Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
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www.academicjournals.com

OPEN ACCESS Research Journal of Phytochemistry

ISSN 1819-3471
DOI: 10.3923/rjphyto.2021.1.13

Review Article
A Critical Review on Potential Pharmacological Activity and Pharmacokinetic Perspective of Swertiamarin

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Abstract

Swertiamarin is a secoiridoid glycoside found extensively in *Enicostemma littorale* and *Swertia chirata* belonging to the family of Gentianaceae, which has been reported to cure many diseases including diabetes, hypertension, atherosclerosis, arthritis, malaria and abdominal ulcers. The present review aimed to compile up-to-date information on the progress made in the protective role of swertiamarin and its metabolites such as gentianine and erythrocentaurin in diabetes mellitus and related complications to provide a guide for future research on this bioactive molecule. Information on the swertiamarin was collected from major scientific databases (Pubmed, Springer, Google Scholar and Web of Science) for publication in1974-2020. In this review, the role of swertiamarin and its metabolites in the management of diabetes mellitus and related complications was discussed. Swertiamarin and its metabolites reported exhibiting a wide range of biological activities such as antidiabetic, anti-atherosclerotic, anti-inflammatory and antioxidant effects. These activities were mainly due to their effect on various signalling pathways associated with swertiamarin such as PPAR-gene upregulation, P-407-induction, inhibition of HMG-Co A reductase, LDL oxidation, lipid peroxidation markers and stimulation of antioxidant enzymes. Swertiamarin and its metabolites exhibit a wide range of biological activities. This review presents evidence supporting the point of view that swertiamarin should be considered a potential therapeutic agent for the management of diabetes mellitus and related complications, giving rise to novel applications in their prevention and treatment.

Key words: Swertiamarin, diabetes mellitus, gentianaceae, *Enicostemma littorale*, LDL oxidation

Citation: Ahamad, J., S. Uthirapathy, E.T. Anwer, M.S.M. Ameen, A. Samad and S.R. Mir, 2021. A critical review on potential pharmacological activity and pharmacokinetic perspective of swertiamarin. Res. J. Phytochem., 15: 1-13.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease that has a significant impact on the health, quality of life and life expectancy of patients as well as on the healthcare system. It is associated with several other metabolic abnormalities such as obesity, hypertension and dyslipidaemia, which contributes to the very high rate of cardiovascular morbidity and mortality[1]. There is an explosive increase in the number of people diagnosed with diabetes worldwide. India is one of the 6 countries of the International Diabetes Federation (IDF) South-East Asia (SEA) region. The 415 M people have diabetes in the world and 78 M people in the SEA region, by 2040 this will rise to 140 M. There were 69.1 M cases of diabetes in India in 2015 (IDF)[1]. Epidemiological studies have demonstrated that obesity associated with inflammation, hypertension and other metabolic aberrations are a major risk factor for diabetes mellitus and cardiovascular disease. Cardiovascular complications are the leading cause of morbidity and mortality in patients with type 2 diabetes[2,3]. The relationship between tissue insulin resistance and compensatory hyperinsulinemia cause early atherosclerosis and an increased cardiovascular risk for the non-insulin-dependent diabetic patient[4]. Sowers *et al*.[5] suggest diabetic patient with cardiovascular complication has a high mortality rate and hypertension is commonly found in diabetes patients than non-diabetes patients. Accelerated atherosclerotic vascular disease is the leading cause of mortality in patients with diabetes mellitus. The increases of oxidative injury in diabetes mellitus patients associated with a weakened defence due to reduced endogenous antioxidants[6].

Current therapy to alleviate diabetes mellitus and related complications (such as cardiovascular complications, diabetic retinopathy, diabetic neuropathy and diabetic nephropathy) is not optimal and thus efforts have been made to develop effective and better drugs, which led to the discovery of natural agents. Herbal remedies are natural products derived from plants and plant products that have been traditionally used to treat various diseases[7]. During the last decade, the use of traditional medicine has expanded globally and it's gaining popularity. Herbs have been widely used for therapeutic purpose due to the expensive and unaffordable of the conventional drug in the rural area because herbal remedies are readily available and cheap. According to the WHO, herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries. The WHO Expert Committee on diabetes has recommended that traditional medicinal herbs be systematically investigated[8].

Many researchers are focusing on natural products and their secondary metabolites in search of new drugs for the management of diabetes mellitus and related complications. Among these secondary metabolites, swertiamarin a secoiridoid glycoside, the main component of plants such as *Enicostemma littorale* has attracted more attention due to its ability to exert beneficial effects in various pathological conditions. The *E. littorale* locally known as *Chota-chiretta* in India is a commonly used Ayurvedic medicine for the treatment of diabetes[9]. It is a glabrous perennial herb and found throughout India. The *E. littorale* is known for several medicinal uses including antidiabetic, hypolipidemic and antipyretic. It is also reported as a digestive, liver tonic, urinary astringent and anti-periodic. It is useful in dyspepsia, flatulence, colic, abdominal ulcers, constipation, intermittent fevers, malaise and pruritus[10]. However, to the best of our knowledge, to date, systemic studies to understand the molecular basis of diabetes and related complications preventing properties of the active constituent of *E. littorale* (swertiamarin) has not been reported. Hence, the present review aimed to compile up-to-date information on the progress made in the protective role of swertiamarin in diabetes mellitus and related complications to provide a guide for future research on this bioactive molecule. Hence, this study based on the collection of research articles related to swertiamarin from various scientific databases and a systemic review of its potential pharmacological role in the management of diabetes mellitus and related cardiovascular complications.

METHODOLOGY

The information on the swertiamarin in diabetes mellitus and related complications were collected from several databases such as Science direct, Pubmed, NCBI, Springer and Google scholar etc., from 1974-2016. Some information also collected from official websites, such as IDF and WHO. The keywords used for the searching, such as swertiamarin and diabetes mellitus, swertiamarin and antihyperlipidaemic activity, swertiamarin and antioxidant activity, swertiamarin and anti-inflammatory activity, swertiamarin and nephropathy and metabolism and pharmacokinetics of swertiamarin.

Potential pharmacological activity of swertiamarin: Swertiamarin (Fig. 1) is a secoiridoid glycoside derived from loganic acid through mevalonic acid pathway. It is distributed predominantly among the members of Gentianaceae, mainly *Enicostemma littorale* Blume and *Swertia chirata* Roxb. Some specific activities have been reported for swertiamarin

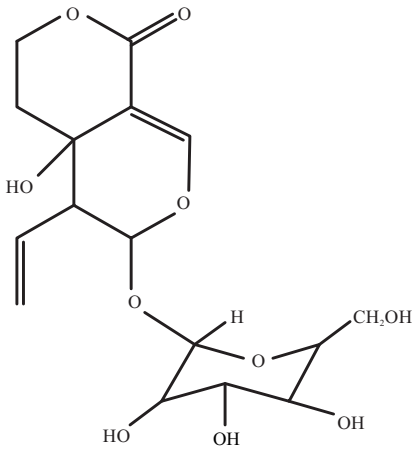

Fig. 1: Chemical structure of swertiamarin[11]

such as anticholinergic, antihyperlipidemic, insulinotropic, antinociceptive, antioxidant and hepatoprotective[10]. This review provides an overview of the swertiamarin role in the management and treatment of diabetes mellitus and related complications.

E. littorale has a long history of human use in traditional medicine throughout India. There are a plethora of reports of experimental and clinical pieces of evidence related to various pharmacological activities of *E. littorale* and swertiamarin. Up-dated information summarized in Table 1 (potential pharmacological activity of swertiamarin in pre-clinical study) and Table 2 (clinical efficacy of *E. littorale* in diabetic patient).

Antidiabetic activity: The authors establish the effectiveness of swertiamarin in achieving glucose homeostasis via inhibition of carbohydrate metabolizing enzymes by *in vitro* and *in vivo* studies. Swertiamarin was effective in inhibiting α-amylase and α-glucosidase with IC_{50} values of 1.29±0.25 and 0.84±0.11 mg mL^{-1}, respectively. The studies in starch and sucrose challenged mice showed that swertiamarin effectively restricted the increase in the peak Blood Glucose Level (BGL). The increase in peak BGL was 49 and 57 mg dL^{-1} only in the treatment groups compared to 70 and 80 mg dL^{-1} in untreated groups after 30 min in starch and sucrose-fed mice, respectively[11]. Same group isolated erythrocentaurin from the ethyl acetate fraction of *E. littorale* using medium-pressure liquid chromatography. The method consisted of a simple step gradient from 10-20% ethyl acetate in n-hexane. Isolated erythrocentaurin was evaluated for α-amylase inhibition activity, it exhibited a concentration-dependent α-amylase inhibition (IC_{50} 1.67±0.28 mg mL^{-1})[12]. Dhanavathy *et al.*[13] have investigated the effect of swertiamarin in diabetic Wister rat. Swertiamarin at 15, 25, 50 mg kg^{-1} b.wt., in 28 days, has significantly reduced fasting blood glucose, glycated haemoglobin (HbA1c), total cholesterol, triglycerides, LDL and increase the plasma insulin, HDL levels and body weight as compared to STZ-induced diabetic rats. The pancreas of swertiamarin treated diabetic rats showed regeneration of islets when compared to STZ-induced diabetic rats as confirmed by immunohistological studies.

Phoboo *et al.*[14] have reported the antidiabetic activity of swertiamarin isolated from *Swertia chirayita.* The crude aqueous and 12% ethanolic extracts of *Swertia chirayita* showed a moderate-to-high positive correlation between antioxidant activity and total phenolic content and moderate-to-high α-glucosidase inhibitory activity. The hexane fraction of *Swertia chirayita* has local healing and antihyperglycemic potential due to the presence of three main phytochemicals mainly swertiamarin, mangiferin and amarogentin. Swertiamarin has shown to modulate 5-HT_2 receptor and hypolipidemic potential in animal models of depression, diabetes and obesity[15]. *In-silico* studies performed by Vaidya *et al.*[16] reported that swertiamarin, secoiridoid glycoside has been shown to possess antidiabetic activity through inhibiting glycogen phosphorylase-a at pyridoxal phosphate binding site with its docking energy of -7.01 kcal moL^{-1}. Swertiamarin and its derivatives confer anti-diabetic and anti-hyperlipidemic effects[17]. Maroo *et al.*[18] reported the dose-dependent blood-glucose-lowering effect of the aqueous extract of *E. littorale* in alloxan-induced diabetic rats. A dose of 1.5 g dry plant equivalent extract per 100 g body weight resulted in a significant increase in serum insulin levels without producing any toxic effects as compared to diabetic control rats. The effect was mainly attributed regeneration of β-cells in the pancreas and stimulating insulin release[19]. An aqueous extract of *E. littorale* when administered to streptozotocin (STZ)-induced neonatal NIDDM rats in the dose of 2 g kg^{-1} orally for 6 weeks, resulted in a significant decrease in fasting BGL in glucose fed rats. Insulin sensitivity was found to be significantly increased after treatment with *E. littorale* along with a decrease in elevated cholesterol, triglyceride and creatinine values of NIDDM rats[20]. A herbo-mineral formulation containing *E. littorale* exhibited antihyperglycemic and antioxidant activities when administered orally to STZ-induced diabetic rats at doses of 100 and 200 mg kg^{-1} continuously for 45 days. Significant improvement in blood glucose tolerance was also observed[21].

Clinical efficacy in the diabetic patient: The fresh juice of *E. littorale* (1-5 ounce three times a day along with the diabetic diet) produced a fall in fasting blood sugar in 17 cases

Table 1: Potential pharmacological activity of Swertiamarin in a pre-clinical study

Drug/Sources	Model	*In vivo*/ *In vitro*	Dose	Effect	References
Swertiamarin *(E. littorale)*	Anti-diabetes (Type 1 diabetes Wistar rats	*In vivo*	15, 25 and 50 mg kg^{-1} b.wt., (28)	Significantly decrease in fasting sugar, HbA1c, LDL and increase in body weight, plasma insulin and HDL. Also, regeneration of islets was observed	Dhanavathy *et al.*[13]
Swertiamarin (*S. chirayita*)	Anti-diabetes (Crude aqueous and 12% ethanol solution extracts)	*In vitro*	-	Both extracts have moderate-to-high α-glucosidase inhibitory activity	Phoboo *et al.*[14]
Swertiamarin *(E. littorale)*	Anti-diabetes (STZ-induced diabetic rats)	*In vivo*	2 g kg^{-1} (6 weeks)	Fasting and fed glucose and insulin levels were significantly decreased. Also, the insulin sensitivity index was significantly increased	Babu and Prince[21]
Swertiamarin *(E. littorale)*	Anti-diabetes (Zucker fa/fa rat)	*In vivo*	75 mg kg^{-1} day (28 days)	Swertiamarin significantly reduced serum glucose, MMP-3 and 9 and urea	Vaidya and Giri[30]
Swertiamarin *(E. littorale)*	Anti-diabetes (STZ-induced diabetic nephropathy in Sprague Dawley rats)	*In vivo*	*E. littorale* 1g kg^{-1} and swertiamarin 50 mg kg^{-1} (3 weeks)	Significant decrease in the levels of serum glucose, urea, creatinine, cholesterol, SGPT and SGOT in rats treated with aqueous extract of *E. littorale* and swertiamarin.	Sonawane *et al.*[41]
Swertiamarin *(E. littorale)*	Atherosclerosis (Hypercholesterolemic diet)	*In vivo*	50 and 75 mg kg^{-1}	Inhibit the biosynthesis of hepatic cholesterol by blocking HMG CoA reductase and trigger an over-expression of hepatic LDL receptors. Decreased ratio of serum cholesterol to HDL-C	Vaidya *et al.*[26]
Swertiamarin *(E. littorale)*	Atherosclerosis (Poloxamer-407 induced hyperlipidemia rat)	*In vivo*	50 mg kg^{-1}	Decrease serum cholesterol levels and oxidation of LDL. Reduction of the ratio of LDL to HDL cholesterol	Vaidya and Rajani[27]
Swertiamarin *(E. littorale)*	Atherosclerosis (Zucker fa/fa rat)	*In vivo*	75 mg kg^{-1} day (28 days)	Serum cholesterol and triglyceride are reduced.	Vaidya and Giri[30]
Swertiamarin *(E. littorale)*	Atherosclerosis (Hypercholesterolemic diet)	*In vivo*	1.5 g dry plant equivalent 100 g^{-1} b.wt., day	Decrease in the level of triglycerides and LDL and increase in HDL-C	Vasu *et al.*[35]
Swertiamarin *(E. littorale)*	Hypertension (high fructose-fed rats)	*In vivo*	1.5 g dry plant equivalent extract (100 g^{-1} b.wt., day)	Swertiamarin improved insulin resistance, along with reduced hypertriglyceridemia, hypertension, platelet aggregability, blood coagulation, serum enzymes (CK-MB, SGOT, LDH and SGPT) and vascular reactivity in HF fed rats	Bhatt *et al.*[31]
Swertiamarin (*E. axillare*)	Antioxidant (D-GaIN hepatotoxicity model)	*In vivo*	100 and 200 mg kg^{-1} b.wt.,	Increased the levels of SOD, GSH and decreased the levels of TBARS in serum, liver and kidney caused by D-GaIN.	Jaishree and Badami[34]
Swertiamarin (*E. axillare*)	Anti-inflammatory (Freund's Complete Adjuvant induced Arthritis in Sprague Dawley rats)	*In vivo*	2, 5, 10 mg kg^{-1} b.wt.,	Swertiamarin significantly inhibited the levels of paw thickness, lysosomal enzymes and increased the body weight. Besides, it decreased the release of proinflammatory cytokines (IL-1, TNF, IL-6) and proangiogenic enzymes (MMPs, iNOS, PGE2, PPARγ and COX-2) and increases the levels of anti-inflammatory cytokines (IL-10; IL-4)	Saravanan *et al.*[37]
Swertiamarin (*E. axillare*)	Anti-inflammatory (FLS isolated from AA rats and cultured with IL-1β)	*In vitro*	10–50 µg mL^{-1}	mRNA and protein expression levels of caspase 3, Proinflammatory mediators(TNFα, IL-6, PGE2, COX-2, iNOS, MMPs) and osteoclastogenic mediator (RANKL) were decreased through reduction in the proliferation of FLS and NO production	Saravanan *et al.*[38]

Where, STZ: Streptozotocin; HbA1c: Hemoglobin A1c, LDL: Low density lipoprotein, MMP-3: Matrix metalloproteinase-3, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic-oxaloacetic transaminase, HMG CoA: β-Hydroxy β-methylglutaryl-CoA, CK-MB: Creatine kinase-MB, SOD: Superoxide dismutase, GSH: Glutathione, D-GaIN: D-Galactosamine induced, IL: Interleukin, TNF: Tumor necrosis factor, iNOS: Inducible nitric oxide synthase, PGE2: Prostaglandin E_2, PPARγ: Peroxisome proliferator-activated receptors, COX-2: Cyclooxygenase-2, FLS: Fibroblast-like synoviocytes, AA rats: Adjuvant-induced arthritis rats, RANKL: and Receptor activator of nuclear factor kappa-B ligand

Table 2: Clinical efficacy of *E. littorale* in diabetic patient

Therapeutic agent	Study models	Dose and duration of study	Outcome	References
E. littorale	Type 2 diabetes (n=17)	The fresh juice of *E. littorale* (1-5 ounce three times a day along with the diabetic diet)	Treatment with fresh juice of *E. littorale* significantly decreases fasting blood sugar of diabetic patients	Thirumalai[22]
E. littorale	Type 2 diabetes (n=20)	Aqueous extract of the *E. littorale* (5 g twice a day, 30 min before meal) for two months	Significant decrease in fasting blood glucose, postprandial blood sugar, glycosylated haemoglobin, serum cholesterol, triglyceride, LDL, VLDL, levels and a significant increase in serum insulin, HDL levels were observed. The antioxidant parameters of the patients also improved	Vasu *et al.*[23]
E. littorale	Type 2 diabetes (n=84)	250 mg per pill twice daily for 3 months	Blood glucose and serum insulin levels were significantly decreased. Reduced urine sugar, systolic and diastolic pressure, as well as pulse rate, was observed	Upadhyay and Goyal[24]
E. littorale	Type 2 diabetes (n=50)	Aqueous extract of *E. littorale* (500 mg) 3-times a day for a period of 3-months with follow up of 1-month	Significant decrease in fasting blood glucose, postprandial blood sugar, serum cholesterol, triglyceride, LDL, VLDL, levels and the significant increase in HDL levels were observed	Mir *et al.*[25]

Where, *Enicostemma littorale*, n: Number, LDL: Low-density lipoprotein, VLDL: Very-low-density lipoprotein, HDL: High-density lipoprotein

of diabetic patients[22]. In another study, the antidiabetic efficacy of the aqueous extract of the *E. littorale* was examined in newly diagnosed NIDDM patients taking only the extract and was administered as two divided doses, 30 min before the meal as 5 g aqueous extract per single dose. Out of the 20 patients who volunteered, 11 completed the 2 month trial and a decrease in fasting and postprandial blood glucose and glycosylated haemoglobin levels were observed along with an improvement in the antioxidant parameters of the patients. There was also an increase in serum insulin levels in 7 patients after extract treatment as compared to levels before treatment. Serum total cholesterol and serum triglyceride levels were decreased significantly with a significant increase in serum HDL cholesterol levels[23]. Upadhyay and Goyal[24] demonstrated the efficacy of *E. littorale* (250 mg pilL^{-1}) twice a day for 3 months in preventing various complications arising in type 2 diabetic patient (n = 84). At the end of the treatment, blood glucose and serum insulin levels of type 2 diabetic patients were significantly reduced from 205±10.2-150±6.8 mg dL^{-1} and 99.8±8.6-73.2±6.3 MCU mL^{-1}, respectively. It also significantly reduced urine sugar, systolic blood pressure as well as pulse rate. Also, treatment significantly reduced serum creatinine, cholesterol and triglyceride levels along with a significant increase in serum HDL levels. These results suggest that *E. littorale* provided amelioration of the hyperglycemic and hyperinsulinaemic conditions of diabetic patients. The therapeutic potential of standardized aqueous extract of *E. littorale* was evaluated in metabolic syndrome patients (n = 50). Patients were randomized to either placebo or aqueous extract of *E. littorale* (500 m three times a day in addition to ongoing conventional treatment for 3 months with follow up o 1 month). The *E. littorale* treatment showed a significant decline in fasting blood sugar, postprandial blood sugar, serum cholesterol, triglyceride, LDL, VLDL levels and the significant increase in HDL levels. These results suggest that *E. littorale* exhibit excellent hypoglycemic and hypolipidemic potential in metabolic syndrome patients[25].

Cardioprotective and antihyperlipidemic activity: Swertiamarin has been reported to have high anti-atherogenic, cholesterol-lowering potential and inhibition of HMG-Co-A reductase[26]. The antihyperlipidaemic effect of swertiamarin is carried out in a rat model of hyperlipidaemia induced by P-407 (Poloxamer-407), a non-ionic surfactant. Studies have reported that swertiamarin decreases serum cholesterol levels and reduced the oxidation of LDL. Besides, swertiamarin has also shown to have the ability in increasing HDL levels and reduction in the ratio of LDL/HDL cholesterol[27].

Swertiamarin (50 mg kg^{-1}, i.p.) administered once a day for 6 weeks resulted in significant reductions in serum triglycerides, cholesterol and low-density lipoprotein levels in diabetic animals. Serum fasting glucose was significantly decreased, moreover, the insulin sensitivity index was significantly increased in swertiamarin treated animals[28]. Swertiamarin treatment is reported to have no significant effect on adipogenesis or the mRNA expression of PPAR-g and GLUT-4, however, there was a significant increase in the mRNA expression of adiponectin. Treatment with gentianine, active metabolite of swertiamarin significantly increased adipogenesis, which was associated with a significant increase in the mRNA expression of PPAR-g, GLUT-4 and adiponectin[29].

The non-fasting glucose level in Zucker rats was significantly reduced with the treatment of swertiamarin but AUCglucose showed small changes. The serum cholesterol and triglyceride also reduced in swertiamarin treated rats. Insulin can activate the lipoprotein lipase and hydrolyses triglycerides. Therefore, insulin deficiency causes the inactivation of lipoprotein lipase and result in dyslipidemia. When swertiamarin is used at 75 mg kg^{-1} day i.p., for 28 days, the serum glucose, cholesterol, triglycerides, non-esterified free fatty acid, urea and matrix metalloproteinase 3 and 9 (MMP 3 and MMP 9) has been shown significant reduction than untreated Zucker fa/fa rat and these results suggest that the decrease in serum MMP 9 and MMP 3 levels are one of the possible mechanism responsible for the improvement of these complications by swertiamarin[30]. The aqueous extract of *E. littorale* showed a cardioprotective and antihypertensive effect in fructose-fed rats. High fructose-fed rats treated with *E. littorale* showed improved insulin resistance, along with reduced hypertriglyceridemia, hypertension, platelet agreeability, blood coagulation, serum enzymes (CK-MB, SGOT, LDH and SGPT) and vascular reactivity[31]. The aerial part of *E. littorale* showed a hypolipidaemic effect in p-dimethylamino benzene induced hepatotoxic animals[32].

Antioxidant activity: In an *in vitro* study, swertiamarin showed antioxidant activity by scavenging hydroxyl radicals, H_2O_2 superoxide radicals and inhibiting lipid peroxidation and nitric oxide radical[33]. Furthermore, studies conducted by Jaishree and Badami[34], by using D-galactosamine induced acute liver damage in rats. The D-galactosamine caused significant hepatotoxicity by alteration of several hepatic parameters. It also caused significant lipid peroxidation and reduced the levels of antioxidant defence mechanisms. The elevated levels of CAT, SOD and GSH and reduced levels of thiobarbituric acid reactive substances were observed in serum, liver and kidney after treatment with swertiamarin (100 and 200 mg kg^{-1} b.wt.) for 8 days before D-galactosamine. Other than that, D-galactosamine intoxicated rats have been shown to reduce hepatic damage with bile duct proliferation with swertiamarin treatment. These observations suggest that swertiamarin restored these antioxidant mechanisms in the damage caused by D-galactosamine. The aqueous extract of *E. littorale* at a dose of 1.5 g per 100 g b.wt., decreased the activities of erythrocyte catalase and superoxide dismutase and lipid peroxidation levels, with the increase in glutathione levels in hypercholesterolaemic rats[35]. Oral administration of aqueous extract of the whole plant (1 and 2 g kg^{-1}, 45 days) significantly decreased blood glucose, thiobarbituric acid reactive substances, hydroperoxides and increased glutathione, superoxide dismutase, catalase and glutathione peroxidase in liver, kidney and pancreas of alloxan diabetic rats[36].

Anti-inflammatory activity: Swertiamarin (100 and 200 mg kg^{-1} b.wt.,) showed antiedematogenic activity in carrageenan, formalin and histamine-induced paw oedema in albino rats[33]. Saravaran *et al.*[37] demonstrated the anti-arthritic efficacy of swertiamarin in adjuvant arthritis animal model by the estimation of biochemical parameters, proinflammatory cytokines and enzymes along with histopathological and radiographic observations. They reported that swertiamarin (2, 5, 10 mg kg^{-1} b.wt.) significantly abolished inflammation by regulating toxic substances like lysosomal enzymes, free radicals, bone destructive enzymes and paw thickness of arthritic animals. The histopathological and radiological evidence supported the curative effect of swertiamarin on bone destruction and confirmed the recovery of treated animals through balancing inflammatory mediators in the arthritic joint. The treatment significantly diminished the protein levels of p65 NF-Kb, p-IκBα, a p-STAT3 and p-JAK2 transcription factor in animals as well as LPS induced RAW 264.7 macrophage cells. The release of proinflammatory cytokines (IL-1, TNF, IL-6) and proangiogenic enzymes (MMPs, iNOS, PGE2, PPARγ and COX-2) were decreased and significantly increased the levels of anti-inflammatory proteins (IL-10, IL-4) in the swertiamarin treated groups when compared to the disease group. These results suggest that the swertiamarin inhibited the development of arthritis by modulating NF-KB/IκB, JAK2/STAT3 signalling and thus it acts as an anti-arthritic agent.

Saravaran *et al.*[38] in another study evaluated the anti-inflammatory potential of swertiamarin in IL-1β induced Fibroblast Like Synoviocytes (FLS) which was isolated from Freund's Complete Adjuvant Induced Arthritic (AIA) rats. Both

the normal FLS and AA-FLS were used for the experiment and its efficacy was estimated in terms of mRNA and protein expression levels of inflammatory and osteoclastogenesis mediators. Results indicated that IL-1β induced cell proliferation (149.46±13.73%) and NO production (162.03±11.03%) in AA-FLS was abolished after treatment with swertiamarin. Swertiamarin also significantly modulated the expression of apoptotic marker (caspase 3), proinflammation mediators (TNF-α, IL-6, PGE2, COX-2, iNOS, MMPs) and osteoclastogenic mediator (RANKL) at both the mRNA and protein levels. Further, treatment with swertiamarin inhibited the levels of p38 MAPKα in a dose-dependent manner and also significantly attenuated the release of the same in a time-dependent mode. This finding suggests that treatment with swertiamarin attenuated IL-1β induced FLS and it revealed anti-inflammatory potential by attending aggressive FLS.

In vivo and *in vitro* study on immunomodulatory activity of swertiamarin (2, 5, 10 mg kg^{-1} b.wt.) was further conducted by Saravaran *et al.*[39] *In vivo* activity was estimated in Sheep Red Blood Cell (SRBC) model by the assessment of Hemagglutinating Antibody (HA) titer, effect on organ weight, Plaque-Forming Cells (PFC) assay, Delayed-Type Hypersensitivity (DTH) reaction and quantitative hemolysis of SRBC. Whereas, the *in vitro* studies were done on isolated splenocytes, neutrophils and peritoneal macrophages. In an *in vivo* study, swertiamarin treated animal showed a significant increase in HA titer, PFC and weight of the spleen and thymus, however, the immune-inflammatory reaction was reduced in the DTH reaction. In an *in vitro* study, mRNA and protein levels of Th2-mediated cytokines (IL-4 and IL-10) are elevated, whereas the levels of Th1-mediated cytokines (TNF-α, IL-1β and IL-6) in Con A-stimulated splenocytes were reduced with swertiamarin treatment. Further, swertiamarin inhibited the release of free radicals significantly in phytohemagglutinin-induced neutrophils and also ameliorated the mRNA and protein expression of proinflammatory cytokines (TNF-α, IL-1β and IL-6) in lipopolysaccharide (LPS)-induced macrophages and these results confirmed that swertiamarin acted as an anti-inflammatory mediator. The *E. littorale* was found to exert 54 and 30% anti-inflammatory activity in carrageenan-induced acute inflammation and chronic inflammation of cotton pellet granuloma respectively. The alcoholic extract was found to be effective on human erythrocyte membrane stabilization and inhibition of cobra venom phospholipase A_2 and the effect was found to be comparable to the standard anti-inflammatory drug, hydrocortisone[40].

Swertiamarin in diabetic nephropathy: Diabetic nephropathy was assessed by serum urea, creatinine, lipid profile and water intake levels. The aqueous extract of *E. littorale* treatment significantly prevented the loss in body weight, water intake and food consumption. Swertiamarin significantly revered the elevated glucose level in STZ induced diabetic rats. Also, aqueous extract of *E. littorale* can inhibit HMG-CoA reductase action which is the limiting factor of the LDL metabolism. Treatment with aqueous extract of *E. littorale* (1 g kg^{-1}, p.o.) and swertiamarin (50 mg kg^{-1}, p.o.) daily for 3 weeks in type 1 diabetic nephropathy complications in Sprague-Dawley rats significantly decreased serum urea and creatinine and other parameters associated with the development of diabetic nephropathy in type 1 diabetic rats. Besides, considerable improvement in the histology of glomerular function has been observed in animals treated with the aqueous extract of *E. littorale* and swertiamarin. Besides that, SGPT and SGOT levels were decreased in type 1 diabetes rats treated with an aqueous extract of *E. littorale*[41].

Metabolism and pharmacokinetic perspective of swertiamarin: The metabolism of swertiamarin conducted by using human intestinal bacteria (*Proteus mirabilis*). This bacterium enables the swertiamarin to metabolize into gentianine, erythrocentaurin and 5-(hydroxymethyl)-isochroman-1-one[42] as presented in Fig. 2.

Wang *et al.*[43] investigated the metabolic fate of swertiamarin in Wister rats by using liquid chromatography/ion trap mass spectrometry. The results showed two new metabolites of swertiamarin (1), (R)-gentinol (4a) and (S)-gentnol (4b) in rat plasma together with known metabolite gentianine (2). The authors also prepared (R)-gentinol (4a) and (S)-gentnol (4b) metabolites from stereoselective reduction of gentianone (3) which is and oxidation product of gentianine (2) as presented in Fig. 3.

Li *et al.*[44] demonstrated that swertiamarin showed rapid absorption, elimination and high concentrations were found in the liver and kidneys, indicating that swertiamarin was rapidly metabolized in the liver and eliminated by the kidneys. By using LC-ESI-MS/MS method the plasma concentration-time profile of swertiamarin (20 and 50 mg kg^{-1}) has been obtained in SD-rats and the pharmacokinetic parameters have been calculated. Study results showed that swertiamarin was rapidly absorbed into blood circulation after administration. Based upon results, swertiamarin required 0.95 hrs to reach peak concentration (1920.1 mg mL^{-1}) and half-life was less than 1.50 hrs. Wang *et al.*[45] reported that swertiamarin was biotransformed to erythrocentaurin and 3,4-dihydro-5-

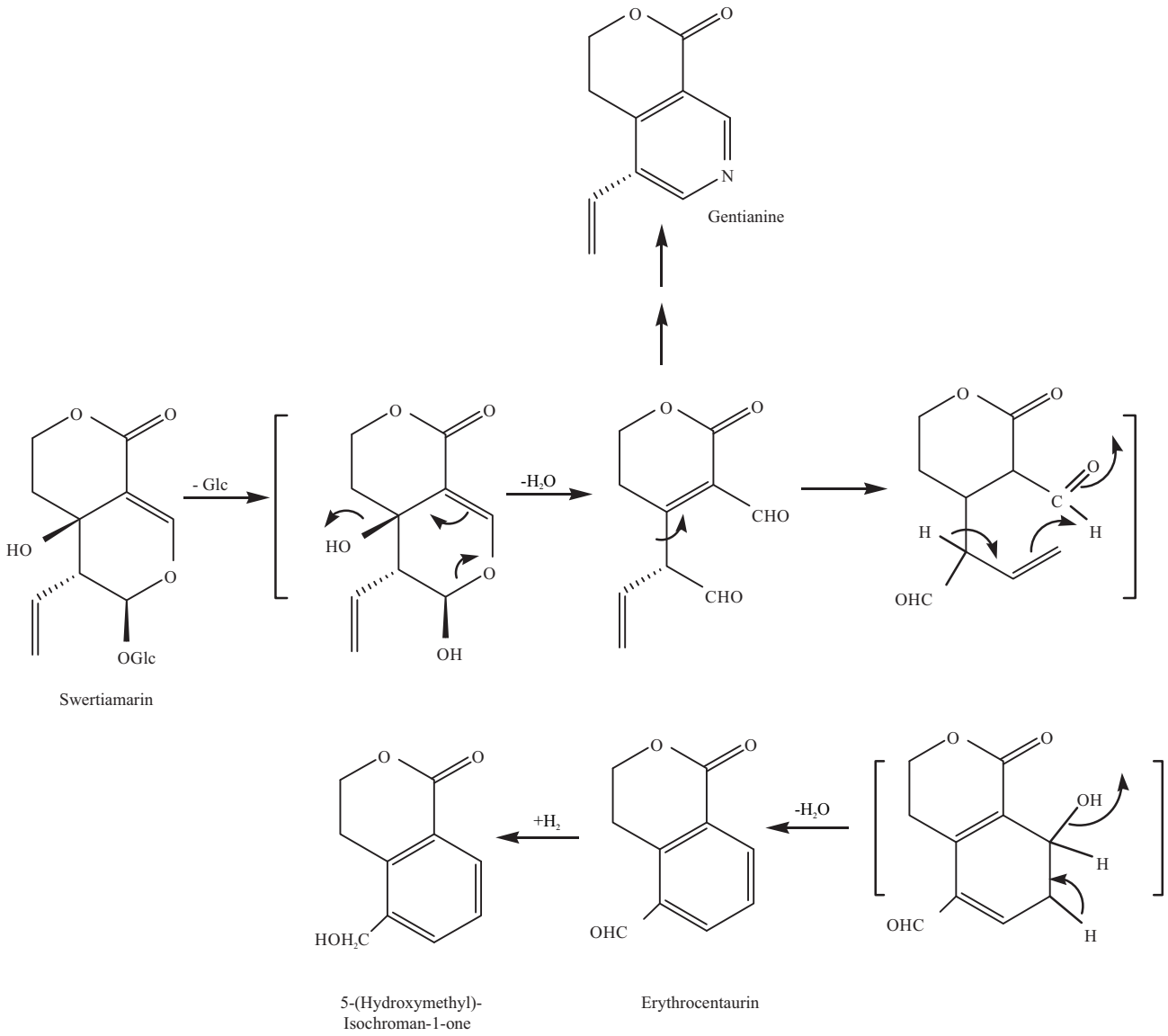

Fig. 2: Metabolites of swertiamarin[42]

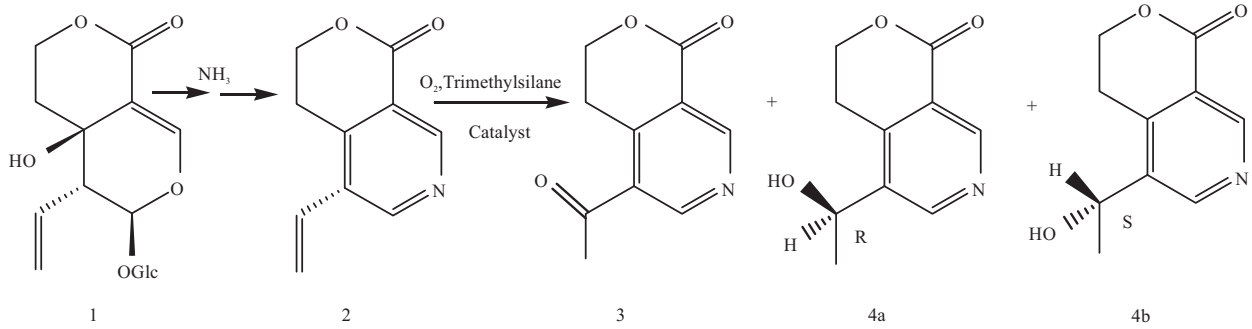

Fig. 3: Preparation of gentianine (2), gentianone (3), (R)-gentinol (4a) and (S)-gentnol (4b) from swertiamarin (1)[43]

(hydroxymethyl) isochroman-1-one by human intestinal bacteria. Xu *et al.*[46] studied the pharmacokinetics of swertiamarin alone and co-administration with oleanolic acid. In the previous study, it has shown swertiamarin is rapidly absorbed into blood circulation. When swertiamarin co-administered with oleanolic acid, both peak plasma concentration and AUC were reduced while both clearance and apparent volume of administration were increased. This indicates oleanolic acid could reduce the bioavailability of rats due to drug-drug interaction.

An *in vivo* metabolism study of swertiamarin has been performed by Wu *et al.*[47] by using 2,4-dinitrophenylhydrazine derivatization. The main metabolite of swertiamarin which is erythrocentaurin has been detected after oral administration of swertiamarin. This study proposed that swertiamarin undergoes hydrolysis to give aglycone by bacterial β-glucosidase, which is readily converted to erythrocentaurin. Vaidya *et al.*[26] revealed that gentianine is an active metabolite of swertiamarin that possesses a pharmacophoric moiety. They explored whether the antidiabetic effect of swertiamarin is due to gentianine, using 3T3-L1 cells. They found that gentianine significantly increased adipogenesis by upregulating the mRNA expression of PPAR-γ, GLUT-4 and adiponectin while swertiamarin treatment had no significant effect on adipogenesis however, there was a significant increase in the mRNA expression of adiponectin. These results suggest that antidiabetic effect of swertiamarin in *in vivo* is possibly due to active metabolite gentianine.

Quantitative estimation of swertiamarin in different samples: Given the importance of *E. littorale* in traditional systems of medicine, several analytical methods have been reported for the estimation of its bioactive components (swertiamarin) and its quality assessment. An HPLC method for estimation of swertiamarin in methanolic extract of *E. littorale* and marketed formulations was developed and validated. The mobile phase composed of methanol and waters (90:10 v/v) with the retention time of 10.15±1.52 min. The method was found precise and accurate for the estimation of swertiamarin in methanolic extract and marketed formulation[48].

An HPLC-ESI-MS method has been reported for the simultaneous determination of four iridoid glycosides in a formulated preparation composed of 10 ingredient herbs, with iridoids as the main bioactive components. The sample preparation for quantification comprised of a simple ultrasonic extraction and the satisfactory chromatographic separation of the four structurally similar iridoid glycosides was affected in less than 3 min on a C18 column (3 µm, 100×2.0 mm), using an elution system of 10% methanol[49]. The HPTLC densitometry method has been developed for the analysis of swertiamarin in methanolic extract of *E. littorale* and commercial formulations. The method was employed with a high degree of precision and accuracy for the estimation of swertiamarin in methanolic extract of *E. littorale* and commercial formulations[50]. An LC-MS/MS method was developed for the simultaneous estimation of two bioactive markers, mangiferin and amarogentin along with three other components, amaroswerin, sweroside and swertiamarin in rat plasma using kutkoside as an internal standard. The method was successfully applied to generate a pharmacokinetic profile of markers as well as to detect other components in plasma after intravenous dose administration of herbal preparation containing secoiridoids in male Sprague-Dawley rats[51].

An HPLC method has been reported for the simultaneous determination of swertiamarin and its two metabolites, erythrocentaurin and (5Z)-5-ethylidene-8-hydroxy-3,4,5,6,7,8-hexahydro-1H-pyrano [3,4-c] pyridin-1-one (M1) in *Aspergillus niger*. The chromatographic separation was achieved on a C18 ODS column by gradient elution with 0.04% formic acid in water and 0.04% formic acid in acetonitrile as the gradient mixtures. The retention times of swertiamarin, erythrocentaurin and M1 were 14.6, 16.8 and 24.8 min, respectively. The method was applied for the quantification of swertiamarin and its two metabolites during the fermentation process and the evaluation of the bioavailabilities[52]. Chia-Ming L *et al.*[53] developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous determination of gentiopicroside, geniposide, baicalin and swertiamarin in rat plasma. To avoid the stress caused by restraint or anaesthesia, a freely moving rat model was used to investigate the pharmacokinetics of herbal medicine after the administration of a traditional Chinese herbal prescription of Long-Dan-Xie-Gan-Tang (10 g kg^{-1}, p.o.). Analytes were separated by a C18 column with a gradient system of methanol-water containing 1 mM ammonium acetate with 0.1% formic acid. The linear ranges were 10-500 ng mL^{-1} for gentiopicroside, geniposide and baicalin and 5-250 ng mL^{-1} for swertiamarin in biological samples. The swertiamarin showed the lowest level of AUC (2.5±0.1 min µg mL^{-1}) and fell below the lower limit of quantification (LLOQ) at 300 min in the concentration-time curves after LDXGT administration. It was assumed that this low level in rat plasma was due to either the low content in LDXGT (0.22 mg g^{-1}) or the first-pass effect. The authors also develop a simple, precise, rapid and reliable HPTLC method for the estimation of swertiamarin in traditional bitters and formulations. Aluminium-backed silica gel 60 F_{254} plates

Table 3: Analytical methodology for the quantitative estimation of swertiamarin and related compound

Type of sample	Analytical methodology	Mobile phase	Compound estimated	References
Hydro-alcoholic extracts of *E. littorale*, *S. chirata* and formulations	HPTLC	Ethyl acetate-methanol-water (80:15:5, v/v)	Swertiamarin	Ahamad *et al.*[54]
Methanolic extract of *E. littorale*	HPLC-UV	Methanol: waters (90:10 v/v)	Swertiamarin	Alam *et al.*[48]
Formulation	HPLC-ESI-MS	10% methanol	Iridoid glycosides	Yang *et al.*[49]
S. chirata in rat plasma	LC-ESI-MS/MS	Methanol: water (25:75 v/v, containing 0.1% acetic acid)	Swertiamarin	Li *et al.*[44]
Methanolic extract of *E. littorale* and commercial formulations	HPTLC	Ethyl acetate: methanol: water (77:15:8 % v/v)	Swertiamarin	Alam *et al.*[50]
Formulation containing *S. chirata* in rat plasma	LC-MS/MS	Gradient system of acetonitrile: ammonium acetate buffer 0.5 Mm, pH 3	Swertiamarin, mangiferin and amarogentin	Suryawanshi *et al.*[51]
In *Aspergillus niger* ferments	HPLC-UV	Gradient system of Formic acid (0.04%) in water and formic acid (0.04%) in acetonitrile	Swertiamarin erythrocentaurin	Jun *et al.*[52]
The formulation in rat plasma	LC-MS/MS	Gradient system of methanol-water containing 1 mM ammonium acetate with 0.1% formic acid	Gentiopicroside, geniposide, baicalin and swertiamarin	Chia-Ming *et al.*[53]
Hydro-alcoholic extract of *E. littorale*	HPLV-UV	Methanol-water (80:20)	Swertiamarin	Ahmad *et al.*[55]

Where, HPTLC: High-performance thin-layer chromatography, HPLC: High-performance liquid chromatography, HPLC-ESI-MS: High-performance liquid chromatography-Electrospray ionization mass spectrometry, LC-MS/MS: Liquid chromatography-tandem mass spectrometry

(20×10 cm) was used as stationary phase and ethyl acetate-methanol-water (80:15:5, v/v) as the mobile phase. Densitometric quantification was carried out at 246 nm. Regression analysis of the calibration plot showed a good linear relationship between peak-area vs. swertiamarin concentration. Linearity was found to be in the range of 200–1200 ng per band. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The limit of detection (LOD) and quantification (LOQ) for swertiamarin was found to be ~38 and ~115 ng per band, respectively. The developed method was found to be linear (r2 = 0.9981), precise (Relative Standard Deviation [RSD] $\leq$1.42% and $\leq$1.83% for intra-day and inter-day precision), accurate (mean recovery ranged from 97.69±1.46-100.03± 0.61%), specific and robust[54]. The results showed a good correlation with data obtained from HPLC analysis used as the reference method[55]. Table 3 summarizes the analytical methodology for quantitative estimation of swertiamarin and related compound.

CONCLUSION

E. littorale is a rich source of chemically novel compounds such as swertiamarin and needs exhaustive screening against new targets in future. As exemplified in this study, swertiamarin has been shown to exert beneficial effects in several models of diabetes mellitus and cardiovascular diseases. The mechanism by which swertiamarin reduces diabetes includes a reduction in blood glucose, HbA1c, total cholesterol, triglycerides, LDL levels and elevation in body weight and plasma insulin, HDL levels as well an increase the insulin sensitivity index. It also decreases the SGOT, SGPT and serum ALP activities. Further, it significantly decreased serum urea and creatinine and other parameters associated with the development of diabetes mellitus.

SIGNIFICANCE STATEMENT

This study discovered the swertiamarin a secoiridoid glycoside from *E. littorale* that can be beneficial for the management of diabetes and related complications. This study will help the researchers to uncover the critical areas of phytopharmacology and pharmacokinetic perspective of swertiamarin that many researchers were not able to explore.

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