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Abstract. Bio-flavonoids comprise a group of phenolic secondary plant metabolites that are widespread in nature. Major flavonoids that have well categorized structures and well defined structure function-relationships are: flavans, flavanones, flavones, flavonols, flavanols, flavanonols, cetechins, anthocyanidins and isoflavones. Bio-flavonoids are well-known for their multi-directional biological activities including anti-diabetic efficacy. Numerous studies have been carried out to explore their potential role in the treatment of diabetes. A good number of studies have already demonstrated the hypoglycemic effects of flavonoids using different experimental models and treatments - the drug candidates have been shown to exert such beneficial effects against the disease manifestation, either through their capacity to avoid glucose absorption or to improve glucose tolerance. It has also been demonstrated that flavonoids can act per se as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms, to attenuate the diabetic complications; besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism pathway. As a result, bio-flavonoids are now-a-days regarded as promising and significantly attractive natural substances to enrich the current therapy options against diabetes.
The purpose of this resume is to represent promising anti-diabetic flavonoid candidates highlighting their absorption and metabolism along with their mode of action in regulating diabetic symptoms.

1. Introduction

Diabetes mellitus is the most prevalent metabolic syndrome world-wide with an incidence varying between 1 to 8% [1,2]. The disease arises when insufficient insulin is produced, or when the available insulin does not function properly. Thus diabetes is characterized by hyperglycaemia (elevation in blood sugar levels) resulting in various short-term metabolic changes in lipid and protein metabolism and long-term irreversible vascular changes. The long-term manifestation of diabetes can result in the development of some complications, broadly classified as microvascular or macrovascular disease. Microvascular complications include neuropathy (nerve damage), nephropathy (renal disease) and vision disorders (retinopathy, glaucoma, cataract and corneal diseases), while macrovascular complications include heart disease, stroke and peripheral vascular disease, which can lead to ulcers, gangrene and amputation [3]. These complications are also found in non-diabetic population, but have a two to five-fold increase in diabetic subjects [4]. The last century has seen a rapid increase in the global prevalence of coronary artery disease (CAD) [5,6].

Current estimates from different countries in Europe and the United States have shown that diabetes and its complications account for 8-16% of the total health costs for society and this will increase dramatically unless major efforts are made to prevent the ongoing epidemic. There are two major categories of diabetes - insulin dependent diabetes mellitus (IDDM, Type 1 diabetes mellitus) and non-insulin dependent diabetes mellitus (NIDDM, Type-2 diabetes mellitus). Type 1 diabetes occurs due to almost 95% destructions of β-cells of islets of Langerhans in the endocrine pancreas caused by an autoimmune process, usually leading to absolute insulin deficiency, this type has an early onset, most often between the ages of 10 and 16 yrs. Insulin resistance in peripheral tissue and an insulin secretive defect of the β-cells characterizes Type-2 diabetes mellitus (NIDDM). It is the most common form of diabetes mellitus constituting above 90% of the diabetic population and highly associated with a family history of diabetes, older age, obesity and lack of exercise [3]. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025 [7]. The World Health Organization (WHO) has predicted that the major burden will occur in the developing countries, there will be a 42% increase from 51 to 72 million in the developed countries while 170% increase from 84 to
228 million, in the developing countries [8]. Prevalence of the complications is greater among the lower socio-economic people due to lack of good control of glycaemia and hypertension and also due to behavioral factors. The direct and indirect costs involved in the treatment of the chronic disease especially when associated with the vascular complications are enormous. The overall global scenario urges to implement cost-effective and at the same time efficacious preventive measures against diabetes to reduce the high morbidity and mortality [4].

2. Currently available therapies

Currently available therapies for diabetes include insulin and various oral anti-diabetic agents such as sulfonylureas, biguanides, \( \alpha \)-glucosidase inhibitors, and glinides, which are used as monotherapy or in combination to achieve better glycemic regulation. Many of these oral anti-diabetic agents suffer from various adverse effects, thus, managing diabetes without any side effects is still a challenge to the workers [9], and hence the search for more effective and safer therapeutic agents in eradicating diabetic syndromes has continued to be an important area of investigation. Both fasting and postprandial impaired glucose tolerance are associated with an increased risk of developing Type-2 diabetes mellitus and therefore form an important target group for interventions aimed at preventing diabetes [10]. The pharmacological agents with the greatest effect on postprandial hyperglycemia include insulin lispro, amylin analogues, and \( \alpha \)-glucosidase inhibitors. In hyperglycemia associated with diabetes, the use of aldose reductase inhibitors has been reported for the treatment of diabetic complications [11]. Aldose reductase as a key enzyme in the polyol pathway has been reported to catalyze the reduction of glucose to sorbitol. Sorbitol does not readily diffuse across cell membranes, and the intracellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as peripheral neuropathy, retinopathy, and cataracts [12]. A recent study reported that aldose reductase may also be involved with another signal transduction pathway in the pathogenesis of diabetic nephropathy [13].

3. Back to the plant kingdom

The use of ethnobotanicals has long folkloric history for the treatment of blood sugar abnormalities. In the India, indigenous remedies have been used in the treatment of diabetes since the time of Charaka and Sushruta (6th century B.C.) [14]. Plants have always been exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly
from them. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential [15]. Many of such plants have exhibited anti-diabetic activity when assessed using presently available experimental techniques [17-20]. It may be mentioned in this connection that the discovery of widely used hypoglycaemic drug, metformin came from the traditional approach of using *Galega officinalis*. In spite of all these, the indigenous system has not yet gained enough momentum in the scientific community. The reasons may be many including lack of belief among the practitioners of conventional medicine over alternative medicine, alternative form of medicine are not very well-defined and natural drug may vary tremendously in content, quality and safety. To cope with severe problems associated with using of synthetic anti-diabetic drugs, there is a need to look for more efficacious drugs with lesser side effects and also of low cost. It is the high time to turn our attention to the plant kingdom in search of natural drugs for diabetes following an integrated approach and using correct procedures. The hypoglycemic effect of several plants used as anti-diabetic remedies has already been confirmed, and the mechanisms of hypoglycemic activity of these plants are being studied; if even a single plant material stands the acid-test of efficacy comparable to commonly used synthetic oral drugs already marketed, it will herald the discovery of cheap and relatively nontoxic drug.

4. Purpose of the present review

A number of review articles on the uses of various plants (different parts of plant materials, crude extracts, herbal formulations, etc.) as anti-diabetic agents have been published time to time [22-26]. Naturally occurring chemotypes of varying structural skeletons have also been reported to possess anti-diabetic properties [27,28], and the purpose of this resume is to represent promising anti-diabetic bio-flavonoids highlighting their absorption and metabolism along with mode of action in regulating diabetic symptoms.

5. Anti-diabetic bio-flavonoids of promise

Bio-flavonoids comprise a group of phenolic secondary plant metabolites that are widespread in nature. Major flavonoids that have well categorized structures and well defined structure function-relationships are: flavans, flavanones, flavones, flavonols, flavanols, flavanonols, catechins, anthocyanidins and isoflavones. Bio-flavonoids are well-known for their multi-directional biological activities including anti-diabetic efficacy [29-32]. Numerous studies have been carried out to explore their potential role in the treatment of diabetes [27,28,33]. A good number of studies have already demonstrated the
hypoglycemic effects of flavonoids using different experimental models and treatments - the drug candidates have been shown to exert such beneficial effects against the disease manifestation, either through their capacity to avoid glucose absorption or to improve glucose tolerance. It has also been demonstrated that flavonoids can act per se as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms, to attenuate the diabetic complications; besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism pathway. As a result, bio-flavonoids are now-a-days regarded as promising and significantly attractive natural substances to enrich the current therapy options against diabetes. This present section embodies the information on promising anti-diabetic efficacies of certain bio-flavonoids.

Choi et al. [34] demonstrated that intraperitoneal administration of prunin (naringenin 7-O-β-D-glucoside) produces a significant hypoglycemic effect in diabetic rats. Anti-hyperglycemic effects have also been demonstrated for various flavonoids including chrysin and its derivatives, silymarin, isoquercetrin and rutin [35-37]. Long-term studies carried out with rutin orally administered to diabetic rats showed that it decreased the plasma glucose levels by up to 60% when compared to the control group. However, oral administration of rutin to normal rats did not show any significant effect on fasting plasma glucose levels [38]. Chronic treatment with hesperidin and naringin was found to lower the blood glucose level of db/db mice compared with the control group [39].

Myrciacitrins I, II, III, IV and V (1-5) isolated from the dried leaves of Myrcia multiflora DC. (family: Myrtaceae) were reported to possess significant rat lens aldose reductase inhibitory activity [40], the IC50 values for the flavonoids 1-5 were determined as 3.2 x 10⁻⁶, 1.5 x 10⁻⁵, 4.6 x 10⁻⁵, 7.9 x 10⁻⁷, 1.6 x 10⁻⁵ and 1.3 x 10⁻⁵ M, respectively [40,41]. Hence, myrciacitrin IV (4) exhibited the most potent activity, although it had less activity than epalrestat, a commercially available synthetic aldose reductase inhibitor (IC₅₀ = 7.2 x 10⁻⁸ M) [40].

Kawabata et al. [42] isolated five 6-hydroxy-flavonoids (6-10) from the methanol extract of Origanum majorana L. (family: Lamiaceae) leaves and studied their α-glucosidase enzyme inhibitory activity, three of these flavonoids: 6-hydroxyapigenin (scutellarein) (6), 6-hydroxyapigenin-7-O-β-D-glucopyranoside (7), 6-hydroxyluteolin-7-O-β-D-glucopyranoside (8) are previously known [43-47], and the other two feruloylglucosides namely, 6-hydroxyapigenin-7-O-(6-O-feruloyl)-β-D-glucopyranoside (9) and 6-hydroxyluteolin-7-O-(6-O-feruloyl)-β-D-glucopyranoside (10) are novel compounds. All the isolates showed rat intestinal α-glucosidase inhibitory activity, at an equal concentration of 500 μM, the flavonoid candidates 6-10
inhibited the enzyme activity by 81%, 44%, 55%, 25% and 26%, respectively.

The respective IC\textsubscript{50} values for 6-10 were determined as 12, >500, 300, >500 and >500 \(\mu\text{M}\). Another flavonoid, 6-hydroxyluteolin (11) [48], was also found to exhibit potent \(\alpha\)-glucosidase inhibitory activity (92% inhibition at a concentration of 500 \(\mu\text{M}\)) with an IC\textsubscript{50} value of 10 \(\mu\text{M}\) [42]. The same group [49] also evaluated 5,6,7-trihydroxyflavone (baicalein, 12), the flavonoid constituent of \textit{Scutellaria baicalensis}, as an important inhibitor against rat intestinal \(\alpha\)-glucosidase (IC\textsubscript{50} = 32 \(\mu\text{M}\)).

The investigators also observed that apigenin (5,7,4\text-quote)-trihydroxyflavone, 13) and luteolin (5,7,3\text-quote,4\text-quote-tetrahydroxyflavone, 14), both lacking the 6-hydroxyl substituent, showed negligible activity (12% and 22% inhibition at 500 \(\mu\text{M}\), respectively) in the \(\alpha\)-glucosidase inhibitory assay. From their study, the present investigators suggested that 5,6,7-trihydroxyflavone skeleton is crucial for high \(\alpha\)-glucosidase inhibitory activity regardless of B-ring hydroxylation, in addition, glycosation of 7-hydroxyl substituent as well as acylation of the sugar reduces the enzyme inhibitory activity [49].

Haraguchi \textit{et al.} [50] isolated \(C\)-glucosidic flavone derivative named as isoaffineyin (5,7,4\text-quote,5\text-quote-pentahydroxyflavone-6-\(C\)-glucoside, 15) from \textit{Manikara indica} (family: Sapotaceae), the flavonoid candidate exerted promising inhibition against porcine lens aldose reductase activity with an IC\textsubscript{50} value of 4.6 \(\mu\text{M}\) (epalrestat was used as positive control, IC\textsubscript{50} = 0.87 \(\mu\text{M}\)).
The genistein derivatives (16-19) isolated from an EtOAc-soluble partition of the MeOH extract of a branch of *Tetracera scandens* (family: Dilleniaceae) were evaluated to possess promising activities on Type-2 diabetes mellitus treatment since the test compounds significantly stimulated the uptake of glucose, adenosine monophosphate-activated kinase (AMPK), glucose transport protein-4 (GLUT4) and GLUT1 mRNA expressions and protein tyrosine phosphatase 1B (PTP1B) inhibition in L6 myotubes [51]. The IC$_{50}$ values for isofavonoids 16-19 in inhibiting PTP1B activities were
determined as $31.75 \pm 0.27$, $28.13 \pm 0.19$, $20.63 \pm 0.17$ and $37.52 \pm 0.31 \mu M$, respectively (ursolic acid was used as positive control with $IC_{50}$ value of $5.13 \pm 0.45 \mu M$). No muscle cell toxicity was reported with compounds 17-19, while compound 16 reduced muscle cell viability with $IC_{50}$ value of $18.69 \pm 0.19 \mu M$. The investigators, thus, demonstrated that the isoflavonoids constituents (16-19) of *T. scandens* stimulate glucose-uptake in basal and insulin-stimulated L6 myotubes in a dose-dependent manner - AMPK activation, GLUT4 and GLUT1 expressions and PTP1B inhibition by these bioactive constituents appeared to be involved in the mechanism of the stimulation of basal and insulin-responsive glucose-uptake. Hence, compounds 16-19 may be possible candidates of a novel therapeutic strategy for Type-2 diabetes mellitus treatment, although further studies will be required to clarify the molecular mechanism of these bioactive constituents [51].

Isoorientin (20), isolated from the water and butanolic extracts of *Cecropia obtusifolia* (family: Ceropiaceae), exhibited potent hypoglycemic activity comparable to that of glibenclamide at a dose of 3 mg/kg body weight in diabetic rats [52].

Kim et al. [53] isolated a new flavonol glycoside, quercetin 3-*$\alpha$-L-arabinopyranosyl-(1\(\rightarrow\)2)-*$\beta$-D-glucopyranoside (21) along with the known flavonoid glycosides such as kaempferol 3-*$\beta$-D-glucopyranoside
(astragalin) (22a) and quercetin 3-\(\text{-}\)O\(\beta\)-D-glucopyranoside (isoquercetin) (22b) from the leaves of *Eucommia ulmoides* (family: Eucommiaceae), these flavonoid constituents were found to be glycation inhibitors having comparable activity to that of aminoguanidine, a known glycation inhibitor. The IC\(_{50}\) values for the test compounds 21, 22a and 22b were determined as: 2.95 \(\times\) 10\(^{-7}\), 4.86 \(\times\) 10\(^{-7}\), and 3.20 \(\times\) 10\(^{-7}\) M, respectively (aminoguanidine was used as positive control, IC\(_{50}\) = 4.45 \(\times\) 10\(^{-7}\) M) [53].

Tabopda *et al.* [54] reported that six unusual C-4\(^{\prime}\)-prenylated flavonols, dorsilurins F-K (23-28), isolated from the roots of *Dorstenia psilurus* (family: Moraceae), were found to exhibit glycosidase enzyme inhibitory activity against \(\alpha\)-glucosidase, \(\beta\)-glucosidase, and \(\alpha\)-mannosidase. Compound 23, with three unmodified prenyl groups, showed the best \(\alpha\)-glucosidase inhibitory activity (IC\(_{50}\) 4.13 \(\mu\)M), while compound 28, with only one unmodified prenyl group, showed the least \(\alpha\)-glucosidase inhibitory activity (IC\(_{50}\) 43.95 \(\mu\)M). Thus, it was suggested that \(\alpha\)-glucosidase inhibitory activity of the compounds increased with the number of unmodified prenylated groups present. These compounds (23-28) showed very weak enzyme inhibitory activities against \(\beta\)-glucosidase and \(\alpha\)-mannosidase [54].
Two dihydroflavonol glycosides such as engeletin (29) and astilbin (30), isolated from the leaves of *Stelechocarpus cauliflorus* (family: Annonaceae), exhibited inhibitory activity against a recombinant human aldose reductase, the inhibitory activity of 29 (IC$_{50}$ = 1.16 μM) was found to be twice that of quercetin (positive control, IC$_{50}$ = 2.48 μM), and 23 times greater than that of 30 (IC$_{50}$ = 26.7 μM) [55].

Flavonoid glycosides (FG 1 and FG 2), isolated from *Phyllanthus fracternus* (family: Euphorbiaceae), at a dose of 100 mg/kg p.o. were found to be hypoglycaemic in alloxanised rats (20 and 25%) at 3 hrs, however, no
blood sugar lowering was observed in normal rats [56]. A neoflavonoid, coutareagenin [5-hydroxy-7-methoxy-4-(3,4-dihydroxyphenyl)-2H-benzo-1-pyran-2-one] isolated from the bark of *Hintonia latiflora* (family: Rubiaceae), exhibited promising anti-diabetic efficacy in streptozotocin-induced Wistar rats as well as in menopausal diabetic women [57,58].

Kaempferol-3,7-\(\alpha\)-dirhamnopyranoside (kaempferitrin, 31), isolated from the *n*-butanol fraction of the leaves of *Bauhinia forficata* (family: Leguminosae), exhibited significant hypoglycemic effect in normal and alloxan-induced diabetic rats on oral administration. In normal rats, reduction in blood glucose level was noticed only with the higher dose of 31 (200 mg/kg) at 1 h after treatment, whenever such efficacy of the test compound in diabetic rats was evident at all doses administered (50, 100, and 200 mg/kg), and this profile was found to be maintained throughout the period studied for both higher doses. However, in glucose-fed hyperglycemic normal rats, kaempferitrin could not down-regulate blood glucose levels [59]. Kaempferol-3-neohesperidoside, a glycosylated flavonoid structurally very similar to kaempferitrin, was also shown to demonstrate promising hypoglycemic effect in both oral and intraperitoneal treatments in diabetic rats, in addition, kaempferol-3-neohesperidoside-VO(IV) complex showed potent hypoglycemic efficacy throughout the post-treatment period studied when compared with zero time [60]. When complexed with vanadium, quercetin also demonstrated much promising insulin-enhancing activity in STZ-diabetic mice with no effect on the blood glucose level of normal mice, which is in agreement with the results for kaempferitrin and kaempferol-3-neohesperidoside-VO(IV) complexes [60,61]. Quercetin itself was evaluated to possess anti-diabetic effect by reducing the blood glucose level of diabetic rats in 8-10 days of treatment [62], in the same study by Vessal and his group, the test compound exerted no effect on the glucose tolerance curve either in normoglycemic or in STZ-diabetic rats [62]. These results support the views of Shetty *et al.* [63] for hypoglycemic effects of quercetin in diabetic rats.

Three prenylated flavanones (33-35) isolated from stem barks of *Erythrina abyssinica* (family: Leguminosae) exhibited inhibitory activity against protein tyrosine phosphatase 1B (PTP1B) in dose-dependent manner with IC\(_{50}\) values >60, 18.9±1.9 and 15.7±0.4 \(\mu\)M, respectively [64], hence, the flavanone (32) bearing a 2,2-dimethylpyran moiety on B ring is less potent than the other two (33 & 34) in the series. The investigators, thus, suggested that substitution of prenyl groups on flavonoids may be important for *in vitro* PTP1B inhibitory activity and cyclization between a hydroxy group and the prenyl group in B ring without prenyl or methoxy groups may reduce the activity [64]. One more isoprenyl flavonoid (35) isolated from the root barks of *Erythrina mildbraedii* were also found to exhibit inhibitory
activity against PTP1B enzyme in dose-dependent manner with IC$_{50}$ values 21.2±1.6 μM. The present investigators argued that substitution of isoprenyl groups on ring-B might be important for PTP1B inhibitory activity in vitro, and introduction of one more hydroxyl group to C-5 of ring-A or one of the isoprenyl groups in ring-B might be responsible for a loss of such activity [65].

Isorhamnetin 3-O-β-D-glucoside (36) isolated from the ethylacetate fraction of *Salicornia herbacea* (family: Chenopodiaceae) was evaluated to possess significant inhibitory activity against rat lens aldose reductase (RLAR) in vitro with an IC$_{50}$ value of 1.4 mM, which is similar to that of tetramethyleneglutaric acid (IC$_{50}$ = 1.7 mM) [66]. The flavonol glycoside (36), when administered orally at 25 mg/kg in streptozotocin (STZ)-induced diabetic rats, caused not only a significant inhibition of serum glucose concentration but also sorbitol accumulation in the lenses, red blood cells (RBC), and sciatic nerves, thereby, advocating the test compound from *S. herbacea* as a leading compound for further study as a new drug for the prevention and/or treatment of diabetes and its complications [66].

Luteolin 6-C-(6″-O-trans-caffeoylglucoside) (37) isolated from *Phyllostachys nigra* (family: Gramineae) showed inhibitory efficacy against advanced glycation end products (AGEs), hence, this compound could be offered as a leading compound for its further study towards development of new natural products drug for diabetic complications [67]. Jang et al. [68] reported two flavan-3-ol derivatives (38 and 39) from the roots of *Actinidia arguta* (family: Actinidiaceae) that were found to exhibit inhibitory activity in vitro on the formation of advanced glycation end products with IC$_{50}$ values of 13.5 and 17.9 μg/mL, respectively.

Few more advanced glycation end products (AGEs) inhibitors such as the dihydroflavonol glycosides (40 and 41) [55], isoflavone C-glucosides (42 and 43) [69] and the 2,3-dioxygenated flavanone erigeroflavanone (44) have also been reported [70]. The isoflavone C-glucosides (42 and 43) isolated from the roots of *Pueraria iobata* (family: Pueraria) showed more potent in vitro inhibitory activity against AGEs formation with IC$_{50}$ values 8.7 and 24.9 μg/mL, respectively [69]. The present investigators [69] suggested that the compound (42) is worthy of consideration as a therapeutic agent for diabetic complications or related diseases. Yoo et al. isolated the 2,3-dioxygenated flavanone, erigeroflavanone (44) from the flowers of *Erigeron annuus* (family: Asteraceae/Compositae), and evaluated its inhibitory activity against AGEs formation with an IC$_{50}$ value 22.7 μM [70].

A flavone xylopyranoside, 4′,5-dihydroxy-6,7-dimethoxyflavone-3-O-β-D-xylopyranoside (45), isolated from the roots of *Euphorbia leucophylla* (family: Euphorbiaceae) by Satyanarayana et al., was found to reduce the
blood glucose levels (BGLs) and increase the serum insulin levels in normal and diabetic rats [71]. One flavone \([1''(R)-5,4',1''-\text{trihydroxy}-6,7-(3'',3''-\text{dimethylchromano})\text{flavone}, 46]\) and one flavanone \([(2S)-4'-\text{O-methyl-6-methyl-8-prenylnaringenin}, 47]\) both isolated \textit{Eysenhardtia platycarpa} (family: Leguminosae) were evaluated to possess promising anti-

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hyperglycemic activity by decreasing glucose level of streptozotocin (STZ)-induced diabetic rats (31 mg/kg of body weight, \( P < 0.05 \)) [72].

Matsuda et al. [12] examined a variety of flavonoids for their rat lens aldose reductase inhibitory activity to study structure-activity relationships. Among the flavone constituents, 3’,4’-dihydroxyflavone (48), 3’,4’,7-trihydroxyflavone (49), luteolin (50), and luteolin 7-\( \beta \)-D-glucopyranoside (51) were found to possess potent inhibitory activity with IC\(_{50}\) values of 0.37, 0.30, 0.45 and 0.99 \( \mu \)M, the flavonoid glycosides, quercitrin (52), guaijaverin (53) and desmanthin-1 (54) also showed the most potent activity against the enzyme with respective IC\(_{50}\) values of 0.18, 0.18 and 0.082 \( \mu \)M [12]. The activity of desmanthin-1 (54) was equivalent to that of a commercially available synthetic aldose reductase inhibitor, epalrestat (IC\(_{50}\) = 0.072 \( \mu \)M). From their detailed studies, Matsuda et al. suggested the following structural requirements of flavonoids for aldose reductase inhibitory activity - (i) the 5-hydroxyl moiety has no effect, (ii) the 3-hydroxyl and 7-\( \beta \)-glucosyl moieties reduce the activity, (iii) the 2-3 double bond enhances the activity, and (iv) the flavones and flavonols having the catechol type moiety at the B ring (the 3’,4’-dihydroxyl groups) exhibit stronger activities than those of pyrogallol-type moiety (the 3’,4’,5’-trihydroxyl groups) [12].
6. Absorption and metabolism of flavonoids

6.1. Absorption of flavonoids

As far as reports are available, the absorption of dietary flavonoids may be influenced by the matrix in which they are consumed, with enhanced excretion in urine of easily recognized mammalian conjugates observed when presented in foods with a higher fat content [73-78] - although certain reports are there in contrast to [79-83]. However, an important factor in the absorption efficiency of flavonoid glycosides in the intestine is the sugar moiety, as demonstrated for quercetin glycosides, its aglycone and rutin supplements in healthy ileostomy volunteers [84]. Flavonoid aglycones, being hydrophobic in nature, can be transported across membranes by passive diffusion, whereas in flavonoid glycosides the sugar moiety enhances the hydrophilicity of the flavonoid molecules as a whole, thereby, reducing the possibility of passive transport. Hence, it may be argued that flavonoids are absorbed by active transport [85]. A good number of studies in human and animals are in agreement with the fact that some dietary flavonoids such as flavanols [86], quercetin-3-gluicoside and quercetin-4′-glucoside [87-89] can be absorbed in the small intestine — however, quercetin, quercetin-3-galactoside, quercetin-3-rutinoside (rutin), naringenin-7-glucoside, genistein-7-glucoside and cyanidine-3,5-diglucoside have been found not to be [89,90].

It has been suggested that before absorption flavonoids are cleaved by specific enzymes either in the lumen or inside the cells of the gut. Lactase-phlorizin hydrolase (LPH) is anchored in the brush-border membrane in the small intestine and catalyzes extracellular hydrolysis of some glucosides [91,92]. Another enzyme, located intracellularly and with broad specificity, is the cytosolic β-glucosidase (CBG). It is found in abundance in the small intestine, liver and kidney of mammals and requires active transport of hydrophilic glucosides into the cells [93]. Concerning LPH activity, it has been shown that the enzyme cleaves some flavonol and isoflavone glycosides such as quercetin-4′-glucoside, quercetin-3-glucoside, quercetin-3,4′-glucoside, 3′-methylquercetin-3-glucoside, genistein-7-glucoside, and
daidzein-7-glucoside. However, quercetin-3-rhamnoglucoside and naringenin-7-rhamnoglucoside (naringin) are not substrates for this enzyme [91,93]. In addition, \( \beta \)-glucosidase activity is reported to act on flavonoid and isoflavone glycosides according to the position and the structure of the sugar moiety attached to the flavonoid aglycone [94]. Mechanism of absorption have still not been completely elucidated but is believed to involve \textit{inter alia} interaction of certain glucosides with the active sugar transporter-1 (SGLT-1) and luminal lactase-phlorizin hydrolysate (LHP), passive diffusion of the more hydrophobic aglycones, or absorption of the glycoside and interaction with cytosolic \( \beta \)-glucosidase (CBG).

\subsection{6.2. Metabolism of flavonoids}

After being absorbed in body, flavonoids undergo three main types of conjugations such as methylation, sulfation and glucuronidation [95-97]. The most important enzymes involved in flavonoids metabolism are catechol-\( O \)-methyltransferase (COMT, EC 2.1.1.6), phenol sulfotransferase (P-PST, SULT, EC 2.8.2.1) and UDP glucuronosyl transferase (UDPGT, UGT, EC 2.4.1.17). Catechol-\( O \)-methyltransferase methylates polyphenols and has the highest activity in the liver and kidneys [98]. Phenol sulfotransferases are cytosolic enzymes that transfer sulfate moieties to hydroxyl groups from substrates such as iodothyronines, phenols and hydroxyarylamines mainly in the liver [96,97,99]. UDP glucuronosyl transferase catalyzes the conjugation of polyphenols to glucuronic acid in endoplasmic reticulum in the intestine, liver and kidney. In humans, the liver has the greatest capacity for glucuronidation while in rats, the highest level of glucuronyl transferase activity was observed in the intestine [99-101]. Conjugation reactions with glucuronic acid and/or sulfate appear to be the most common type of metabolic pathways for the flavonoids first occurring in the gut barrier [85] and these conjugates then reach the liver, where they are further metabolized [81,99,102]. Otake \textit{et al.} [103] showed that hepatic UDP-glucuronosyl transferase isoforms were the main factors responsible for galangin metabolism into two major glucuronides conjugated at the 7- and 3- positions by using human liver microsomes. Also, Vaidyanathan and Walle [104] demonstrated no glucuronidation of \((-\)-epicatechin by human liver and small intestinal microsomes. However, in rats, \((-\)-epicatechin was efficiently metabolized by liver microsomes with formation of two glucuronides. In the same study, the authors concluded that sulfation also occurred in both the liver and intestine in human and rats.

Three \((-\)-epicatechin metabolites such as \((-\)-epicatechin-3\('\)-\( O \)-glucuronide, \( 4'\)-\( O \)-methyl\((-\)-epicatechin-3\('\)-\( O \)-glucuronide, and \( 4'\)-\( O \)-methyl\((-\)-epicatechin-5 or 7-\( O \)-glucuronide have been isolated from human
urine [105], whereas the exact fate of (+)-catechin is not known although there is evidence for the formation of (+)-catechin sulfates, sulfo-glucuronides, and 4′-methylated conjugates in plasma and urine [76,106]. In contrast, (−)-epicatechin gallate and (−)-epigallocatechin gallate appear to be excreted in bile [79,86,107,108]. The (−)-epicatechin gallate is extensively methylated by human liver catechol O-methyl transferase at the 4′-position and to a lesser extent at the 3′-position [109,110], while (−)-epigallocatechin gallate is metabolized first to the 4″-methyl ether and then to the 4′,4″-dimethyl ether [110].

Flavonoid glycosides that are not absorbed in the small intestine along with the conjugated metabolites that are excreted in bile can be metabolized by microflora when they reach the colon. Glycoside flavonoid-hydrolyzing enzymes have been identified in fecal flora cultures. Bokkenheuser et al. [111] recovered three enzyme-producing strains that, using β-glucosidases, α-rhamnosidase, and/or β-galactosidases, were capable of converting rutin to quercetin. Also, it was shown that at least some of the bacterial glycosidases are able to cleave glycosidic bonds and flavonoid-saccharide bonds in the gut [91]. Genistein-7-glucoside and daidzein-7-glucoside have not been found in human plasma [112] but the aglycones have been observed [113]. Human metabolism of isoflavone glycosides produces genistein and daidzein 7-glucuronides and 7-sulfates and 4′,7-diconjugates (including diglucuronides and mixed conjugates), with monoglucuronides predominant [114,115]. The profile of metabolites has been demonstrated in studies with quercetin, rutin, and naringin. The flavonoid metabolism produces aromatic acids such as phenylvaleric, phenylpropionic, phenylacetic and benzoic acids with easy absorption through the colonic barrier [116-118]. Flavonol glycosides and quercetin aglycone have not been convincingly demonstrated in plasma [119-121], although kaempferol aglycone has been detected [122]. The main kaempferol metabolite in human plasma is the 3-glucuronide [122]. The three major metabolites of quercetin are: quercetin-3-glucuronide, quercetin-3′-sulfate, and isorhamnetin-3-glucuronide. Apigenin glucuronides have been detected in urine after volunteers consumed parsley [123], luteolin aglycone administered to volunteers has been detected in plasma as a monoglucuronide accompanied by a trace of unconjugated luteolin [124,125]. Chrysin is transformed primarily to the 7-glucuronide with much smaller yields of the 7-sulfate [126].

Metabolites of flavonoids in general (and also microflora metabolites), aglycones, glycosides and conjugated metabolites which are not absorbed, may follow two pathways of excretion: via the biliary or the urinary route. Large conjugated metabolites are more likely to be eliminated in the bile whereas small conjugates such as monosulfates are preferentially excreted in
urine [100]. When excreted in bile, the flavonoids are passed to the duodenum and metabolized by intestinal bacteria, which results in the production of fragmentation products and/or the hydrolysis of glucurono- or sulfoconjugates [127]. The resulting metabolites which are released may be reabsorbed and enter an enterohepatic cycle or being excreted in feces [128,129]. For each flavonoid, the beneficial effect will be dependent upon their absorption and availability in the body. Thus, these factors should be considered in any interpretation of the potential health effects of flavonoids.

7. Mode of action of flavonoids

Very recently, Cazarolli et al. [130] reviewed on the mode of action of flavonoids including cellular and molecular mechanism. In their review, the authors thoroughly discussed about the various effects of the drug candidates in regulating diabetic syndromes. It has been demonstrated that flavonoid compounds act against diabetes mellitus either through their capacity to avoid glucose absorption or to improve glucose tolerance. In vitro studies have shown that a soybean extract containing the isoflavones genistein and daidzein inhibits glucose absorption into the intestinal brush border membrane vesicles of rabbits [131]. Naringenin was also found to reduce glucose uptake in the intestinal brush border membrane vesicles of diabetic rats to a level similar to that of normal rats [132]. The (−)-epicatechin gallate, myricetin, quercetin, apigenin, (−)-epigallocatechin gallate, and (−)-epigallocatechin demonstrated a marked reduction in glucose absorption, when compared with the control, by competitive inhibition of sodium-dependent glucose transporter-1 [133]. The non-glycosylated flavonoids were shown to reduce glucose absorption under sodium-dependent conditions in vivo and in vitro in animal tissues [134,135]. Besides reducing glucose absorption, another possible mechanism followed by flavonoid compounds to control blood glucose levels is the inhibition of α-glucosidase activity in the intestine. Such inhibitory effects against α-glucosidase activity were observed when luteolin, kaempferol, chrysin and galangin were used both in vitro and in vivo to study the potential role in the absorption and metabolism of carbohydrates [136]. Kim et al. [137] also demonstrated the α-glucosidase inhibitory activity of flavonoids in a study, where it was shown that luteolin, amentoflavone, luteolin 7-O-glucoside and daidzein are the strongest inhibitors of the compounds tested.

It has also been demonstrated that flavonoids can act per se as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms, to attenuate the diabetic complications, besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues,
and regulate the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism pathway. In an experimental study by Liu et al. [138], genistein was found to act directly on pancreatic β-cells, leading to activation of the cAMP/PKA signaling cascade to exert an insulinotropic effect.

Interestingly, it has found that epigallocatechin 3-gallate mimics the effects of insulin on the gene expression reduction of phosphoenolpyruvate carboxykinase and G-6-Pase in the mouse liver [139], like insulin, the drug candidate enhances tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1), mitogen-activated protein kinase, p70s6k, and PI3K activity, and reduces phosphoenolpyruvate carboxykinase gene expression mediated by PI3K [140]. Furthermore, epigallocatechin 3-gallate upregulates glucokinase mRNA expression in the liver of db/db mice [141]. In another study, oral administration of rutin to diabetic rats resulted in a decrease in plasma glucose and increase in insulin levels, and restored the glycogen content and hexokinase activity. The activity of enzymes such as G-6-Pase and fructose-1,6-bisphosphatase significantly decreased in the liver and muscles of rutin-treated diabetic rats [142]. Kaempferol-3-neohesperidoside has been shown to have the efficacy for prompt stimulating of glycogen synthesis in rat soleus muscle by approximately 2.38-fold, it has also been demonstrated that the phosphatidylinositol-3-kinase (PI3K) -glycogen synthase kinase-3 (GSK-3) pathway and mitogen-activated protein kinase (MEK) - protein phosphatase-1 (PP-1) pathway are involved in the stimulatory kaempferol-3-neohesperidoside effect on the glycogen synthesis [143]. Very recently, Cazarolli et al. [144,145] have reported on the mechanism of action of the anti-diabetic effects of apigenin-6-C-β-L-fucopyranoside and apigenin-6-C-(2"-O-α-L-rhamnopyranosyl)-β-L-fucopyranoside – the former drug candidate was evaluated to stimulate insulin secretion and potentiated glucose-induced insulin secretion in hyperglycemic rats, in addition, this flavonoid stimulated glycogen synthesis in rat soleus muscle through mechanisms well known to insulin signal transduction, thereby, establishing the dual effects of apigenin-6-C-β-L-fucopyranoside as an anti-hyperglycemic (insulin secretion) as well as an insulino-mimetic (glycogen synthesis) agent [144]. In another study, the same group of investigators has characterized apigenin-6-C-(2"-O-α-L-rhamnopyranosyl)-β-L-fucopyranoside as both an insulin secretagogue and an insulin-mimetic agent [145].

8. Conclusions

Diabetes mellitus has already emerged as an alarming disease worldwide affecting the public health much. Though presently available therapies
against the disease reduce the sufferings to some extent, still it remains inadequate and at the same time is costly, and also associated with a lot of side effects. Hence, there is an urgent need for search of more efficacious drugs with no or minimum side effects. There has been a growing interest in anti-diabetic agents from natural products, particularly those derived from plants. Flavonoids are naturally occurring phenolic compounds with a broad range of biological activities and the beneficial effects of flavonoids have been studied in relation to diabetes mellitus, either through the inhibition of intestinal $\alpha$-glucosidase enzyme or through their capacity to avoid glucose absorption and/or to improve glucose tolerance. A good number of bio-flavonoids reported over the past 15-20 years discussed in this review clearly demonstrate that these exogenous substances represent an unparalleled source of molecular diversity in relation to the drug discovery process in the treatment of Type-2 diabetes. Although there has been considerable scientific progress over the past few years in unraveling of the effect and mechanism of action of flavonoids, we still need to define the missing steps in the flavonoid-signaling network and elucidate the mechanism of cross-talk based on the complex mechanism of insulin action, in order to provide new insights into the potential role of flavonoids in diabetes treatment. Further study is required concerning safety (assessment of toxic effect) and human trial to develop potential anti-diabetic remedies of choice.

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