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Review Article

Potential of Medicinal Plants to Ameliorate Retinopathy Events in Diabetes: A Systematic Review

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ABSTRACT

Diabetic Retinopathy (DR) is one of the most common diabetic complications which can lead to vision loss if left unattended. Medicinal plants are considered as a treatment option for its lesser side effects. Given the overwhelming number of studies on various medicinal plants using different subjects, this systematic review aims to update the current status of the potential of medicinal plants in ameliorating DR. Literature from the years 2011 to 2020 was retrieved from PubMed, ScienceDirect and Scopus databases using the search terms: Medicinal plants AND (diabetes OR hyper glycaemia) AND retinopathy. The PRISMA guidelines were adhered to for reporting the systematic review, while the SYRCLE tool was used to assess the risk of bias in animal studies. Inclusion and exclusion criteria were established for selecting compatible studies. Based on these criteria, four out of 439 studies were selected: Studies on DR in rats included three or more assays for measuring retinal vascular permeability, VEGF protein and gene expressions and body weight. An additional six studies from a manual search brought the total to ten selected studies. All studied medicinal plants demonstrated potential in ameliorating DR, based on their downregulation of diabetes-induced retinal vascular leakage and VEGF expressions. Medicinal plants with significant potential in attenuating DR included *Zingiber zerumbet* rhizomes and its active ingredient, zerumbone; *Lycium barbarum; Plantaginis semen*; and apocynin. The aqueous extracts of *Radix astragali, Radix angelica sinensis, Panax notoginseng, Lycopus lucidus* Turcz and total lignans from *Fructus arctii* can be further evaluated in future studies.

Keywords: Medicinal plants, Diabetic retinopathy, Retinal vascular permeability, VEGF gene, *Zingiber zerumbet*, *Radix astragali*.

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INTRODUCTION

Diabetes, or Type 2 Diabetes Mellitus (T2DM), is currently considered an epidemic that is expected to affect up to 4 million people globally by 2025.[1] Diabetic Retinopathy (DR) is one of the most common diabetic complications, alongside nephropathy and neurodegeneration and it raises significant clinical concern as it can eventually lead to visual loss without proper management. DR is a clinical manifestation of diabetic microvascular complications, involving alterations to retinal cells that result in the formation of microvascular lesions, inter-retinal edema, exudation, haemorrhage and even new growth of intraocular blood vessels.[2]

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An advanced stage of DR is characterized by retinal neovascularization and vitreous haemorrhage.[3] If untreated, it may progress to tractional retinal detachment, where the retina separates from the underlying retinal pigment epithelium due to scar tissue contraction from damaged retinal blood vessels. The most common conventional treatment for DR is laser photocoagulation, which stabilizes visual acuity but can cause retinal damage and scarring.[2,4] Other treatments include intravitreal pharmacological agents and vitreous surgery. The former, such as anti-VEGF agents, are preferred as they can be applied to both mild and severe stages of DR. Surgical intervention would be the last resort for DR patients if they fail to respond to both laser treatment and pharmacotherapy (Mansour *et al.*, 2020). Although the use of pharmacological agents has become the mainstay therapy for DR, there are studies that demonstrate the insignificant response and improvement in DR patients yielded from anti-VEGE treatment.[4,5] Drugs like thiazolidinediones (an insulin sensitizer) have even been

withdrawn or restricted for use due to their severe side effects.[6] The combination treatment comprising pharmacotherapy and laser therapy is also suggested but there is still lack of clinical evidence to prove its efficacy while concurrently not increasing burden of patients.[7] All these certainly prompt the need to discover new alternative or complementary therapeutic intervention to ameliorate DR in diabetic patients.

When there is no single strategy established for highly effective Diabetic Retinopathy (DR) management owing to high cost and adverse effects of pharmacotherapy and laser therapy on retinal tissue, researchers have resorted to seek medicinal plants for their potent therapeutic and preventive functions against DR to delay as much of its progression.[8-10] This is because natural products are inexpensive and have minimal toxicity that have been widely used in different parts of the world to treat various diseases since centuries ago. Many have shifted their focus from conventional drug discovery to identification of natural products to ameliorate the diabetes-induced pathological changes in retina.[11,12] There are also different plant-derived bioactive compounds identified to demonstrate potentials in treating or preventing DR that are desired to reduce the incidence of this complication in diabetic patients.[11-15]

There are many primary research studies investigating the therapeutic effects of medicinal plants or natural products, of *in vivo* and/ or *in vitro* study design on diabetes and its complications in human, animals and/ or cell lines. However, there is very limited centralized discussion to integrate and elucidate the types and functional status of medicinal plants or natural products in amelioration of Diabetic Retinopathy (DR) in different study models together with their underlying therapeutic mechanisms.

Therefore, this systematic review aimed to summarize the phytochemical properties of medicinal plants and to collect evidence on the potential therapeutic effects of medicinal plants or natural products on DR in preclinical studies on animals as well as clinical trials. In other words, this systematic review aims to establish a constructive database on diverse therapeutic properties of various natural products and medicinal plants in amelioration of DR in different experimental models based on various outcome measures, as a direction for future research.

MATERIALS AND METHODS

This systematic review was carried out and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[16] The PRISMA checklist was utilized to streamline the reporting process and improve the quality of the protocol. The study explicitly identifies itself as a systematic review in the title. An abstract is included, summarizing the primary objectives, methods and results of the review. The review's objective is clearly and succinctly stated. Inclusion and exclusion criteria, as well as methods for grouping studies in the synthesis, are specified. Documentation includes all consulted databases, registers, websites, organizations and reference lists. Comprehensive search strategies, including any filters and limits, are presented for all sources. Methods for determining study inclusion, including the number of reviewers and records screened, are explained. All sought outcomes are listed and defined. The process for assessing risk of bias in included studies, including tools used, number of reviewers and independence, is detailed.

Data Source

A systematic search was conducted using four database search engines, namely PubMed, Science Direct, Scopus and Google Scholar. Manual search was performed using references of included articles to obtain additional studies and information related to the search outcomes.

Search Strategy

The literature search was conducted through catalogued descriptors in MeSH (Medical Subject Headings) in English. The literature searches for each step starting from identification of studies, screening, eligibility and inclusion of studies was presented in a flowchart as shown in Figure 1, along with the inclusion and exclusion criteria used for study selection.

Study Selection

The full-text article for each study was independently screened, studied and analysed to determine the eligibility of studies to be selected.

Inclusion Criteria and Exclusion Criteria

Studies conducted over the past ten years (January 2011 to June 2020) were considered. We included studies that utilized rats as study subjects and conducted at least three of the following assays: retinal vascular permeability, Western blot analysis for VEGF, Real-Time Polymerase Chain Reaction (RT-PCR) for VEGF and body weight measurement. We excluded duplicate studies, case reports, reviews, editorials, surveys and those not involving medicinal plants from this systematic review. Additionally, phytochemical studies, *in vitro* studies, human studies, studies unrelated to Diabetic Retinopathy (DR) and studies not employing common assays for investigating DR amelioration were also excluded.

Data Extraction and Quality Assessment

Each of the searches was independently conducted by three reviewers. Disagreements were resolved by a designated coordinator. Following removal of duplicates, the titles and abstracts were independently analysed by the three reviewers to exclude studies not meeting the inclusion criteria. Data from the selected studies were extracted and tabulated using a standardized format. Any discrepancies or disagreements were adjudicated by a fourth reviewer to determine adherence to the

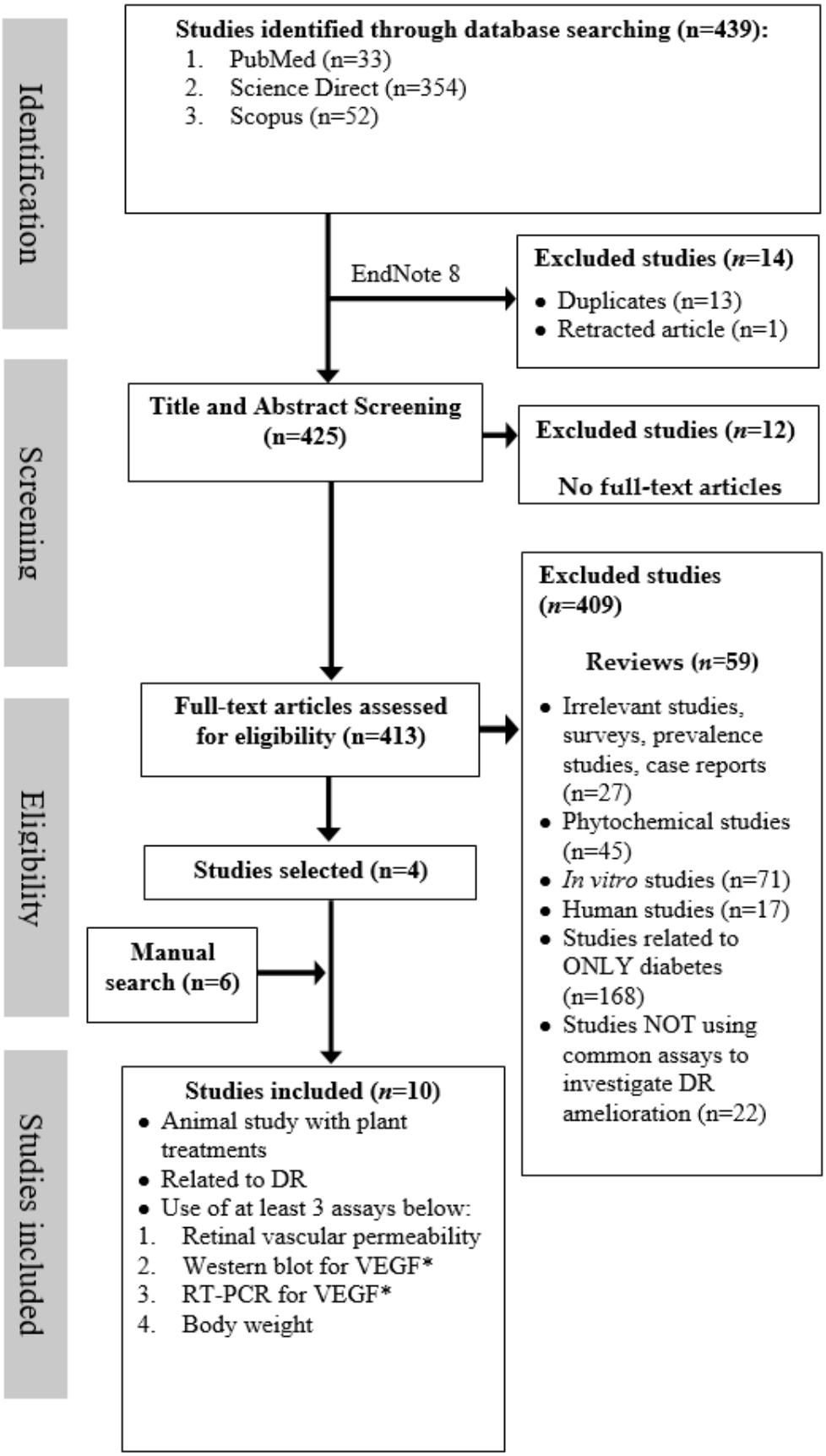

Figure 1: Flowchart of systematic literature search (VEGF*: vascular endothelial growth factor).

inclusion and exclusion criteria. The study subjects used, special treatment given, plant extraction methods and methods used for each assay with the corresponding findings were collected from the 10 selected studies.

Animal intervention studies differ from Randomized Controlled Trials (RCTs) in several aspects. Therefore, the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE)'s Risk of Bias (RoB) tool, was utilized for assessing the risk of bias in the selected studies. This approach ensured consistency and minimized discrepancies in evaluating the methodological aspects of all 10 chosen studies.[16,17] The quality of the article was assessed based on the following criteria: (1) Was the allocation sequence adequately generated and applied? (2) Were the groups similar at baseline or were they adjusted for confounders in the analysis? (3) Were the animals randomly housed during the experiment? (4) Was incomplete outcome data adequately addressed? (5) Are reports of the study free of selective outcome reporting? Each question was independently evaluated by three reviewers and categorized as 'high,' 'low,' or 'unclear risk of bias.' Discrepancies among the reviewers were identified and resolved through consensus.

RESULTS

All the relevant information included for all 10 selected studies were summarized accordingly in Supplementary Table 1.

Amelioration of Retinal Vascular Permeability

Among the 7 studies evaluating the rat retinal vascular permeability by Evans Blue (EB) dye injection, all showed amelioration in EB leakage in retinas of treated diabetic rats, with different levels of significance at $p<0.05$, $p<0.01$ or $p<0.001$ in treatment groups as compared to diabetic control model and normal rats. Four out of the 5 studies comprising positive control group (treated with calcium dobesilate, CD or fenofibric acid, FA) revealed greater amelioration of EB leakage in retinas of diabetic rats by plant treatment in comparison to synthetic drug treatment.[18-21]

Based on the study outcome by Liu *et al.* (2016), zerumbone (isolated from *Zingiber zerumbet* Rhizomes, ZZR) of higher dose (40 mg/kg/day) was considered to exhibit high potential in amelioration of blood retinal barrier disruption by demonstrating 58.1% ($p<0.01$) of reduction in EB leakage in retinas of STZ (streptozotocin)-diabetic rats.[20] As reported by Hong *et al.* (2016) and Tzeng *et al.* (2015), EEZZR (Ethanol Extract of *Zingiber zerumbet* Rhizomes) or ZZRext (*Zingiber zerumbet* rhizome ethanol extracts) also demonstrated high potential in amelioration of retinal vascular leakage induced by diabetes.[19-21] RRP (aqueous extract of *Radix Astragali, Radix angelica sinensis* and *Panax notoginseng*), as reported by Gao *et al.* (2013), also exhibited almost similar effect of about 53.2% reduction in EB leakage. The same effect was also found to be very significant in the study of Zhang *et al.* (2020) that TLFA-H (total lignans from *Fructus arctii* at high dose of 1.38 g/kg/day) treatment reduced the 3-fold increase in EB leakage of 18-19 ng/mg ($p<0.001$) seen in the diabetic control rats by approximately 52.6-55.6% to 8-9 ng/mg ($p<0.001$) in treatment group. The remaining two plant treatments-LT (*Lycopus lucidus* turcz) extract and PSEE (*Plantaginis semen* ethanol extract) in the studies by Liu *et al.* (2019) and Tzeng *et al.* (2016) demonstrated moderate and low potential, respectively but with dose-dependent manner in amelioration of EB leakage in diabetic rats.[22,23]

Western Blot Analysis for Vascular Endothelial Growth Factor (VEGF) Protein Expression

Vascular Endothelial Growth Factor (VEGF) is known to be an important angiogenic factor associated with Diabetic Retinopathy (DR) pathogenesis and thus, can be used to study the underlying mechanism behind vascular leakage into retinas. Among the 6 studies involving Western blot for analysis of VEGF protein expression, all demonstrated significant reduction in VEGF protein level in diabetic rats' retinas following treatment as compared to diabetic model. Three of the studies by Gao *et al.* (2013), Yang *et al.* (2020) and Yao *et al.* (2018) validated the high potential of the studied medicinal plants in ameliorating DR by means of reducing the tendency of VEGF in mediating increased retinal vascular permeability.[18,24,25]

The medicinal plants used included RRP (aqueous extract of *Radix Astragali, Radix angelica sinensis* and *Panax notoginseng*) treatment for 12 weeks, Essential Oil extract from Fructus *Alpiniae zerumbet* (EOFAZ) treatment for 8 weeks and *Lycium barbarum* Polysaccharides (LBP) treatment for 20 weeks respectively on diabetic rats and cell samples. RRP particularly restored VEGF relative density in diabetic rats' retinas from 2.0-2.5 units ($p<0.05$) in diabetic control to 1.0-1.5 units ($p<0.05$), compared to ~1 unit in normal rats. LBP, particularly of 400 mg/kg, downregulated VEGF protein level in diabetic rats' retinas. EOFAZ and Apocynin (16 mg/kg/d) were also found to give an obvious downregulation of VEGF protein level in diabetic rats ($p<0.05$ versus diabetic model).

The medicinal plant with moderate potential in alleviation of retinal VEGF protein expression at a dose-dependent manner was PSEE (*Plantaginis semen* Ethanol Extract). Another plant treatment possessing potential lower than other plant treatments in downregulating VEGF protein expression was 300 mg/kg EEZZR (Ethanol Extract of *Zingiber zerumbet* Rhizomes).

Real Time-Polymerase Chain Reaction (RT-PCR) for VEGF mRNA Expression

To further confirm Vascular Endothelial Growth Factor (VEGF) expression at molecular level, Real Time-Polymerase Chain Reaction (RT-PCR) is useful for analysis of its mRNA level which firstly involves reverse transcription of retinal total RNA into cDNA followed by the amplification process. All 10 selected

studies performed this assay for quantification of VEGF mRNA level. Different medicinal plants demonstrated low, moderate to high potential in amelioration of VEGF mRNA expression depending on the level of reduction of VEGF mRNA level in diabetic treatment groups. Three of the studies using 3-level dosages of plant treatments yielded a dose-dependent effect in VEGF mRNA reduction.[22,23,26] Tzeng *et al.* (2015) established the amelioration effect of the plant treatment like that of the positive control group using Calcium Dobesilate (CD) treatment,[21] while Liu *et al.* (2016) demonstrated a plant treatment with greater amelioration effect in VEGF expression as compared to that of Fenofibric Acid (FA) treatment.[20]

According to Tzeng, *et al.* (2016), PSEE gave a moderate effect in amelioration of VEGF gene expression. EEZZR,[23] as examined by Hong *et al.* (2016), demonstrated 30.8% ($p<0.05$) of reduction in VEGF mRNA level in diabetic rat retinas as compared to that in diabetic model,[16] which was considered to exhibit lower potential in improving retinal vascular leakage when compared to zerumbone and PSEE. The remaining five studies demonstrated rather low potential in alleviation of increased VEGF expression induced by diabetes.[18,20,21,24,26]

Body Weight Improvement

When the development of diabetes is characterized by decrease in body weight, an increase in body weight following treatment of medicinal plants indicates the amelioration of diabetic condition. Of the 10 selected studies within years 2011-2020, 60% (n=6) of them demonstrated positive result in alleviation of body weight loss induced by diabetes. Three of the studies by Hong *et al.* (2016), Tzeng *et al.* (2015) and Tzeng *et al.* (2016) demonstrated insignificant body weight reduction in diabetic rats while the other three studies by Liu *et al.* (2016), Liu *et al.* (2019) and Wang *et al.* (2019) demonstrated obvious increment in body weight following treatment. The remaining 40% (n=4) of the selected studies by Gao *et al.* (2013), Yang *et al.* (2020), Yao *et al.* (2018) and Zhang *et al.* (2020) did not demonstrate significant difference in body weights between control and treatment groups. This indicated that treatments involved in these studies had no effect on alleviation of body weight reduction caused by hyperglycaemia.

DISCUSSION

Most studies investigating the treatment for Diabetic Retinopathy (DR),[27-32] as well as the 10 selected animal studies involved the assays to assess retinal vascular permeability together with protein and gene expressions of Vascular Endothelial Growth Factor (VEGF) in the diabetic study subjects besides monitoring other parameters such as body weight.

Inflammatory response because of oxidative stress and protein glycation implicated by hyperglycaemia can be generated through signaling of various pro-inflammatory cytokines and lead to leukostasis. These events can include several biochemical pathways.[1,33,34] Occlusion of the retinal capillaries followed by retinal ischaemia might then occur which stimulate the release of several growth factors and cytokines. This is where VEGF, one of the important angiogenic factors, plays a key role in mediating the increased retinal vascular permeability and angiogenesis.[35-37] The resulting disruption of Blood-Retinal Barrier (BRB) which later increases the permeability of serum constituents into the neural tissues signifies the hallmark of DR.[38]

In the selected studies, the breakdown of BRB or retinal vascular permeability was quantified as the concentration of Evans Blue (EB) dye in rat dry retinas. The potential of plant treatments used in the studies was based on the high, moderate and low extent of EB dye reduction in treated diabetic rats as compared to normal rats and diabetic controls. According to all 7 studies performing this assay on the Streptozotocin (STZ)-induced diabetic rats, all used medicinal plants demonstrated potential in attenuation of BRB disruption. Of the five studies compared against synthetic drug, four of them demonstrated high potential of medicinal plants in attenuation of DR by causing greater amelioration of retinal vascular permeability than that of synthetic drugs (calcium dobesilate, CD and fenofibric acid, FA). These plants included: RRP (aqueous extract of *Radix Astragali*, Radix *Angelica sinensis* and *Panax notoginseng*),[18] EEZZR (ethanolic extract of *Zingiber zerumbet* rhizomes) or ZZRext (*Zingiber zerumbet* Rhizome ethanol extracts)[19,21] and zerumbone studied.[20] Besides that, as reported by Zhang *et al.* (2020), TLFA (total lignans of *Fructus arctii*) also demonstrated highly significant reduction of vascular permeability at its highest dose of 1.38 g/kg/day.[26]

The two selected studies by Hong *et al.* (2016) and Tzeng *et al.* (2015) examining the same type of plant-ethanol extracts of *Zingiber zerumbet* rhizomes, together with the study by Liu *et al.* (2016) on zerumbone (an important phytochemical of *Zingiber zerumbet* rhizomes, shared the similar outcome that they achieved greater attenuation of DR than drugs (CD and FA). In fact, *Zingiber zerumbet* rhizomes rich in medicinal properties have long been extensively studied for its anti-cancer, anti-microbial, anti-inflammatory and antioxidant properties but not for attenuation of diabetic complications (Vasant, *et al.*, 2017). Many studies with positive outcomes as reviewed by Chen *et al.* (2013) and Haque *et al.* (2019) have postulated its protection against diabetic microvascular complications.[39,40]

RRP is a modified Dang Gui Bu Xue Tang (DBT) comprising *Astragali Radix* (AR) or "huang-qi" and *Angelicae sinensis* Radix (ASR) or "dang-gui" combined with bitter ginger-*Panax notoginseng*. It was newly found by Gao *et al.* (2013) to significantly reduce retinal vascular leakage by 53.2% through inhibiting the expression of various pro-inflammatory factors and chemokines. Its anti-diabetic effect against the complications has also been studied since a decade ago that it not only exerts anti-inflammatory effect in diabetic atherosclerosis of rat model

as reported by Zhang *et al.* (2006) but also antioxidant property in amelioration of diabetic nephropathy according to Tzeng *et al.* (2013) and Zhang *et al.* (2019).[32,41] *P. notoginseng* with its different ginsenoside (Rg1, Rb1, Rd, Re14) and notoginsenoside (R1) constituents also exhibited anti-inflammatory and antioxidant properties.[15,42] This could most likely explain the reason RRP exhibited high potential in attenuation of retinal vascular leakage, based on the inhibitory effect of each plant included in RRP on the inflammatory processes and oxidative stress induced by diabetes. For TLFA, in the study by Zhang *et al.* (2020), its highest dose at 1.38g/kg/day reduced the 3-fold increase in EB. The *Lycopus lucidus* turcz (LT) aqueous extract and *Plantaginis semen* ethanol extract (PSEE) used in the selected studies were found to exhibit significant but lower potential than other plants aforementioned in amelioration of retinal vascular leakage. LT extract was first studied for DR attenuation by Liu, *et al.* (2019) and was found to decrease BRB permeability in diabetic rats through amelioration of inflammation and angiogenesis via p38-MAPK/NF-κB signalling pathway. [22] This finding matches with a previous study by Lee *et al.* (2008) which demonstrated the inhibitory effect of the aqueous extract of LT leaves on vascular inflammatory process induced by high glucose in Human Umbilical Vein Endothelial Cells (HUVEC).[43] Its protective effects on rat retinas demonstrated in the selected study[23] can be supported by a recent study conducted by Lei, *et al.* (2018).[44]

Among the studies, there were six plants being assessed for both VEGF protein and mRNA expressions. All the studied plants demonstrated potential in suppression on the overall expression of VEGF in diabetic rat retinas. In one of the selected studies, Gao *et al.* (2013) reported that RRP demonstrated significant downregulation of increased VEGF expression levels with its protein levels being restored close to normal in treated diabetic rats.[18] A recent study by Xie *et al.* (2020) also found that Ginsenoside Re (Re), an active ingredient of *P. notoginseng*, demonstrated inhibition on VEGF expression responsible for hyperglycaemia-induced retinal angiogenesis via "the Phosphoinositide 3-Kinase (PI3K)/AKT mediated Hypoxia-Inducible Factor-1-alpha (HIF-1α)/VEGF signal pathway" which in turn downregulated oxidative stress precipitating DR.[45]

In the selected study by Yao *et al.* (2018), *Lycium barbarum* also reduced the overall increased VEGF expression with its protein expression close to its normal value.[25] For this, Wang *et al.* (2017) have established similar outcome that it was effective in decreasing the oxidative damage of retinal nerve cells in diabetic rats via decreasing VEGF protein and mRNA levels.[46] A recent study by Wang *et al.* (2019) even demonstrated the downregulation of VEGF-induced retinal vascular hyperpermeability and angiogenesis by LBP.[47] As for the Essential Oil from *Fructus Alpiniae zerumbet* (EOFAZ) in the selected study by Yang *et al.* (2020), was found to restore VEGF protein expression back to normal level and significant reduction of VEGF gene expression in diabetic treatment group.[47]

According to the selected study by Wang *et al.* (2019), apocynin ("a natural organic compound isolated from *Picrorhiza kurroa*") was found to give an obvious downregulation of both VEGF protein and gene expressions in diabetic rats. The ability of apocynin in preserving BRB and normalizing VEGF expression has already been demonstrated earlier.[48,49]

According to Tzeng *et al.* (2016),[23] PSEE (*P. semen* ethanol extract) of highest dose (300 mg/kg) demonstrated significant lowering effect on VEGF protein and gene expressions. This was also reported by Lei, *et al.* (2018).[44]

As for LT (*Lycopus lucidus* Turcz) extract and TLFA (total lignans of *Fructus alpiniae*) among the studied plants, Liu, *et al.* (2019) and Zhang, *et al.* (2020) also successfully demonstrated the lowering effect of increased VEGF expression induced by diabetes in HRECs (human retinal endothelial cells) and diabetic rat retinas, respectively.[22,26] It was the first time both medicinal plants were evaluated for potential in DR amelioration. Therefore, more scientific studies are needed for elucidation of the mechanism in association with the lowering effect of increased VEGF expression in diabetic condition.

Lastly, unexplained weight loss is considered a classic symptom of diabetes with no planned diet or diuretic treatment.[50,51] Based on the results for body weight reported by the authors among the selected studies, zerumbone, LT extract and apocynin were found to give obvious body weight gain in treated diabetic rats. However, when compared to previous existing findings, apocynin did not cause obvious body weight alterations in the animal subjects as observed in other studies.[52,53] Thus, more evidence is needed to support the outcomes of zerumbone, LT extract and apocynin in amelioration of body weight loss induced by diabetes.

As for the two studies on ZZR ethanol extract by Hong *et al.* (2016) and Tzeng *et al.* (2015) and one study on *P. semen* Ethanol Extract (PSEE) by Tzeng *et al.* (2016), all demonstrated insignificant body weight reduction following treatment in diabetic rats.[19,21,23] This indicates that these plants prevented further body weight loss caused by hyperglycaemia. The findings on ZZR ethanol extract were equivalent to a previous study.[54] For *P. semen*, not much literature focuses on the evaluation of its effect in amelioration of body weight.[55,56]

As for the remaining 4 plant treatments namely LBP, TLFA, EOFAZ and aqueous extract of RRP, all did not demonstrate significant difference in body weight between diabetic control and treatment group. This finding somehow contradicted with

the previous findings regarding each of them. *Lycium barbarum* L. root bark extract significantly increased body weight of diabetic mice 4 weeks after treatment.[57] *Lycium barbarum* Polysaccharide (LBP) of 500 mg/kg was also reported by Du *et al.* (2016) to specifically cause 40.5% body weight increment in diet-STZ-induced diabetic rats.[58] But LBP was found to be unsuccessful in increasing the lowered body weight induced by diabetes in the latest study by Lei *et al.* (2020). For TLFA, previous studies mostly demonstrated its two main anti-diabetic effects: hypoglycaemic activity and inhibition on weight gain.[59,60] This opposed to the negative outcome in body weight amelioration demonstrated in the latest study included in this review.[26]

To ensure the reliability of the diabetic animal models used for study, the type and duration of treatment for diabetic induction in the selected studies were screened to establish the stability of the diabetic models. All the 10 selected animal studies included male rats of different stocks as study subjects, namely Wistar, Sprague-Dawley (SD) and Goto-Kakizaki rats. Streptozotocin (STZ) was used for diabetic induction in rats in 8 of the selected studies while the remaining two studies by Yang *et al.* (2020) and Zhang *et al.* (2020) on EOFAZ and TLFA respectively introduced high-fat-sugar diet (HFSD) with low-dose STZ of 25 mg/kg and 30 mg/kg for diabetic induction.[24,26] The use of HFSD followed by multiple low-dose STZ injections in rats has been found to best mimic the natural development of T2DM in human, with 25 mg/kg to be the optimal dose of STZ as higher dose of 35 mg/kg and above is more likely to resemble T1DM.[60] Since the selected studies did not use STZ for long-term studies and the remaining two studies adopted better way for diabetic induction, the diabetic models established in all selected studies are considered reliable and thus, the results generated were deemed valid.

CONCLUSION

In conclusion, all medicinal plants included in the selected studies demonstrated potential in ameliorating diabetic retinopathy by downregulating retinal vascular permeability and reducing the increased VEGF expressions induced by diabetes. Among the studied medicinal plants, zerumbone showed strong potential for its anti-retinopathy effect, significantly improving retinal vascular dysfunction, preventing body weight loss and downregulating increased VEGF expressions induced by diabetes. Further efforts are needed to elucidate the mechanisms behind each therapeutic property of these medicinal plants against DR.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ABBREVIATIONS

DR: Diabetic Retinopathy; **PRISMA:** Preferred Reporting Items for Systematic Reviews and Meta-Analyses; **SYRCLE:** Systematic Review Center for Laboratory Animal Experimentation; **VEGF:** Vascular Endothelial Growth Factor; **T2DM:** Type 2 Diabetes Mellitus; **MeSH:** Medical Subject Headings; **RCTs:** Randomized Controlled Trials; **RoB:** Risk of Bias; **EB:** Evans Blue; **CD:** Calcium Dobesilate; **FA:** Fenofibric Acid; **ZZR:** *Zingiber zerumbet* Rhizomes; **STZ:** Streptozotocin; **EEZZR:** Ethanol Extract of *Zingiber zerumbet* Rhizomes; **ZZRext:** *Zingiber zerumbet* Rhizome Ethanol Extracts; **RRP:** Aqueous Extract of *Radix astragali, Radix angelica sinensis* and *Panax notoginseng*; **TLFA-H:** Total Lignans from *Fructus arctii* at High Dose; **LT:** *Lycopus lucidus* Turcz; **PSEE:** *Plantaginis semen* Ethanol Extract; **EOFAZ:** Essential Oil Extract from Fructus *Alpiniae zerumbet*; **LBP:** *Lycium barbarum* Polysaccharides; **RT-PCR:** Real Time-Polymerase Chain Reaction; **BRB:** Blood-Retinal Barrier; **TLFA:** Total Lignans of *Fructus arctii*; **DBT:** Dang Gui Bu Xue Tang; **AR:** Astragali Radix; **ASR:** *Angelicae Sinensis Radix*; **LT:** Lucidus Turcz; **HUVEC:** Human Umbilical Vein Endothelial Cells; **PI3K:** Phosphoinositide 3-Kinase; **HIF-1α:** Hypoxia-Inducible Factor-1-Alpha; **HREC:** Human Retinal Endothelial Cells; **HFSD:** High-Fat-Sugar Diet.

AUTHOR CONTRIBUTIONS

Conceptualization, PHY and SLZY; Introduction: ZIA, methodology, MA.; quality assessments, PHY and SLZY, Results: DA and ZXN, Discussion: PHY and SLZY. All authors have read and agreed to the published version of the manuscript.

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Supplementary Table 1: Summary of the selected studies

No.	Sample Model	Treatment	Plant Processing	Assays			
				Retinal vascular permeability by Evans blue dye (ng/mg or ng/g)	**Western blot for VEGF (arbitrary units)**	**Real Time-PCR for VEGF (Threshold cycle, Ct values)**	**Body weight (g)**
(1) Gao *et al*., 2013	Male Goto- Kakizaki (GK) rats & Wistar counterparts (Shanghai, China) housed in room with controlled temperature and humidity exposed to a 12h light/dark cycle provided with unrestricted amount of rodent chow and water 8-week- old male Sprague-Dawley (SD) rats	Male Goto- Kakizaki (GK) rats & Wistar counterparts At 28 weeks of age, the rats were allocated randomly into 4 groups: Normal vehicle (=water) treated Wistar rats (CONTROL) Vehicle treated GK rats GK rats treated with 4g/kg bw/d RRP GK rats treated with 200mg/kg/ bw/d calcium dobesilate (CD) (POSITIVE CONTROL) Vehicle, RRP and CD oral administration for 12 weeks (9-10am, once daily by gavage) Male Sprague- Dawley (SD) rats of 8 weeks old T1DM induction by 60 mg/kg streptozotocin (STZ) - single intraperitoneal injection Age-matched control rats received an equal volume of vehicle (0.01 M citrate buffer, pH 4.5) Blood gluose measurement from tail vein 48hrs after STZ injection (BG>20mmol/L=diabetic) The rats were divided into 3 groups: normal SD rats (control, n=10) Diabetic rats (Diabetic, n=10) Diabetic rats with RRP treatment (4 g/kg body weight/d) (RRP, n= 10)	Aqueous extract of RRP (*Radix Astragali*, *Radix Angelica sinensis* & *Panax notoginseng*) Dang Gui Bu Xue Tang (DBT) containing aqueous extract of *Radix Astragali* and *Radix Angelica sinensis* modified with the addition of *Panax notoginseng* provided by the First Affiliated Hospital of Xiamen University, China Compound identification by HPLC for standardization Spherex C-18 analytical column (mobile phase: 0.2% formic acid in water (A) & methanol (B)) Mobile phase gradient elution programmed as: 95–80% A (0–40 minutes), 65% B (40–50 minutes), 100% B (50–55 minutes), and 95% A (55–60 minutes) Column temperature: 35°C; flow rate: 1mL/min; sample injection volume: 10μL Results of HPLC: At abs of 203nm, 4 compounds identified in RRP: Calycosin Ginsenoside Rg1 Ligustilide Ginsenoside Rb1	**Methods**			
				Evans blue (EB) dye dissolved in saline (30mg/mL). Dissolved EB dye was filtered, and injected at 45 mg/kg through tail vein in 10 seconds. Allow dye to circulate for 2hrs. Rats anaesthetized with pentobartital (40mg/kg bw). Rats' chest was opened to perform cardiac perfusion via the left ventricle with 1% para-formaldehyde in citrate buffer (0.05 M, pH 3.5) under a constant pressure of 120mmHg. Retina dissected, fully dried at 4°C and weighed. Dye extraction via incubation in 150μL formamide for 18hrs at 70°C Centrifugation of extract at 14000rpm, 25°C for 60min. Abs measured using 100μL of supernatant at 620nm and 740nm. Concentration of EB (ng/mg protein) in the extracts calculated from a std curve and normalized by dry retinal weight (mg)	Retinas were dissected and sonicated in a lysis buffer containing 50 mmol/L Tris (pH 7.6), 150 mmol/L NaCl, 5 mmol/L EDTA, 1% Triton X-100, 0.1% SDS, 0.5% deoxycholate, and a protease inhibitor cocktail.. Centrifugation of lysate at 14,000 rpm at 4°C for 10min. Protein concentrations were determined using supernatants by Bradford method. Separation of retinal proteins by SDS-PAGE and transferred to polyvinylidene fluoride membrane Membranes were blotted overnight at 4°C with anti-VEGF (1:500) after blocking. Horseradish peroxidase (HRP)-linked anti-rabbit or mouse (1: 10,000) was used for secondary detection. Immuno- reactivity was visualized using an ECL (Enhanced chemi- luminescence) kit and Kodak X-OMAT film. Quantification of band intensities using a gel documentation system with Quantity One software (Bio-Rad). Relative gene expression was normalized by β-actin	Extraction of total RNA using RNA simple Total RNA Kit Total RNA was reverse transcribed to cDNA with primeScript RT reagent kit using gDNA eraser in a thermal cycler Real time quantitative PCR was performed with the SYBR Premix Ex Taq real-time PCR kit in a Light Cycler 480 System VEGF primers: sense, 5'-ACA GGG AAG ACA ATG GGA TGA-3'; antisense, 5'-GGG CCA GGG ATG GGT TT-3' Threshold cycle (Ct) values of target genes were normalized with α-actin of the same sample and expressed as relative to controls	Measurement performed twice weekly Findings:
				Findings			
				RRP attenuated retinal vascular permeability. The retinal blood vessel permeability in **Diabetic group** significantly increased (12.1±4.5 ng/mg) compared with that in control group (3.2±1.4 ng/mg) ($p < 0.001$) showing an impaired BRB in diabetes. **CD treatment** significantly decreased retinal vascular permeability (6.9±1.0 ng/mg, $p<0.05$) **RRP** reversed the retinal vascular permeability to a further extent (5.9±2.4 ng/mg, $p< 0.01$). Similar to GK rats, compared with the control SD rats, diabetic SD rats had more leaked FITC-labeled albumin in their retina. **RRP treatment** significantly reduced retinal vascular leakage	By Western blot analysis, retinas of control SD rats demonstrated significant increase in VEGF level in diabetic condition, and RRP treatment greatly reversed this change ($p<0.05$ versus diabetic rats)	In diabetic group, retinal mRNA levels of VEGF were significantly increased (1.5- to 3.5-fold) compared to control rats ($p < 0.05$) RRP restored increased VEGF mRNA expression levels to control values in the diabetic treatment group	RRP caused no change to body weight after 12-week treatment There is no difference in body weight among groups: **Control:** 409.0±31.2g **Diabetic:** 411.0±47.3g **CD group:** 409.8±32.1g **RRP group:** 411.2±33.0g

No.	Sample Model	Treatment	Plant Processing	Assays			
				Retinal vascular permeability by Evans blue dye (ng/mg or ng/g)	**Western blot for VEGF (arbitrary units)**	**Real Time-PCR for VEGF (Threshold cycle, Ct values)**	**Body weight (g)**
(2) Hong *et al*., 2016	Male Wistar rats (8-10 weeks of age, 200-250g), n=40	WEEK 1 A single dose of 60mg/kg STZ IV injection WEEK 2 After 1 week, rats developing non-FBG level >350mg/dL, polyuria and glucosuria were considered diabetic 3-month treatment Allocation into 5 groups (n=8 per group): Oral EEZZR-200 (200mg/kg in 1.5mL/kg distilled water), once per day for 3 months Oral EEZZR-300 (300mg/kg in distilled water), once per day for 3 months Oral CD daily dose at 500mg/kg for 12 weeks (POSITIVE CONTROL) Vehicle- treated (1.5mL/kg distilled water) STZ-diabetic rats (CONTROL) Vehicle treated (1.5mL/kg distilled water) normal rats (CONTROL) END OF EXPERIMENT After 18-hr fasting and anaesthetized by IP injection of 60mg/kg sodium pentobarbital blood collection from abdominal aorta Removal of rat eyes, washed with cold normal saline for eye homogenate preparation and histo- pathological examinations	Ethanol extract of *Zingiber zerumbet* rhizomes (EEZZR) of 200 & 300mg/kg ZZR extracted by maceration and air-dried 5kg of pulverized ZZR was added to 10L of 95% ethanol at RT for 7 days Occasional shaking was applied Evaporation of EEZZR to dryness under reduced pressure to totally remove alcohol, followed by lyophilization Yielding of ~583g of dry residue (w/w yield: 11.7%) Storage at -20°C until use and suspended in distilled water Analysis was done using LC/MS/MS (liquid chromatography (LC) tandem mass spectrometry): (LC) using HPLC apparatus with 2 micropumps and a UV/Vis (Ultraviolet- Visible) detector; (MS/MS) using triple quadrupole mass spectrometer	**Methods**			
				EB dye was firstly dissolved in saline (30mg/mL), filtered, injected with 45mg/kg dose through tail vein within 10s After dye circulation for 2hrs, anaes-thetization using pentobarbital at 40mg/kg bw was given, chest cavity was opened to allow cardiac perfusion through left ventricle Dissection of retina: fully dried at 4°C and weighed Extraction of EB dye by incubation at 70°C in 150µL formamide for 18hrs Abs (620nm and 740nm) measurement using 100µL supernatant Calculation of EB dye concentration (ng/mg protein) from a standard curve Concentration of EB dye normalized by dry retina weight (mg)	Preparation of retina homogenate in western lysis buffer (30mmol/L Tris–HCl, pH 7.4, 250 mmol/L Na_3VO_4, 5mmol/L EDTA, 250mmol/L sucrose, 1% Triton X-100 with protease inhibitor and phosphatase inhibitor cocktail). Centrifugation of lysate at 14,000xg for 10 min at 4°C and the supernatant was collected. Separation of tissue lysates containing 40–50mg protein on 8–15% SDS-polyacrylamide gels Transfer of separated lysate onto nitro-cellulose membranes 5% skim milk in Tris-buffered saline (TBS) supplemented with 0.1% Tween-20 (TBST) was used for blocking the membrane for 1hr. Lysates were then incubated with primary antibodies against vascular endothelial growth factor (VEGF). Antibodies used at dilution of 1:1000 (After 3X Washing with TBST) incubation with secondary antibodies conjugated with horseradish peroxidase (HRP) for 1hr at RT. (After extra 3X washing with TBST) visualization of immunoreactive bands. Quantification of band densities. Enhanced chemi- luminescence for visualization of immunoreactive bands. Densitometric analysis for quantifying band densities using ATTO Densitograph Software	Extraction of total RNA from rat retinas using TRIzol reagent Reverse transcription. Heating of 1µg of total RNA per sample and 8.5µg/µL random hexamer primers at 65°C for 5min Quenching on ice. Addition of 500 µmol/L each of dATP, dTTP, dCTP, and dGTP, 10 mmol/L DTT, 20 mmol/l Tris–HCl (pH 8.4), 50 mmol/l KCl, 5 mmol/l $MgCl_2$, 40 units of recombinant ribonuclease inhibitor and 100 units reverse transcriptase Samples subjected to DNase treatment for 20min at 37°C in thermal cycler and held at 4°C PCR. Measurement of mRNA expressions by quantitative real-time reverse transcription PCR in a fluorescent temperature Lightcycler 480. Primer sequence for VEGF used: 5′-GCGGGCTGCTGCAATG-3′ (forward) and 5′-TGCAACGCGAGTCTGTGTTT-3′ (reverse). PCR protocol: 95°C for 5min, followed by 45 cycles of 95°C for 5s, 58°C for 15s, and 72°C for 20s. Dissociation curves run after amplification for identification of specific PCR products. mRNA expression were normalized to β-actin mRNA levels. Calculation of mRNA expression by delta-delta Ct method	Body weight was monitored throughout the 12-week treatment
				Findings			**Findings**
				The retinal blood vessel permeability in STZ-diabetic rats significantly increased (13.2±1.6 ng/mg) compared with that in normal control group (2.8 ±1.5 ng/mg (p<0.01). Treatment of STZ-diabetic rats with CD for 12 weeks decreased retinal vascular permeability (7.8 ±1.4 ng/mg) (p<0.05 versus both vehicle- treated normal rats and STZ- diabetic rats). 200mg/kg/day EEZZR reversed the retinal vascular permeability (p<0.01 versus vehicle-treated normal rats; p<0.05 versus vehicle-treated STZ-diabetic rats) 300 mg/kg/day EEZZR reversed the retinal vascular permeability to a further extent (6.2 ±1.2 ng/mg) (p<0.05 versus both vehicle- treated normal rats and STZ- diabetic rats)]	The retinal expression of VEGF demonstrated significant elevation in the STZ-diabetic rats compared with those in normal rats at protein levels EEZZR-300 downregulated retinal VEGF proteins with a decrease of 35.7%, when compared with those observed in the vehicle- treated STZ-diabetic rats (p<0.05) VEGF-to- PEDF (pigment epithelium- derived factor, eye protective protein) ratio increased in 3.1 fold in STZ-diabetic rats, which was downregulated by EEZZR-300 (61.4% decrease, p<0.01 versus vehicle-treated STZ-diabetic rats)	The retinal expression of VEGF was significantly increased in the STZ-diabetic rats compared with those in normal rats at mRNA levels EEZZR-300 downregulated retinal VEGF mRNA expression with a decrease of 30.8%, when compared with those observed in vehicle-treated STZ-diabetic rats (p<0.05)	During 12-week treatment, significant weight loss is seen in STZ-diabetic rats Body weight reduction became not obvious in rats receiving EEZZR or CD treatment Body weight at the end of 12-week treatment: Normal rats (vehicle- treated) - 321.4±12.6g (p<0.01 versus diabetic control) STZ- diabetic rats (vehicle-treated) - 216.3±14.7g (p<0.01 versus normal control) EEZZR- 200 treated STZ-diabetic rats - 262.3±11.2g (p<0.01 versus normal control; p<0.05 versus diabetic control) EEZZR- 300 treated STZ-diabetic rats - 281.8±13.4g (p<0.05 versus normal control; p<0.01 versus diabetic control) CD-treated STZ-diabetic rats - 257.7±15.3g (p<0.01 versus normal control; p<0.05 versus diabetic control)]

No.	Sample Model	Treatment	Plant Processing	Assays			
				Retinal vascular permeability by Evans blue dye (ng/mg or ng/g)	**Western blot for VEGF (arbitrary units)**	**Real Time-PCR for VEGF (Threshold cycle, Ct values)**	**Body weight (g)**
(3) Liu *et al*., 2016	Male Wistar rats (250-250g) housed two per cage under controlled 20-25℃, humidity (50%±5%) Exposed to lighting (12h light/ dark cycle) Provided with food and water ad libitum	**WEEK 1** STZ induction of diabetes by IV injection at dose 60mg/kg Vehicle treatment: sterile saline 0.9%, pH7.4) **WEEK 2** After one week, rats were deemed diabetic by having: Non fasting blood glucose levels>350mg/dL Polyuria Glucosuria **WEEK 3 - 3-month treatment** Allocation to 5 groups: Oral 20mg/kg zerumbone (≥98%) in 1.5mL/kg distilled water Oral 40mg/kg zerumbone (≥98%) in 1.5mL/kg distilled water Vehicle- treated normal rats Vehicle- treated STZ- diabetic rats Oral fenofibric acid (FA) of 100mg/kg/day (purity≥98%) **END OF TREATMENT** Rats were weighed, fasted overnight and anesthetized using an IP injection of sodium pentobarbital (60 mg/kg) They were sacrificed followed by blood collection from abdominal aorta Removal of rat eyes and isolation of retinae	Zerumbone (≥98%; Sigma-Aldrich, Inc.) of 20 mg/kg and 40 mg/kg, each in 1.5mL distilled water	**Methods**			
				EB dye was firstly dissolved in normal saline at 45mg/mL Injection of EB dye of 45mg/kg through tail vein of anaesthetized rats for 10s After 2-hr circulation, their chest was opened to allow cardiac perfusion via left ventricle, with 1% para-formaldehyde in citrate buffer (0.05 mol/L, pH 3.5) under a constant pressure of 120 mmHg Dissection of retinas to be air-dried at 4°C Dry retinas were weighed and incubated in 150µL formamide for 18hrs at 70 ◦C for EB dye extraction Ultra- centrifugation of extract at 14,000rpm for 60min Measurement of abs at 620nm and 740nm using 100µL of supernatant Calculation of EB concentration in the extracts from a standard curve by normalizing the weight of dry retinas	–	Extraction of rat retina total RNA using a Trizol reagent. RNA Quantification by A260 and visualization by agarose gel electrophoresis using ethidium bromide **Reverse transcription** 1 µg of total RNA per sample and 8.5µg/µL random hexamer primers were heated at 65℃ for 5 min and then quenched on ice . This mixture was combined with 500 µmol/L each of dATP, dTTP, dCTP, and dGTP, 10 mmol/L DTT, 20 mmol/L Tris-HCl (pH 8.4), 50mmol/L KCl, 5mmol/L $MgCl_2$, 40 units of RNase recombinant ribonuclease inhibitor and 100 units reverse transcriptase. Samples were treated with DNase at 37℃ for 20 min in a GeneAmp 9700 Thermal Cycler and held at 4℃ **PCR** Measurement of mRNA expression by quantitative real time-PCR in a fluorescent temperature Lightcycler 480. Primer sequence used: 5'-ACAGGGAAGACAATGGGATGA -3'(forward) and 5'-GGGCCAGGGATGGGTTT-3' (reverse) for VEGF. PCR cycling protocol: 95℃ for 5 min, followed by 45 cycles of 95℃ for 5s, 58℃ for 15s, and 72℃ for 20s . After amplification, dissociation curves were run for identifying PCR products mRNA expression normalized to β-actin mRNA levels and calculated by delta-delta Ct method	Body weight measurement after 3 months of treatment
				Findings			
				Increased leakage was observed in the retinas of STZ-diabetic rats Zerumbone-20 and -40 treatment for 3 months decreased retinal EB dye accumulation in retina by 31.8% ($p<0.05$ versus vehicle treated STZ-diabetic rats) and 58.1% ($p<0.01$ versus vehicle treated STZ-diabetic rats) respectively Fenofibric acid (FA) treatment decreased retinal vascular leakage by 56.7% in STZ-diabetic rats ($p<0.01$ versus vehicle treated STZ diabetic rats)	–	Retinal VEGF mRNA expression was significantly increased in STZ-diabetic rats compared to normal rats Retinal VEGF mRNA expression was decreased by zerumbone-40 to 67.7% as compared to STZ-diabetic rats ($p<0.05$)	Body weights of the STZ- diabetic rats were lower ($p<0.01$) than those of the normal rats Body weight of zerumbone-40 treated STZ-diabetic rats were 25.9% higher than STZ diabetic rats ($p<0.05$). Similar results were seen in positive control group (STZ diabetic rats treated with fenofibric acid) ($p<0.01$ versus vehicle treated normal rats; $p<0.05$ versus vehicle treated STZ-diabetic rats)

No.	Sample Model	Treatment	Plant Processing	Assays			
				Retinal vascular permeability by Evans blue dye (ng/mg or ng/g)	**Western blot for VEGF (arbitrary units)**	**Real Time-PCR for VEGF (Threshold cycle, Ct values)**	**Body weight (g)**
(4) Liu *et al*., 2019	Male Sprague Dawley (SD) rats (8-week old, 220-250g), n=30 Rats raised in a suitable environment with 24±3℃ Exposed to 12-hr light/dark cycle Food and water supply were given to allow acclimatiza- tion to the environment for at least 3 days before experiment Human retinal micro-vascular endothelial cells (HRECs) cultured in RMPI supple-mented with 10% foetal bovine serum at 37℃ in 5% CO_2	SD Rats All rats (n=30) were randomly allocated into control (n=5) and diabetic retinopathy (DR) (n=25) groups <u>WEEK 1-5</u> Control rats fed with ordinary diet; DR rats fed with high-fat diet for 5 weeks DR rats were given STZ IP injection (35mg/kg); control rats were injected with 35mg/kg natrium citricum buffer solution (0.1mol/L, pH4.5) <u>WEEK 6</u> Both groups of rats were fed with their corresponding diets Blood glucose (measured using caudal vein blood) reaching 16.7mmol/L or higher represented DR model <u>18-WEEK TREATMENT (6 groups, n=5 per group)</u> Control rats DR model rats LT 3mg/kg LT 6mg/kg LT 12mg/kg Calcium Doxium (CD) 150mg/kg (positive control) <u>END OF TREATMENT</u> Rats were sacrificed by abdominal aortic method Removal of eyes and isolation of retinas Retinas was frozen in liquid nitrogen and stored at -80℃ Human retinal micro- vascular endothelial cells (HRECs) Cells treated with glucose at different doses and assigned to 6 groups: Negative control (5mmol/L) High glucose (HG) model (30mmol/L) HG with 3mg/kg LT group HG with 6mg/kg LT group HG with 12mg/kg LT group HG with CD group	*Lycopus lucidus* turcz (LT) extract 100g dried LT was added with 10 times the amount of distilled water and incubated at 100℃ for 2hrs Addition of 8 times the amount of water for 1.5hr Centrifugation of mixture Concentration of combined supernatants to 100mL using rotary vacuum evaporator Aqueous suspension processed into a freeze-dried powder using a vacuum freeze dryer, stored at -80℃. For treatment, LT powder was dissolved in deionized distilled water and filtered through a 0.22μm disk filter	**Methods**			
				● IP Injection of EB dye (2% EB dissolved in phosphate buffered saline, PBS) ● After 2-hr circulation, injection of PBS into left ventricle for cardiac perfusion for EB extraction ● Dissection of retinas and weighed, and completely dried ● Dry retinas treated with 120μL formamide at 70℃ for 18hrs to collect EB dye ● The extract was centrifuged at 10,000g 2X at 4℃ for 1hr ● Abs of the supernatant at 620nm was measured ● Concentration of EB dye in each extract was analysed using a standard curve ● Each sample was measured 3 times	–	Trizol reagent was used for total RNA extraction from HRECs **<u>Reverse transcription</u>** RNA was reverse- transcribed to cDNA using QuantiTect RT kit **<u>Real time-PCR</u>** PCR cycling protocol: 95℃ for 5 min and then 40 cycles of 94℃ for 15s, 55℃ for 20s, 72℃ for 20s, followed by 72℃ for 7 min Gene expression normalized to GAPDH (endogenous control) Primer sequences used: VEGF, forward 5'-GGGCCTCGGTTCCAGAAG-3' and reverse 5'-AACTTCACCACTTCATGGGCT-3' Expression levels were analysed following the $2^{-\Delta\Delta Cq}$ calculation method	Body weight of rats was measured every 4 week
				Findings			
				● BRB permeability was elevated in the DR model group compared to that of control group ● LT 6mg/kg group specifically decreased BRB permeability in diabetic rats (lower permeability than DR model group) ($p<0.001$ versus DR model; $p<0.01$ versus positive control)	–	● mRNA expression level of VEGF was increased in HG-induced HRECs ● Elevated mRNA level was reduced after LT treatment in a dose- dependent manner (3mg/kg LT: $p<0.01$ versus positive control; 6mg/kg LT: $p<0.05$ versus DR model; 12mg/kg LT: $p<0.001$ versus DR model)	LT treatment gave obvious increase in body weight of treated diabetes rats compared to untreated rats

No.	Sample Model	Treatment	Plant Processing	Assays			
				Retinal vascular permeability by Evans blue dye (ng/mg or ng/g)	Western blot for VEGF (arbitrary units)	Real Time-PCR for VEGF (Threshold cycle, Ct values)	Body weight (g)
(5) Tzeng *et al*., 2016	Male Wistar rats (8-10 week old, 200-250g), n=40	**WEEK 1** 60mg/kg IV injection of STZ for diabetes induction **WEEK 2** **Criteria for diabetes:** After 1 week of STZ injection, non-fasting blood glucose more than 350mg/dL Polyuria Glucosuria **TREATMENT** 200mg/kg or 300mg/kg doses of ZZRext were mixed with 1.5mL/kg distilled water **WEEK 4 - 3-month treatment** 2 weeks after STZ injection, rats were allocated into 5 groups: Oral ZZRext 200mg/kg treatment group (n=8) Oral ZZRext 300mg/kg treatment (n=8) Oral Calcium dobesilate (CD) 500mg/kg treatment group (n=8) *Vehicle treated normal rats (n=8) *Vehicle treated STZ-diabetic rats *Vehicle = 1.5mL/kg distilled water **END OF TREATMENT** Rats were anaesthetized using IP injection of sodium pentobarbital (60mg/kg) after 18-hr fasting Blood collection from abdominal aorta Removal of rat eyes, flash frozen in liquid nitrogen and kept for -80°C storage (for biochemical analysis) Eyes were formalin fixed for histological analysis	*Zingiber zerumbet* rhizome ethanol extract (ZZRext) Coarse powder preparation by comminution and filtration (20-40 mesh) 20g powder was ground in a 95% (v/v) ethanol solution using a mixer Extraction of samples for 3 days with vigorous shaking Isolation of filtrate by membrane filtration for removal of macro- and micro- molecular components Dry weight of ZZRext yielded 12.3% ZZRext was concentrated using rotary- vacuum evaporator at 50°C and was later freeze- dried	**Methods**			
				EB dye was first dissolved in saline (30mg/mL), filtered and injected through tail vein at 45mg/kg dosage within 10s After 2-hr circulation, rats were anaesthetized with 40mg/kg/bw pentobarbital Their chest cavity was then opened for cardiac perfusion via left ventricle with 1% para- formaldehyde in citrate buffer (0.05mol/L, pH3.5) under constant pressure of 120mmHg Dissection of retinas, weighing and and drying at 4°C EB dye extraction by incubation in 150µL formamide for 18hrs at 70 ◦C Abs measurement using 100µL supernatant at 620 and 740nm Concentration of EB in the extracts was calculated from a standard curve and normalized by dry retina weight	–	Extraction of rat retina total RNA using a Trizol reagent. RNA Quantification by A260 and visualization by agarose gel electrophoresis using ethidium bromide **Reverse transcription** 1 µg of total RNA per sample and 8.5µg/µL random hexamer primers were heated at 65°C for 5 min and then quenched on ice This mixture was combined with 500 µmol/L each of dATP, dTTP, dCTP, and dGTP, 10 mmol/L DTT, 20 mmol/L Tris-HCl (pH 8.4), 50mmol/L KCl, 5mmol/L $MgCl_2$, 40 units of RNase recombinant ribonuclease inhibitor and 100 units reverse transcriptase Samples were subjected to DNase treatment at 37°C for 20 min in a GeneAmp 9700 Thermal Cycler and then held at 4°C **PCR** Measurement of mRNA expression by quantitative real time-PCR in a fluorescent temperature Lightcycler 480 Primer sequence used: 5'-ACAGGGAAGACAATGGGATGA -3'(forward) and 5'-GGGCCAGGGATGGGTTT-3' (reverse) for VEGF PCR cycling protocol: 95°C for 5 min, followed by 45 cycles of 95°C for 5s, 58°C for 15s, and 72°C for 20s. Dissociation curves run after amplification to identify PCR products. mRNA expression normalized to β-actin mRNA levels and calculated according to the delta-delta Ct method	Body weight measured throughout the whole experiment
				Findings			
				STZ-diabetic rats showed increased EB dye leakage in the retinas STZ-diabetic rats with treatment had higher retinal vascular permeability than normal control but lower retinal vascular leakage than untreated STZ-diabetic rats. Treatment of STZ-diabetic rats with ZZRext for 3 months decreased retinal vascular permeability dose- dependently ($p<0.05$ versus vehicle treated normal rat and STZ-diabetic rats). CD treatment (positive control) yielded a 42.6% ±2.3% decrease in retinal vascular permeability in STZ diabetic rats ($p<0.05$ versus vehicle treated STZ-diabetic rats). ZZRext-300 yielded greater reduction in EB dye leakage than CD treatment group	–	STZ-diabetic rats had higher retinal mRNA VEGF as compared to controls ($p<0.05$) mRNA expression of VEGF in STZ-diabetic rats were lower than those vehicle-treated counterparts CD treatment reduced retinal mRNA VEGF level in STZ- diabetic rats, as compared to vehicle-treated counterparts ($p<0.05$ versus vehicle treated STZ-diabetic rats) Highest dose of ZZRext (300mg/kg) produced same effect as CD treatment ($p<0.05$ versus vehicle treated STZ- diabetic rats)	Weight gain in STZ-diabetic rats was significantly less compared to normal rats Body weight reduction was not obvious in ZZRext and CD treatment groups Vehicle treated normal rats (337.1±11.9g) Vehicle treated STZ- diabetic rats (217.1±13.9g), $p<0.05$ versus normal rats. ZZRext-200 treatment (265.4±10.6g), $p<0.05$ versus normal and STZ- diabetic rats ZZRext-300 treatment (285.9±12.7g), $p<0.05$ versus normal and STZ- diabetic rats CD treatment (270.6±14.5g), $p<0.05$ versus normal and STZ- diabetic rats

No.	Sample Model	Treatment	Plant Processing	Assays			
				Retinal vascular permeability by Evans blue dye (ng/mg or ng/g)	**Western blot for VEGF (arbitrary units)**	**Real Time-PCR for VEGF (Threshold cycle, Ct values)**	**Body weight (g)**
(6) Tzeng *et al*., 2016	Male Wistar rats (8-week old, 200-250g), n=50 housed two per cage under controlled temperature (20-25℃), humidity (50%±5%) Exposed to lighting (12-hr light/dark cycle) Provided with food and water ad libitum	IV injection of STZ (60mg/kg) for induction of diabetes Vehicle treatment: sterile saline 0.9% at pH7.4 **Criteria for diabetes:** After 1 week of STZ injection, non-fasting blood glucose more than 350mg/dL Polyuria Glucosuria **TREATMENT** 100, 200, or 300 mg/kg PSEE was mixed with 1.5mL/kg distilled water for use Vehicle = 1.5mL/kg distilled water **TREATMENT for 8 weeks** 5 groups (n=10 per group) Vehicle- treated normal rats Vehicle treated STZ diabetic rats PSEE 100mg/kg treatment in STZ diabetic rats PSEE 200mg/kg treatment in STZ diabetic rats PSEE 300mg/kg treatment in STZ diabetic rats **END OF TREATMENT** Rats were weighed, fasted overnight and anesthetized using an IP injection of sodium pentobarbital (60 mg/kg) They were sacrificed followed by blood collection from abdominal aorta Removal of rat eyes and isolation of retinae	*Plantaginis semen* (10kg) ground into a 40-mesh powder extracted in 95% ethanol (5 volumes of ethanol) in a stainless steel extraction tank at RT for 24hrs Filtration of ethanol mixture through funnels and centrifugation at 1350xg, at 4℃ for 20min (repeated thrice) Removal of precipitate Concentration of ethanol extract using rotary evaporator to produce *Plantaginis semen* ethanol extract (PSEE) Evaporation of PSEE under reduced pressure conditions to eliminate alcohol Lyophilization of PSEE yielded ~1328g dry residue (w/w yield: 13.2%) PSEE kept at -20℃ until use	**Methods**			
				EB was firstly dissolved in saline (45mg/mL), filtered, injected with 45mg/kg dose through tail vein within 10s. After 2-hr dye circulation, anaesthetization with pentobarbital (40mg/kg bw) was given Their chest cavity was opened to allow cardiac perfusion via left ventricle (with 1% para- formaldehyde in citrate buffer (0.05 mol/L, pH 3.5) under a constant pressure of 120 mmHg) Dissection of retina: fully dried at 4℃ and weighed Extraction of EB dye by incubation at 70℃ in 150µL formamide for 18hrs Abs (620nm and 740nm) measurement using 100µL supernatant Concentration of EB (ng/mg protein): calculated from a standard curve and normalized by total protein concentration in the retinal tissues	Right and left retinas were pooled as one sample, which were homogenized in 1mL ice cold hypotonic buffer A (10 mmol/L of HEPES, 10 mmol/L of KCl, 2 mmol/L of $MgCl_2$, 1mmol/L of dithiothreitol, 0.1 mmol/L of EDTA, and 0.1 mmol/L of phenylmethyl-sulfonylfluoride; pH 7.8). A solution of 80 µL of 10% Nonidet P-40 was added to the homogenates, and the mixture was centrifuged for 2 min at 14,000xg at 4℃. Determination of protein concentration of each sample using a Bio-Rad protein assay kit Bovine serum albumin (BSA) used as a standard. Electrophoresis of tissue lysates containing 40-50mg protein through 8%, 12% and 15% SDS-polyacrylamide gel Separated proteins were electro-phoretically transferred to a nitrocellulose membrane 5% skim milk solution was used for blocking of membrane for 1hr Overnight incubation of membrane with primary antibodies to VEGF at 4℃. Antibodies used at dilution of 1:1000. After 3X 5min washes in Tris-buffered saline with Tween (TBST; 20 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, and 0.05% Tween 20), membranes were incubated with the appropriate peroxidase- conjugated secondary antibodies. After 3X wash in TBST, membranes visualized on X-ray film Band densities were determined using ATTO Densitograph Software and quantified as the ratio to β-actin. The mean value for samples was adjusted to a value of 1.0 from the vehicle- treated normal rats on each immunoblot, expressed in densitometry units. All experimental sample values were expressed to this adjusted mean value	Extraction of rat retina total RNA using a Trizol reagent Right and left retinas were pooled as one sample RNA quantification by measuring abs at 260nm and visualization by agarose gel electrophoresis using ethidium bromide **Reverse transcription** 1 µg of total RNA per sample and 8.5µg/µL random hexamer primers were heated at 65℃ for 5 min and then quenched on ice This mixture was combined with 500 µmol/L each of dATP, dTTP, dCTP, and dGTP, 10 mmol/L DTT, 20 mmol/L Tris-HCl (pH 8.4), 50mmol/L KCl, 5mmol/L $MgCl_2$, 40 units of RNase recombinant ribonuclease inhibitor and 100 units reverse transcriptase Samples were treated with DNase at 37℃ for 20min in a GeneAmp 9700 Thermal Cycler and held at 4℃ **PCR** Measurement of mRNA expression by quantitative real time-PCR in a fluorescent temperature Lightcycler 480 Primer sequence used: 5'-ACAGGGAAGACAATGGGATGA -3'(forward) and 5'-GGGCCAGGGATGGGTTT-3' (reverse) for VEGF PCR cycling protocol: 95℃ for 5 min, followed by 45 cycles of 95℃ for 5s, 58℃ for 15s, and 72℃ for 20s Dissociation curves run after amplification to identify PCR products mRNA expression normalized to β-actin mRNA levels and calculated according to the delta-delta Ct method	Measurement of body weight at the end of experiment
							Findings
				Findings			Body weight of diabetic rats was significantly less than that of the normal rats Body weight reduction was not obvious in diabetic rats receiving PSEE treatment as compared to vehicle treated normal and diabetic rats Vehicle treated normal rats: 353.6±10.3g ($p<0.01$ versus vehicle treated STZ- diabetic rats) Vehicle treated STZ- diabetic rats: 181.7±8.9g ($p<0.01$ versus vehicle treated normal rats) PSEE 100: 208.3±9.3g ($p<0.01$ versus normal rats; $p<0.05$ versus STZ- diabetic rats) PSEE 200: 228.7±11.3g ($p<0.01$ versus normal rats; $p<0.05$ versus STZ- diabetic rats) PSEE 300: 239.6±10.7g ($p<0.01$ versus normal rats; $p<0.05$ versus STZ- diabetic rats)
				Increased leakages of EB dye were observed in retinas of STZ diabetic rats ($p<0.01$ versus vehicle treated normal rats) PSEE 100, 200 and 300 treatments for 8 weeks decreased retinal EB dye accumulation by 21.7% ($p<0.05$), 29.5% ($p<0.05$) and 36.4% ($p<0.01$) respectively	Protein expression of VEGF was significantly increased in the STZ-diabetic rats ($p<0.01$) compared to that of normal rats 300mg/kg/day PSEE treatment has decreased VEGF mRNA level by 45.3%, as compared to vehicle treated STZ-diabetic rats ($p<0.01$)	mRNA expression of VEGF was significantly increased in the STZ-diabetic rats compared to that of normal rats 300mg/kg/day PSEE treatment has decreased VEGF mRNA level by 54.3%, as compared to vehicle treated STZ-diabetic rats ($p<0.01$)	

No.	Sample Model	Treatment	Plant Processing	Assays			
				Retinal vascular permeability by Evans blue dye (ng/mg or ng/g)	**Western blot for VEGF (arbitrary units)**	**Real Time-PCR for VEGF (Threshold cycle, Ct values)**	**Body weight (g)**
(7) Wang *et al*., 2019	Male Wistar-NIN rats with average body weight of 230±23g, n=45	**Diabetes induction** IP injection of STZ at 32mg/kg bw diluted in 0.1M citrate buffer **Normal control** 0.1M citrate buffer **AFTER 72HRS** Diabetes is defined as blood glucose more than 150mg/dL **12-WEEK TREATMENT** 1. Normal control (n=15) 2. DM group: fed with AIN-93 diet (n=15) 3. DM+apocynin group: rats fed with AIN-93 diet and 16mg/kg/day apocynin (n=15) - Blood samples from retro-orbital plexus collected once a week for analysis **END OF TREATMENT** Dissection of retinas from eye after 12-week of apocynin treatment In each group of 15 rats, retinas from 12 rats were stored at -80℃ for biochemistry analysis and retinas from the remaining 3 rats were fixed for histological detection	Apocynin of 16mg/kg/day (methoxy-subtituted catechol extracted from the root of a medicinal herb - *Picrorhiza kurroa*)	**Methods**			
				–	Western blot performed by standard method Band intensity was quantified using Image J software Anti-VEGF was used	Tri-reagent was used to isolate retinal total RNA **Reverse Transcription** Use of High Capacity cDNA Reverse Transcription kit to reverse transcribe total RNA to cDNA **qRT-PCR** performed with use of SYBR green master mix in triplicates primers for VEGF: Forward, GGC TCA CTT CCA GAA ACA CG; Reverse, GTG CTC TTG CAG AAT CTA GTG G mRNA expression normalized to β-actin mRNA levels and calculated with accord to delta-delta Ct method	Body weight of rats were measured during the whole experimental period
				Findings			
				-	Apocynin attenuated VEGF expression in diabetic rats' retinas Diabetic rats treated with apocynin demonstrate an obvious lower level of VEGF protein as compared to untreated diabetic rats ($p<0.05$)	Apocynin attenuated VEGF expression in diabetic rats' retinas mRNA level of VEGF was upregulated in retinas of diabetic rats but was decreased by apocynin treatment ($p<0.05$ versus control group; $p<0.01$ versus DM group)	Decline in body weight in DM rats from 300g to below 200g ($p<0.01$ versus control group) Apocynin treatment caused the declined body weight to be up-regulated (above 200g) ($p<0.05$ versus both control and DM groups)

No.	Sample Model	Treatment	Plant Processing	Assays			
				Retinal vascular permeability by Evans blue dye (ng/mg or ng/g)	Western blot for VEGF (arbitrary units)	Real Time-PCR for VEGF (Threshold cycle, Ct values)	Body weight (g)
(8) Yang *et al.*, 2020	**Male Sprague Dawley rats (3-month-old), n=32** maintained in a pathogen-free facility (23 °C±2 °C, 50%±15% relative humidity Exposed to 12:12 h light/dark cycle Provided with free access to water and regular chow or high fat and high sucrose diet (HFSD) For fasting experiments, rats were fasted for 16 h from 6pm to 10am **Rat Müller glial-derived cell line (RMCs)** cultured in Dulbecco's modified Eagle's medium (DMEM) supple- mented with 10% FBS and 1% penicillin/ streptomycin Cell culture was maintained in the atmosphere of 95% humidity and 5% CO_2 at 37°C RMCs proliferation model reproduced by high glucose (HG, 30mM) for 48hrs, and 5.5mM glucose medium as the control	**Male Sprague Dawley rats (3-month -old)** **<u>WEEK 12</u>** HFSD-fed rats were given IP injection of STZ (25mg/kg bw) **<u>WEEK 13</u>** After 1 week of STZ injection, rats having blood glucose consistently >16.7mM were considered diabetic **<u>TREATMENT (for 8 weeks)</u>** 0.18m/kg EOFAZ dissolved in 0.5% Tween 80, diluted with normal saline by IG administration Rats were fed with regular diet and allocated into 4 groups: Control group (n=8), normal saline intra- gastrical (IG) administration EOFAZ group (n=8), 0.18m/kg EOFAZ dissolved in 0.5% Tween 80, diluted with normal saline, IG administration STZ+DM group (n=8), IG normal saline STZ+DM+ EOFAZ group (n=8) **Rat Müller glial-derived cell line (RMCs)** pre-treatment with EOFAZ, RGZ (Rosiglitazone), GW9662 and KN93 for 1 h Followed by direct exposure to HG in 1.5% FBS for 48 hrs Cells in the control group were incubated with DMEM with 1.5% FBS for the next 48hrs The mannitol group of 24.5 mM was used to exclude the osmosis impact on RMCs	Essential oil extract from Fructus *Alpiniae zerumbet* (EOFAZ) Extraction of essential oil from FAZ via steam distillation technology Drying of essential oil with anhydrous sodium sulfate and storage at -20°C Total yield is ~1.3% By gas chromatography and mass spectrometry, essential oil is made up of: α-Pinene, Camphene, β-Pinene, β-Myrcene, o-Cymol, β- Phellandrene, 1,8-Cineole, Linalool, Camphor, (−)-Borneol, 4-Terpineol, (−)-α- Terpineol, trans- Caryophyllene, Nerolidol, Caryo- phyllene oxide, α-Cadinol, t-Muurolol	**Methods**			
				–	Homogenization of Rat Müller glial-derived cell line (RMCs) and whole retina Separation of total proteins (30-50µg) by 10% SDS-PAGE gel and transferred to PVDF (Polyvinylidene difluoride) membrane 5% bovine serum albumin (BSA) in TBST buffer was used to block the membranes for 1hr Overnight incubation of membranes with primary antibody at 4°C, followed by HRP- conjugated secondary antibody Antibody used was at dilution of 1:1000 Digital images of blots were from Syngene Gel Imaging System Quantification of VEGF proteins via densitometry and was expressed as relative to β-actin	Total RNA extracted from Rat Müller glial-derived cell line (RMCs) by TaKaRa MiniBEST universal RNA extraction kit Reverse transcription of mRNA to cDNA Real-time PCR: cDNA amplificated using SYBR Green PCR reagents with primers of VEGF specific amplification: (forward) 5'-CGGAGAGCAACGTCACTATG-3'; (reverse) GGTCTGCATTCACATCTGCT Analysis using comparative cycle threshold method ($2^{-\Delta\Delta Ct}$) Target gene expression normalized to β-actin expression	Measurement of body weight on weekly basis
				Findings			
				–	**EOFAZ inhibited retinal Müller gliosis in diabetic rats** VEGF transcription level was significantly increased in the retinas of diabetic rats ($p<0.05$ versus normal control) VEGF expression was restored close to normal after EOFAZ treatment ($p<0.05$ versus diabetic rats) **EOFAZ ameliorated HG-induced RMCs gliosis** Transcription of VEGF was obviously enhanced in HG-treated RMCs ($p<0.01$ versus normal control) EOFAZ negatively regulated VEGF protein level ($p<0.01$ versus HG-treated rats) in RMCs through inhibition of HG-induced aberrant VEGF upregulation	**EOFAZ ameliorated HG-induced RMCs gliosis** VEGF mRNA expression level was significantly increased in HG-treated RMCs ($p<0.01$ versus normal control) EOFAZ negatively regulated VEGF mRNA level ($p<0.01$ versus HG-treated group) through inhibition of HG-induced upregulation of VEGF	After STZ injection at week 12, body weights of DM rats decreased significantly EOFAZ treatment (weeks 14-21) did not ameliorate decrease in body weight of diabetic rats There was no significant difference in body weights between STZ+DM and STZ+DM+ EOFAZ group

No.	Sample Model	Treatment	Plant Processing	Assays			
				Retinal vascular permeability by Evans blue dye (ng/mg or ng/g)	Western blot for VEGF (arbitrary units)	Real Time-PCR for VEGF (Threshold cycle, Ct values)	Body weight (g)
(9) Yao *et al.*, 2018	Male Sprague- Dawley rats (250±20g) **WEEK 1** housed at RT (22-25℃) and 45% humidity Exposed to 12-hr light/ dark cycles Provided with free access to food and water	**WEEK 2** Rats fasted for 12hrs and given IP STZ injection (45mg/kg; 0.45% STZ solution with 0.1mmol/L citrate buffer, pH4.5) To protect rats from severe hypoglycaemic effects of pancreatic insulin release, rats were provided with 10% glucose solution 6hrs after STZ injection and lasted for next 24hrs Control rats were injected with citrate buffer **WEEK 3** Rats having fasting blood glucose >16.7mmol/L were considered diabetic **TREATMENT (next 20 weeks)** Allocation into 4 groups: Normo- glycaemic control Diabetic control LBP 200 (LBP 200 mg/kg/d) LBP 400 (LBP 400 mg/kg/d) **Retinal tissue preparation** Rats sacrificed by IP injection of 1% pentobarbital sodium (50mg/kg) Removal of right eyes and fixed in 4% para-formaldehyde solution (for histological study) Removal of left eyes and isolation of retina, frozen in liquid nitrogen and stored at -80℃ (for biochemical analysis)	*Lycium barbarum* polysaccharides (LBP) - 200 and 400mg/kg/d	**Methods**			
				–	Homo- genization of retinal tissues on ice using 400µL of RIPA Lysis Bufer containing phenylmethanesulfonyl fuoride (PMSF) Centrifugation of homogenates at 12,000rpm for 5 min at 4℃ Concentration of extracted proteins in supernatant using a protein assay kit SDS-PAGE of samples containing 50µg proteins and transferred to PVDF membranes 5% skimmed milk was used for blocking membranes Incubation of membranes with polyclonal rabbit anti-VEGF antibody (1:1000 dilution) overnight at 4℃ Membranes were washed and incubated with HRP-conjugated secondary antibody (goat anti-rabbit IgG, 1:5000 dilution) for 1hr at RT Bio-Rad image analysis system used for scanning optical density of target bands Quantity One software used for analysis of relative optical density of VEGF	Trizol extraction kit was used to extract total retinal RNA UV abs spectroscopy used to determine quality and quantity of RNA prepared from each sample Reverse transcription of mRNA (2µg) to cDNA in a total reaction volume of 25µL, containing 1 mg Oligo (dT), 5 M-MLV 5 × bufer, and 1.25 dNTP Incubation of mixture at 42℃ for 60min The reaction was stopped by heating at 95℃ for 5 min Primer sequence for VEGF: (F) 5-TAG ACC TCT CAC CGG AAA GAC-3; (R) 5-CAG GAA TCC CAG AAA CAA AAC-3 PCR amplifcation reaction conditions were 94℃ for 2min, 94℃ for 30s, 50℃ for 30s, and 72℃ for 30s (35 cycles) Each run was repeated 3 times, along with 3 non-template negative controls Melting curve analysis to ensure purity of amplified PCR product measurement of fluorescence throughout the process Calculation of Ct values of the samples and mRNA level of VEGF was normalized to β-actin expression	Body weight was recorded every fourth week
				Findings			
				–	VEGF protein expression was increased in diabetic rats ($p<0.01$ versus normoglycaemic rats) LBP200 and LBP400 treatments restored the increased VEGF protein levels back to normoglycemic control level ($p<0.05$ versus diabetic model), with LBP400 treatment giving further reduction in VEGF protein level	VEGF mRNA expression was increased in diabetic rats ($p<0.01$ versus normoglycemic rats) Treatment with LBP reduced the increased VEGF mRNA level caused by diabetes LBP400 gave slightly better effects than LBP200 but such mild difference did not reach a statistical significance	Rapid and significant increase in body weights were seen in control rats at 5 and 10 weeks after the experiments and the body weights were increased continuously and slowly between 10 and 20 weeks The body weights of diabetic rats decreased slightly at 5 weeks and maintained a stable pace from 5-20 weeks, which were significantly lower than normal control rats ($p<0.01$) The body weights of the LBP200 and LBP400 treated diabetic rats were the same as in diabetic rats ($p>0.05$)

No.	Sample Model	Treatment	Plant Processing	Assays			
				Retinal vascular permeability by Evans blue dye (ng/mg or ng/g)	**Western blot for VEGF (arbitrary units)**	**Real Time-PCR for VEGF (Threshold cycle, Ct values)**	**Body weight (g)**
(10) Zhang *et al*., 2020	Male Wistar rats (100-120g body weight), n=58 housed in poly- carbonate cages with a wooden chip mat on the floor, at ambient temperature of 24±2℃ and 60±5% humidity Exposed to a 12-hr dark/ light cycle Provided with tap water and food ad libitum	**DIABETES INDUCTION (4 weeks)** Normal control group (n=8) fed with std commercial rat chow and tap water Model group (n=50) fed with high-fat-sugar diet (HFSD) After 12-hr starvation, model groups were given IP STZ injection (30mg/kg in 0.1mol/L citrate buffer, pH4.4) After 72hrs, they were given second injection at similar dose Controls were given same volume of citrate buffer Rats having FBG >16.7mmol/L were considered diabetic **TREATMENT (Intragastric, IG administration for 9 weeks)** 6 groups (8 rats per group with no. 1-8): Normal control (water) Model control (30% polyethylene glycol 400) TLFA-L (0.35g/kg/d) TLFA-M (0.69g/kg/d) TLFA-H (1.38g/kg/d) Positive control with 150mg/kg/d calcium dobesilate (CD) in water **END OF TREATMENT** Rats were fasted for 18hrs, weighed and anaesthetized with 25% ethyl carbamate (in normal saline, w/w, 1g/kg) They were continually monitored until they had complete lack of response after a foot pinch	Total lignans from *Fructus arctii* (TLFA) Extraction of dried and ground *Fructus arctii* in 95% aqueous ethanol extract (2x80L) for 3hrs at 80℃ Filtration and concentration of liquid ethanol extract in a rotary evaporator at 55℃ under reduced pressure Liquid extract was defatted with petroleum ether (60-90℃), followed by extraction with ethyl acetate (EA) EA extract was concentrated and dried in a vacuum at 55℃, yielding 11.1% (w/w) 8 lignans were identified from TLFA using UV spectrometry: matairesinol, arctigenin, lappaol A, lappaol F, lappaol C, arctignan E, arctiin and lappaol H	**Methods**			
				DR Model Establishment (*same as stated in "Treatment" column*)_Male Wistar rats (n=90) of 100-120g: 16 normal controls (fed with std commercial chow and tap water) and 74 diabetic rats (fed with HFSD) **Before DR model establishment** Normal rats (n=8), subjected to BRB breakdown quantification **After DR model establishment** Diabetic rats (n=8) were subjected to BRB breakdown quantification at the end of 1st, 2nd, 3rd, 4th and 5th months after model establishment **TREATMENT (3 months after model establishment)** 48 diabetic rats were divided into: Model group with 30% polyethylene glycol 400, (n=24). TLFA-L group (0.35g/kg/d), (n=8). TLFA-H group (1.38g/kg/d), (n=8). Positive control treated with 150mg/kg/d CD in water, (n=8) **BRB breakdown quantitation** 3 months after treatment, each group of rats was subjected to BRB breakdown quantitation. EB dye was used with ratio of EB mass to dry retinal mass calculated from spectro- photometer readings. Rats were anaesthetized with 25% ethyl carbonate (in normal saline, w/w, 1g/kg). 3% EB dye (in normal saline, w/w 45mg/kg) were injected via femoral vein of rats. After 2-hr circulation, heart perfusion was performed in rats using normal saline (37℃). Perfusion volume was 10 times the rat's blood volume with flow rate of 66mL/min. Removal of retina at the end of perfusion and drying in 120μL formamide for 18hrs at 70℃ Centrifugation of extract at 10,000xg for 10min. 90μL supernatant used for abs measurement at 623nm	–	Total RNA extraction from fresh retinal tissues **Reverse transcription** Total retinal RNA was reverse transcribed into cDNA The total volume of the reaction system was 20 μL and consisted of 10μL of 2 × RT Reaction Mix, 2μL of RT Enzyme Mix, 5μL of RNA sample, and 3μL of H2O (RNase-Free). The reaction mixture was then mixed, allowed to stand at RT for 10min, incubated at 50℃ for 30min, and stopped by incubation at 85℃ for 5min One μL (2U) of *Escherichia coli* RNase H was added and incubation was continued at 37℃ for 20min **PCR** The target gene was then amplified using real-time PCR using the cDNA as the template FQ-RT-PCR was performed in a total volume of 25μL containing 12.5 μL SYBR® Green ER™ qPCR Super Mix Universal, 0.5 μL of forward primer (10 μM), 0.5 μL of reverse primer (10 μM), 0.5 μL of cDNA, 11 μL of DEPC- treated water Reaction protocols: 50℃ for 2min and 95℃ for 10min, followed by 40 cycles of 95℃ for 15s and 60℃ for 60s VEGF mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method	Measurement of body weight every week throughout the 9-week treatment period
				Findings			

		Rats were sacrificed with removal of eyes Left retinas (of rat no. 1-6) isolated and immersed in Tris for fluorescent quantitative real-time PCR (FQ-RT-PCR) Right retinas (rat no. 3-8) were fixed in 4% para-formaldehyde for paraffin section preparation		EB leakage increased rapidly in the first two months but tailed off gradually indicating DR After 3-month administration, model group exhibited ~3-fold increase in EB leakage into retina ($p<0.001$ versus normal control) TLFA treatment caused lower dye leakage into retina in treatment groups ($p<0.001$ versus model controls), but no significant differences in EB leakage were established between TLFA groups and positive control drug group	–	mRNA expression of VEGF in the retina was significantly increased in model group ($p<0.001$ versus normal control group) TLFA-H ($p<0.001$ versus model control), TLFA-M ($p<0.01$ versus model control) and TLFA-L treatment significantly reduced VEGF mRNA expression in retinal tissues in a dose- dependent manner	Body weights of diabetic rats were significantly lower (<330g) throughout the experiment There was no significant difference in body weights among the diabetic rats with or without treatment Positive control diabetic rats demonstrated higher body weight from week 5 onwards, as compared to the model group and 3 TLFA treatment groups