is no clinical trial registration data for vitexin for any indication for diabetes mellitus, colon or circadian problems. More broadly and in other studies, there are examples that show how easily it is possible to time an intervention during the day – for example, time-restricted feeding studies in overweight type 2 diabetic patients have shown clock-time efficacy of an intervention, but these studies do not fill this compound-specific gap [109] – and that the time of day intervention works can be clinically effective – for example, evening dosing of antihypertensives is now a well-known clinical practice [107]. In the era of customised medicine, personalised circadian phenotyping has started to be made possible by the development of advanced wearable sensors and continuous glucose monitoring, a prerequisite for any precision chronotherapy trial of vitexin in real patients [121,122].

From the above analysis five interrelated research gaps emerge. First, no published experiment has measured clock gene expression after vitexin treatment, nor tested its well documented influence on a circadian dose-response, which is of easy experimentation in existing DSS-colitis and high fat diet mice. Second, there is no combined diabetic plus circulatory model that has been treated with vitexin. Third, the oral bioavailability limitations must be determined specifically for colonic chronotherapy, though of course, the natural colonic delivery properties of vitexin could be beneficial. Fourth, when interacting with other clock-controlled drug-metabolising enzymes, the dose time interaction needs to be characterised [119,90]. Lastly, there is no clinical evidence-base, and the upcoming reference point of the Phase I injectable-vitexin trial will be crucial for first-in-human safety and pharmacokinetic data [88].

8. FUTURE PERSPECTIVES

The highly variable phase aetiology across the population (across chronotypes), by several hours, across beliefs on sleep patterns and across disease status, is a basic essential challenge to any chronotherapeutic intervention [123]. If the purported benefit of a compound is thought to be dependent on dosing in phase with its gut's rhythms, then it is unclear how a population average dosing schedule can provide that benefit. Two technologies now allow for individualised circadian phenotyping to be applied outside specialist sleep laboratories: 1) continuous monitoring of rest-activity rhythms with wearable devices that measure rest-activity and skin-temperature [121,124] and 2) the prediction of the onset of dim-light melatonin, the gold standard for estimating centrally controlled circadian phase, with peripheral blood clock-gene expression profiling from a single blood draw [122,125,126]. It has been previously been explained how to take advantage of an approach combining individual on the basis of the circadian profiles and pharmacokinetic-pharmacodynamic (PK-PD) modelling to optimize the timing of the chemotherapy administration in the context of glioblastoma treatment [127,128]; this methodology could be used in the further research on the compound of flavonoid in the gastrointestinal-metabolic context.

Targeting BMAL1 directly at the cavity of its PASB domain has progressed with the publication of a small molecule that directly targets BMAL1 at its PASB domain cavity, which has the effect of selectively regulating genes rather than 'on/off' the circadian output [129]. Even though BMAL1 has a role in protective barrier gene rhythmicity as well as in promoting an apoptotic cycle of the epithelium in the colon, its crude suppression, but not activation, would prove to be an incomplete therapeutic approach; however, a gene-selective modulator, or a naturally occurring compound having similar context selective activities mediated upstream of the BMAL1 suppressor would be more appropriate to restore colonic BMAL1 rhythms without disrupting its apoptotic gating effect [130,131]. This was complemented by a change to a galactose-modified prodrug strategy to enhance solubility and tissue-targeted delivery of a ROR agonist in a periodontitis model, which has shown in principle to be applicable to those natural compounds which modulate the clock, like vitexin [132].

There are three types of combination therapy to be considered. Vitexin combined with aspirin has shown the best evidence and is supported by studies showing that network pharmacology and molecular docking indicate the combined effects of

potease and inhibition of NF-κB and COX-2 synergistically in colorectal cancer cells, a pathway directly downstream of the TLR4/NF-κB pathway and direct upstream in the coherence of the TLR4/NF-κB axis leading to the colonic injury seen in DMC, however not yet tested. A second, pharmacokinetically-based combination is a combination of vitexin and controlled-release delivery formulations, which have already demonstrated gastrointestinal absorption benefits [88,89]. Currently, the third becomes the least evidence-based, but most mechanistically daring treatment targets vitexin together with a direct modulator of BMAL1 or REV-ERB; the reason is that oxidative, inflammatory and microbial components are targeted by vitexin, while restoration of temporal transcriptional amplitude occurs upstream and is achieved better by a combination of both than by time-restricted feeding only or polyphenol only [134,135].

To implement a future proof-of-concept study, pre-randomisation circadian phenotyping, dosing-time randomisation, and a composite primary endpoint encompassing fecal barrier and inflammatory biomarkers and cGM would be required, exploratory colonic clock-gene endpoints, and systematic pharmacokinetic monitoring of potential CYP3A-mediated interactions [136,106]. The vitexin-aspirin combination is a pragmatic first-in-human approach that incorporates clinical trial safety and experience with the target profile of vitexin.

9. CONCLUSION

This review has collated data from three related areas of colonic research; circadian biology of the colonic mucosa, diabetic colonic pathophysiology and the vascular pharmacology of vitexin and come up with a mechanistic case for vitexin as a candidate chronotherapeutic agent. BMAL1/CLOCK controls rhythmic expression of tight-junctions, renewal of epithelial stem cells and mucosal immunity, drug-metabolising-enzyme activity, and the gut microbiome is rhythmic and coupled to the rhythmic transcription of BMAL1/CLOCK. The architecture is disrupted in type 2 diabetes by simultaneous pathways of hyperglycaemia, oxidative stress, increased NF-κB activation and inflammation, barrier dysfunction, dysbiosis, dysregulation of the clock genes, and mitochondrial dysfunction, which results in unchecked epithelial injury rather than regulated, time-gated epithelial apoptosis, required for maintenance of colonic health.

Considering the interplay of this convergent pathways, addressing multiple convergence areas with a single drug as Vitexin does is therapeutically beneficial, since a single agent targeting a single pathway is able to protect only a part of the process in preclinical studies. In addition to its recently discovered role as a vitamin D receptor agonist, it has another, independent mechanism implicate in gating inflammation to cancer transition in the colonic mucosa.

The chronotherapeutic aspect of this review is in a sense contained within a theoretical framework, but has yet to be substantiated. The effects of vitexin on the expression of BMAL1, CLOCK, PER and CRY have not been quantified; there has been no published work regarding any circadian-dependent variant effects of vitexin's known effects. The argument hinges on the convergence of the targets of Nrf2/NF-κB/AMPK with the proposed nodes by which BMAL1 loss causes colonic injury, how BMAL1 loss provides metabolic stress injuries with unique vulnerabilities, and the microbiota-mediated clock-restoration hypothesis; while plausible enough, the argument is supported to varying degrees by independent evidence streams that must be presented as a falsifiable hypothesis and not as a demonstrated mechanism.

Systemic abnormalities in circadian disruption in patients with diabetic gastrointestinal complications, combining the potential of now available circadian phenotyping tools with ambulatory diabetes care and awareness of the bidirectional modulation of the CYP2C11/CYP3A1 enzyme system by vitexin by the clinician when assessing a potential future combination treatment are some near-term implications of this review before dedicated vitexin trials are finished. The most important research needs are: firstly, direct measurement of the expression of colonic clock genes, after time of day treatment with vitexin, is most tractable; secondly, a combined diabetic-plus-circadian-disruption colonic model to expand studies on vitexin's therapeutic effect in diabetes is the next priority; thirdly, characterization of the time of day pharmacokinetics of vitexin following its administration would be another priority; and lastly, a proof of concept human trial, already established with a colonic biomarker, for therapeutic effect following time of day treatment with vitexin. These dedicated studies are essential to determine the possible clinical benefits of this cadaverous agent and vitexin should be used as a possible chronopharmacological candidate in circadian related colonic disease related to diabetes and considered a well-biologically reasoned promising agent, but not yet validated.

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

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