DISCUSSION

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The macroscopy and microscopy studies were carried out to authenticate the plant *E. littorlae*. The leaf was isobilateral in transverse section. The lower and upper epidermis are made up of tabular cells and covered with thick striated cuticle. Both the epidermis bear anisocytic stomata. The mesophyll consists of 5-9 layers of parenchymatous cells filled with chloroplasts. In the midrib region, the lower epidermis is followed by 1-2 layers of collenchyma. In the centre of midrib, there is a circular vascular strand with central xylem surrounded by phloem. Rosettes of calcium oxalate are rarely seen in the mesophyll cells. All these characteristics are identical to those reported earlier by Prasad and Bhushan (1954).

The stem is squarish in transverse section was and found to have four bulges at four corners. The epidermis is made up of cubical cells and is covered with thick striated cuticle. The epidermis is followed by hypodermis composed 1-2 layers of collenchymatous cells. The space inside the bulges is filled collenchyma. The cortex consists of 5-8 layers of parenchymatous cells. The cortex is followed a ring of endodermis which consists of large barrel-shaped cells. Phloem consists of sieve tissues, companion cells and phloem parenchyma. Cambium consisting of 1-2 layers of cells present in between the phloem and xylem. Xylem consists of vessels, tracheids and parenchyma medullary rays are absent. Central portion is occupied by pith consisting of large parenchymatus cells. The older stem, central portion of pith is obliterated, forming a cavity. Acicular crystals of calcium oxalate are present in the cells of cortex. These characteristics are also identical to those reported earlier by Prasad and Bhushan (1954).

Root, of *E. littorale* studied by us showed epiblema, cortex and stelar region. Epiblema consists of a single layer of parenchymatous cells bulging outside. Cortex consists of 4-11 layers of parenchymatous cells with large intercellular spaces. At the inner margin of the cortex, there is a ring of endodermis consisting of barrel-shaped cells. The stelar region consists of a outer zone of phloem, 1-2 layers of cambium and xylem occuring the rest of the stele. Medullary rays are absent. Acicular crystals of calcium oxalate are seen in the cortical cells. All these micorscopic characteristic details are
identical to those reported by Prasad and Bhushan (1954). Further, the plant was identified and authenticated by Prof. O. P. Saxena, Head, Botany Department, Gujarat University, Ahmedabad, India. The macroscopy and the microcopy study carried out in the present investigation revealed the authencity of the *E. littorale*.

To further authenticate the plant, preliminary phytochemical analysis was carried out. Preliminary phytochemical analysis in our investigation showed the presence of triterpenoids, alkaloids, flavonoids and coumarins in *E. littorale* extract and its different fractions. This is consistent with the phytochemical analysis reported earlier by other investigators. Rai and Thakar (1966) reported the presence of triterpenoids and glycosides in *E. littorale*. The presence of alkaloids and flavonoids was also shown by various workers (Natarajan and Prasad 1972; Retnam and Debritto 1988). Swertiamarin a secoiridoid glycoside was isolated and characterised by Rai and Thakar (1966) and Desai et al (1966). Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening.

Chemoprofiling and marker compound analysis using modern analytical techniques like HPTLC and HPLC have emerged as one of the tool for the quality assessment of the Ayurvedic drugs. HPTLC can be used for the qualitative, semi-quantitative and quantitative phytochemical analysis of herbal drugs and formulations. This includes developing TLC fingerprint profiles and estimation of chemical markers and biomarkers. In the present study for all the extracts HPTLC finger printing was developed using various solvent system.

In our studies we found swertiamarin to be one of the major compounds in extract of *E. littorale*. The yield of swertiamarin was 7.7 % in aqueous extract 5.6 % in ethyl acetate and 29.4 % in n-butanol extract. Thus, swertiamarin can be considered as the marker compound for standardization.

TLC finger printing of the aqueous extract of *E. littorale*, and its fraction ethyl acetate and *n*-butanol was carried out in different solvent system. TLC of aqueous extract showed 7 quenched bands in UV 254 nm at Rf 0.06, 0.20, 0.26, 0.48, 0.62 (swertiamarin), 0.74 and 0.81, Ethyl acetate fraction showed 7 quenched bands at Rf.
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0.04, 0.08, 0.15, 0.22 (swertiamarin), 0.29, 0.35, 0.45, n-butanol fraction showed 8 quenched bands at Rf 0.20, 0.31, 0.51 (swertiamarin), 0.59, 0.65, 0.70, 0.84.

TLC finger print profile at 366 nm of aqueous extract of *E. littorale* showed 5 bands in UV 366 nm at Rf 0.08 (green fluorescent), 0.13 (light yellow), 0.19 (light green fluorescent), 0.27 (light blue) and 0.35 (light blue) n-butanol fraction showed 10 bands in UV 366 nm at Rf 0.10 (green fluorescent), 0.21 (light green), 0.35 (light blue), 0.46 (light blue), 0.56 (light blue), 0.61 (light green fluorescent), 0.66 (blue), 0.74 (blue), 0.79 (blue), and at 0.87 (light blue fluorescent). Ethyl acetate did not show any band.

TLC finger print profile of aqueous extract of *E. littorale* after derivatisation showed 7 bands after derivatisation at Rf 0.11 (orange red), 0.22 (light green), 0.36 (pink), 0.47 (light orange), 0.79 (light yellow), 0.82 (light pink), 0.88 (purple). Ethyl acetate showed 8 bands after derivatisation at Rf 0.24 (light green), 0.27 (pink), 0.29 (violet), 0.35 (blue), 0.37 (purple), 0.50 (blue), 0.51 (blue), and at 0.57 (light pink). n-butanol fraction showed 8 bands after derivatisation at Rf 0.18 (light green), 0.27 (purple), 0.37 (violet), 0.49 (purple), 0.66 (light yellow), 0.75 (light yellow), 0.88 (pink), and at 0.97 (brown). Along with TLC finger print profiles, the presence of swertiamarin in the sample extract was confirmed in co-chromatography (Rf 0.62), and overlay of absorption spectra (λ<sub>max</sub> 240 nm). These TLC finger printing developed for all the extract can be used as one of the tool for the standardization of the plant material of *E. littorale*.

Insulin dependent diabetes mellitus (type 1) was induced by injecting STZ in adult rats. STZ destroys pancreatic beta cells and hypoinsulinemic diabetes is induced (Johansson and Tjalve, 1978). Non-insulin dependent diabetic (type 2) was induced by injection of STZ to 2 day old pups. Neonatal rats treated with STZ (80 to 100 mg/kg) at birth or within the first five days following birth experience severe pancreatic beta cell destruction, accompanied by a decrease in pancreatic insulin stores and a rise in plasma glucose levels (Blondel et al, 1989; Weir et al, 1981). STZ injection to 2 day old pups is reported to induce beta cell injury that is followed by limited regeneration, primarily as a result of ductal budding, rather than mitosis of preexisting beta cells, creating a short term normalization of glycemia. At 6 to 15 weeks of age, the rats are reported to have an impaired glucose disposal rate and significant beta cell secretary dysfunction (Bonnevie-
Nielsen et al, 1981). There have been numerous variations of this model of non insulin dependent diabetes mellitus. There may be several reasons for this variation. In contrast to adult rats treated with STZ, the beta cells of the treated neonates show a tendency to regenerate partially (Wang et al, 1996). Further, following the initial spike in plasma glucose, the STZ treated neonate rat becomes normoglycemic by three weeks of age. In the next few weeks, the beta cell number increases and the extent of regeneration depends on the age at which animal is treated with STZ (Blondel et al, 1989; Weir et al, 1981; Wang et al, 1996; Bonner-Weir et al, 1981; Iwase et al, 1994). It is reported that although 10 week old n2 STZ Wistar rats (i.e. neonate treated with STZ on day 2 of the birth) exhibit normal fed glucose and insulin levels, they are glucose intolerant. By six months of age, a glucose challenge provokes a condition of severe hyperglycemia and hyperinsulinemia in these animals (Schaffer and Wilson 1993). Our experiences on Wistar rats have been such that inspite of keeping controls results have been inconsistent and inter animal variations exists with respect to glucose and insulin levels. Hyperinsulinemia and hypertriglyceridemia were not consistently observed in Wistar rats. Experiments with Sprague Dawley rats showed that hyperglycemia, hyperinsulinemia as well as hypertriglyceridemia were significant and consistent. Inter animal variations were less as compared to Wistar rats. In oral glucose tolerance test it was found that the hyperglycemia was maintained even after 120 min which did not occur in Wistar rats. Hence, we used Sprague Dawley rats in further experiments.

STZ injected to adult rats produced loss of body weight, hyperphagia and polydypsia. The loss of body weight could be due to dehydration and catabolism of fats and proteins (Sevak and Goyal 1996; Umrani and Goyal 2002; Hofteizer and Carpenter 1973). Aqueous extract of *E. littorale* treatment significantly prevented the loss in body weight in type I diabetic rats. There was normal gain in body weight in non-diabetic animals. Treatment with aqueous extract of *E. littorale* prevented polydypsia and polyphagia in type I diabetic rats. STZ-diabetes in type I diabetes produced a significant increase in glucose levels associated with decrease in insulin levels. However, in type II diabetes it showed significant increase in both glucose as well as insulin levels. We also found increase in insulin levels and AUC$_{insulin}$ after glucose load in neonatal STZ-diabetic rats. The high insulin concentration found in neonatal STZ-diabetic rats. It could be due
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to metabolic alterations at extra pancreatic levels. In these rats, the metabolic clearance rate of insulin might have been altered. Insulin degradation following hormone receptor binding (Gliemann and Sonne 1978) and reduced binding of insulin to its receptor have been reported in mild glucose intolerance (Olefsky 1981). Hyperinsulinemia with low hepatic excretion and hypersecretion of beta cells are also reported in mild glucose intolerant obese subjects (Bonora et al., 1983). Therefore, the hyperinsulinemia in neonatal STZ-diabetic rats could be due to either decreased hepatic clearance of insulin or decreased number of insulin receptors, resulting in decreased insulin binding and lowered insulin degradation.

Treatment with aqueous extract of *E. littorale* did not alter insulin levels in non diabetic control or type I diabetic rats. However, it produced a significant decrease in insulin levels in type II diabetic rats as compared to diabetic controls. Treatment with aqueous extract of *E. littorale* showed significant decrease in glucose in both the models of diabetes, suggesting that the decrease in glucose levels may not be due to increase in insulin release by *E. littorale*. Our results do not support the findings of Maroo et al., (2002) who reported an increase in insulin release by *E. littorale*. One of the major reasons of these contradictory results is that the dose used by Maroo et al (2002) was too high 15 g dry plant equivalent extract per kg *i.e* 5 g of extract per kg as compared to 1 g/kg and 2 g/kg used in the present investigation. There dose is rather a non-physiological dose and observations were made within 8 h of treatment.

The effect on glucose levels was not dose dependent with aqueous extract of *E. littorale*. OGTT is a closed loop method that is simple and directly measures the action of endogenous insulin in response to a glucose stimulus (Alford et al., 1971). In this method $AUC_{\text{glucose}}$ (mg/dl.min) indicates insulin stimulated glucose disposal whereas $AUC_{\text{insulin}}$ ($\mu$U/ ml.min) indicates insulin response to glucose stimulus. There was a significant increase in $AUC_{\text{glucose}}$ along with a significant decrease in $AUC_{\text{insulin}}$ in type I diabetic rats as compared to non diabetic control rats. There was a significant increase in $AUC_{\text{glucose}}$ along with a significant increase in $AUC_{\text{insulin}}$ in type II diabetic rats as compared to non diabetic control rats. Treatment with aqueous extract of *E. littorale* did not alter $AUC_{\text{insulin}}$ levels in type I diabetic rats, but there was significant decrease in $AUC_{\text{insulin}}$ levels in type
II diabetic rats. This further confirms that aqueous extract of *E. littorale* produces anti-diabetic action independent of insulin release. Our results rather suggest an increase in insulin sensitivity by *E. littorale* i.e. increase in insulin-mediated glucose disposal. AUC\text{glucose} was found to be significantly decreased in rats treated with aqueous extract of *E. littorale*.

ITT, which represents the response to exogenously administered insulin on blood glucose, has been used to estimate insulin sensitivity (Alford et al., 1971). ITT is a simple, reasonably accurate and rapid method for screening insulin resistance (Grulet et al., 1993). ITT indicates net result of resistance to insulin action at a target level including receptor and post receptor defect. In the present investigation the rate of glucose disposal was significantly decreased in type II control rats as compared with non diabetic control rats.

The insulin sensitivity index $K_{\text{ITT}}$ was found to be significantly lower in neonatal STZ-diabetic rats as compared with controls. This indicates that type II rats are insulin resistant. The specific mechanism underlying the insulin resistant states are heterogeneous and may include a receptor defect (decrease in insulin sensitivity) or post receptor defect (decrease in responsiveness to insulin) or combination of both (Khan, 1978; Crettaz & Jeanrenand, 1980). Treatment with aqueous extract significantly increased $K_{\text{ITT}}$ values.

Facilitative glucose uptake occurs through a family of highly related integral membrane proteins that share significant sequence similarity. Although several lines of evidence suggest the presence of additional glucose transporters, only four members of this gene family have been established functionally. Of the four glucose transporters isoforms, GLUT4 is highly expressed in adipose tissue and striated muscle with lower levels of GLUT1 isoform (Charron et al, 1999). In the basal state GLUT4 cycles slowly between the plasma membrane and one or more intracellular compartments with the vast majority of transporter residing in vesicular compartments within the cell interior (Rea and James, 1997; Kandror and Pilch 1996). Activation of insulin receptor triggers a large increase in the rate of GLUT4 vesicle exocytosis and a smaller decrease in the rate of internalization by endocytosis (Satoh et al, 1993; Jhun et al, 1992). The stimulation of exocytosis by insulin is probably the major step for the GLUT4 translocation because
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Complete inhibition of GLUT4 endocytosis only modestly increases plasma membrane associated GLUT4 protein without affecting the extent of insulin stimulated GLUT4 translocation (Shibata et al., 1995; Kao et al., 1998; Ceresa et al., 1998). In contrast to GLUT4, GLUT1 is localized both to the plasma membrane and intracellular storage sites in the basal state but displays only modest insulin stimulated redistribution to the plasma membrane. Thus, the overall insulin dependent shift in the cellular dynamics of GLUT4 vesicle trafficking results in a net increase of GLUT4 on the cell surface, thereby increasing the rate of glucose uptake (Pessin et al., 1999).

In the present study GLUT4 levels were found to be decreased in cardiomyocytes from STZ-type I diabetic rats. This is consistent with the earlier findings (Garvey et al., 1993; Stanley et al., 1994). Treatment with aqueous extract at 2 g/kg showed increased GLUT4 levels in type I diabetic rats. These results indicate that decrease in glucose in type I diabetic rats may be because of increase in GLUT4 levels.

Earlier studies have shown that in STZ-diabetic rats, insulin deficiency is associated with hypercholesterolemia and hypertriglyceridemia (Rodrigues et al., 1986). Insulin deficiency may be responsible for the dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key enzyme that is rate limiting in the metabolism of cholesterol rich LDL particles. The mechanisms responsible for the development of hypertriglyceridemia in uncontrolled diabetes in humans (possibly in insulin deficient STZ-diabetic rats) are due to a number of metabolic abnormalities that occur sequentially. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, resulting in increased secretion of VLDL-triglyceride from liver (Balasse et al., 1972). If insulin deficiency continues for long time there occurs conversion of acids to ketone and this conversion is inhibited by VLDL-triglycerides (Basso and Havel, 1970). In diabetic rats there is decrease in lipoprotein lipase activity (Nikkila et al., 1977) resulting in impaired clearance of VLDL and chylomicrons from plasma (Bagdade et al., 1968). In our study aqueous extract of *E. littorale* significantly decreased both cholesterol and triglyceride levels in both the models of diabetes. Decrease in triglycerides was found to be greater at 1 g/kg. Triglycerides levels were brought to near normal levels by treatment with *E. littorale*. However, decrease in
cholesterol was relatively less with treatment as compared to decrease in serum triglycerides.

Typical pathological (mesangial thickening) and immunohistochemical changes of diabetic renal disease have been reported both in rats with experimentally induced diabetes (Brown et al., 1982) and in rats with spontaneous diabetes (Cohen et al., 1987). Such changes are also seen in normal kidneys transplanted in diabetic rats (Lee et al., 1974). Islet cell transplantation into diabetic rats which results in normalization of carbohydrate metabolism, also causes reversal of established renal lesions i.e. mesangial thickening and immunohistochemical changes (Mauer et al., 1974) and corrects the increased rate of albumin excretion observed in these animals (Mauer et al., 1978). It has been shown that in rats with either experimental (Fox et al., 1977; Rasch 1979a, Rasch 1980) or spontaneous (Cohen et al., 1987) diabetes, vigorous insulin therapy prevents development of mesangial and glomerular basement membrane thickening. All the functional and structural changes in kidneys resulting from STZ administration in rats can thus be attributed to the altered metabolism in diabetes. Increase in serum creatinine, urea and blood urea nitrogen (BUN) levels has been observed in patients with diabetes (Mulec et al., 1990).

Histologically the major pathology of diabetic kidney is confined to the glomerulus which includes nodular changes in the glomerulus (William 1961). In our studies histological examination of both type I and type II diabetic rats did not show any such changes. However, type I diabetic rats showed multifocal areas of cortical tubular vacuolations with dilatation of tubules especially at the corticomedullary junction. Patches of interstitial mononuclear cell infiltration were observed suggesting presence of moderate degree of chronic inflammatory changes in these animals. Type II diabetic rats showed tubular vacuolations with tubular epithelial hypertrophy. Tubular degeneration evident from the eosinophilic appearance with pyknosis of nuclei, was also observed. These morphological abnormalities in type I and type II diabetic rats were associated with a significant elevation in serum creatinine and urea levels indicating impaired renal function of diabetic animals. These observations are consistent with those reported earlier by Jensen et al. (1981), Bleasel and Yong (1982), Cam et al. (1993) and Dai et al. (1993). Treatment with aqueous of *E. littorale* extract produced not only considerable reduction
in the intensity and incidence of these pathological changes but also significant decrease in elevated serum creatinine and urea levels of diabetic animals. This suggests that *E. littorale* not only is devoid of any toxic effect on kidney but also provides protection from diabetes induced nephrotoxicity.

STZ-diabetic rats have been shown to exhibit an elevated plasma ALT level without morphological changes in liver (Domingo et al., 1991; Cam et al., 1993; Dai et al., 1993). Our investigation also a significant elevation in serum levels of the liver enzymes of both type I and type II diabetic rats was observed. However, no appreciable changes were observed in the hepatic morphology of the diabetic rats. The histopathological characteristic of the liver from type I diabetic rats were comparable to those of control rats. Whereas, type II diabetic rats showed multifocal areas of hepatocellular vacuolations with cellular infiltration. No correlation was observed between the functional and structural changes in the livers of diabetic rats. Similarly, in the current study we found that treatment with aqueous extract of *E. littorale* produced significant decrease in serum GPT and GOT levels in type I and type II diabetic rats.

A aqueous extract of *E. littorale* showed significant effect in both the models of diabetic rats. We decided to go for its fractionation to have a more active fraction and the compound responsible for the antidiabetic activity of *E. littorale*. The aqueous extract was fractionated using the solvents of varying polarity like petroleum ether, toluene, chloroform, ethyl acetate, *n*-butanol. The fraction remained after *n*-butanol fractionation was the residual fraction. The dose dependent activity of all the fractions was carried out in the diabetic rats. Treatment with ethyl acetate (500 mg/kg) and *n*-butanol (500 mg/kg) fractions of *E. littorale* showed activity similar to that of aqueous extract. However, treatment with toluene, chloroform and residual fraction did not show antidiabetic activity.

Fasting glucose levels in type I and type II were significantly decreased by treatment with ethyl acetate and *n*-butanol fractions but not by toluene, chloroform and residual fractions. Treatment with ethyl acetate and *n*-butanol fractions did not show any change in insulin levels of type I diabetic rats however, there was significant decrease in insulin levels in type II diabetic rats. Similarly ethyl acetate and *n*-butanol fractions
produced a significant decrease in $AUC_{glucose}$ values in type I and type II treated groups and a significant decrease in the $AUC_{insulin}$ in type II diabetic rats. $K_{ITT}$ was also significantly increased by ethyl acetate and n-butanol fractions. The elevated cholesterol and triglyceride levels observed in type I and type II diabetic rats were also decreased significantly by these fractions of *E. littorale*. The improvement in glucose and lipid profile was significantly greater in case of n-butanol fraction as compared to other fractions. Treatment with n-butanol fraction showed significant decrease in creatinine, urea, SGPT and SGOT levels as compared to diabetic rats. However ethyl acetate fraction showed significant changes only in creatinine and SGOT levels, and not in the levels of urea, and SGPT as compared to diabetic rats. Treatment with toluene (500 mg/kg), chloroform (500 mg/kg), ethyl acetate (100 mg/kg), n-butanol (100 mg/kg) and residual fractions (500 mg/kg) of *E. littorale* did not produce any effect on glucose, insulin, triglyceride, cholesterol, creatinine, urea, SGPT or SGOT levels as compared to diabetic control rats. Our data suggest the possibility of presence of active constituents in n-butanol and ethyl acetate fractions that may be responsible for antidiabetic activity and associated complications in both the models of diabetes.

In preliminary TLC experiments, swertiamarin was found to be one of the major compounds in *E. littorale* it was 7.7 % in aqueous extract 5.6 % in ethyl acetate and 29.4 % in n-butanol fractions. TLC finger printing of the aqueous extract and its ethyl acetate and n-butanol fraction showed relatively higher percentage of swertiamarin. It was 43.7 % in the aqueous extract where as ethyl acetate and n-butanol fractions showed 10.71 % 52 % respectively. In the present work, a simple, sensitive HPTLC method was developed for the estimation of swertiamarin. Since swertiamarin has solubility in solvents of very varying polarity, it was a challenging task to devise a suitable solvent system to resolve it from a mixture in sample extracts. Of the several solvent systems tried, the solvent system of ethyl acetate-methanol-water (7.7 + 1.5 + 0.5, v/v) provided good separation of swertiamarin ($R_f = 0.62$) from the other components present in the sample extracts. The identity of the band of swertiamarin in the sample extract was confirmed by overlapping its UV absorption spectrum with that of standard swertiamarin. The purity of the swertiamarin band in the sample extracts was confirmed by comparing the absorption spectra of start middle and end position of the band.
The HPTLC method was validated in terms of precision, accuracy and repeatability. The method is specific as it resolved nicely the swertiamarin with $R_f$ value of 0.62, in the presence of other components in samples of *E. littorale*. A linear relationship was obtained within the concentration range of 320-1120 ng/spot for swertiamarin with the correlation coefficient of 0.99. The instrumental precision was studied by repeated scanning of the same spot seven times (% CV = 0.95). Repeatability of the method was tested by analysing the standard solution (320 ng / spot) five times (% CV = 0.09). Variability of method was studied by analysing aliquots of different concentrations on the same day (intra-day precision) and on different days (inter-day precision) and the RSD indicated that the method was precise. Accuracy of the method was determined at two levels (50 % and 100 % addition) by adding a known amount of swertiamarin to the powder of *E. littorale* and the mixture was analysed. The recovery was found to be 101.04 % and 99.22 % at the two levels respectively and the average recovery was 100.13.

As swertiamarin was one of the major component present in more active fraction ethyl acetate and n-butanol we made an attempt to isolate it. Swertiamarin was isolated by fractionation and column chromatography followed by purification by recrystallization. A patent (No 1028/MUM/2003) has been filed for the method of separation. Purity of swertiamarin isolated was confirmed by following methods. First the TLC finger printing. The TLC profile showed a single spot with its $R_f$ value varying from 0.2 and 0.9 in different solvent systems like ethyl acetate : methanol : water (9 : 0.8 : 0.2) showing sweriamarin at $R_f$ 0.32, ethyl acetate : methanol : water (7.7 : 1.5 : 0.5) showing sweriamarin at $R_f$ 0.51 and ethyl acetate : methanol : water (5 : 1.5 : 1) showing sweriamarin at $R_f$ 0.92. The UV absorption spectrum of swertiamarin recorded at start, middle and end positions of the band completely overlapped, and gave an absorption maxima ($\lambda_{\text{max}}$) of 240 nm. Further, the TLC chromatogram also showed a single peak. The isolated sample of swertiamarin was then identified from its spectral data using UV, IR, Mass and NMR which matched well with that of the standard swertiamarin. The IR, UV and NMR spectra of the isolated sample and the standard were superimposable.
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The above spectral data of the isolated sample and the reference standard confirm the identity of the isolated compound as swertiamarin. Swertiamarin is reported to have a CNS depressant and anticholinergic activity (Bhattacharya 1976; Yamahara 1991) this makes swertiamarin as a biomarker. Further, it is an interesting molecule and a simple method for its extraction and isolation would aid in isolation of the compound in sufficient amounts for carrying out experiments to explore its biological activities since most of the Gentianaceae members are important medicinal plants.

A comparative study for the aqueous extract and the swertiamarin isolated in the type I diabetic rats was carried out. Treatment with aqueous extract of E. littorale 2 g/kg or swertiamarin 50 mg/kg did not show significant reduction in the elevated food intake and water intake but produced significant decrease in glucose and AUC<sub>glucose</sub> levels of diabetic rats. The decrease in glucose and AUC<sub>glucose</sub> with aqueous extract was more as compared to swertiamarin. Treatment with aqueous extract of E. littorale or swertiamarin also significantly decreased STZ-induced serum cholesterol, triglycerides, LDL, VLDL and urea elevated levels in diabetic rats. The improvement in the lipid profile with swertiamarin treatment was more as compared to that of aqueous extract. Swertiamarin did not produce a significant decrease in serum creatinine, GPT and GOT levels, however, treatment with aqueous extract showed significant decrease in serum GPT and serum GOT levels. These results suggest that swertiamarin possesses anti-hyperlipemic activity. Some other constituents are likely to be present in aqueous extract of E. littorale that are responsible for activities not observed with swertiamarin.

Aqueous extract of E. littorale showed beneficial effects on lipid profile and the liver functions of diabetic rats. Taking this into consideration we also studied the effect of aqueous extract on high fed diet model of hypercholesteremia also its effect on antioxidant parameters as well as on CCL<sub>4</sub> induced liver injury.

Hypercholesterolemia is one of the most important risk factors for atherosclerosis. (Steinberg et al., 1989; Parthasarathy et al., 1998). It is reported that oxidative modification of LDL appears to be important role in coronary artery disease and atherogensis (Camejo et al., 1976). High cholesterol diet induces significant increase in triglyceride, cholesterol and decrease in HDL-C levels. Treatment with aqueous extract of
*E. littorale* was found to significantly decrease triglyceride, cholesterol and increase HDL-C levels. Increased levels of serum cholesterol, LDL and triglyceride as well as low HDL-C levels are also responsible for the high value of atherogenic index and low HDL-ratio (Arnold et al., 2001). Treatment with aqueous of *E. littorale* extract significantly decreased the atherogenic index.

High cholesterol diet or hypercholesterolemia induces oxidative stress generate free radical and decreases the antioxidant parameters. It is reported that lipid peroxidation of the polyunsaturated fatty acids in LDL starts after decreased levels of endogenous antioxidants such as vitamin E, β-carotene, and lycopin (Esterbauer et al., 1987). In rats fed with high cholesterol diet, there is significant decrease in catalase and glutathione levels and increased lipid peroxidation and superoxide dismutase in the liver. These findings support previous reports of Tsai (1975). In our study, treatment with aqueous extract significantly decreased lipid peroxidation and increased superoxide dismutase, catalase, and reduced glutathione levels. The improvement in antioxidant parameters may be because of presence of triterpenoids and flavonoids in the aqueous extract. It is reported that triterpenoids possess lipid lowering activity (Khanna et al., 1969; Nityanand and Kappor 1973; Pathak et al., 1990; Shaila et al., 1997). Flavonoids are also reported to possess antioxidant activity (Middleton et al., 2000), and free radical scavenging activity of aqueous extract showed the antioxidant of *E. littorale*. The presence of triterpenoids and flavonoids may be responsible for the observed beneficial effects of aqueous extract of *E. littorale* and hence can be recommended to be used as a lipid lowering and antioxidant agent in atherosclerosis.

Iridoids, a widely distributed class of natural product have shown encouraging biological activities including hepatoprotective activity Suparna (1998). Swertiamarin, a secoiridoid glycoside is one of the major compounds present in *E. littorale*. There are no pharmacological data available to substantiate the therapeutic value of *E. littorale* in liver disorders. Also our study in diabetic rats shows that aqueous extract of *E. littorale* decreases AST and ALT levels significantly. Therefore, in the present study, the hepatoprotective effect of the aqueous extract was evaluated using CCl₄-induced liver damage in the mice.
We found that aqueous extract of *E. littorale* produced significant reduction in CCL$_4$ induced increase in AST, ALT also ALP levels. It was also found to preserve the structural integrity of the hepatocellular membrane. All these results suggests that *E. littorale* exerts a protective action against CCL$_4$-induced hepatic damage. The action was prominent at the dose of 500 mg/kg of aqueous extract of *E. littorale*. The marked reduction by aqueous extract of *E. littorale* in CCL$_4$ induced elevated levels of bilirubin in mice further substantiates the hepatoprotective action of *E. littorale*.

It is known that CCl$_4$ toxicity is dependent on one of its highly reactive product the trichloromethyl radical (CCl$_3$). This radical binds covalently to neighbouring proteins and lipids, and initiates lipid peroxidation that causes severe membrane alterations this in turn causes leaking of transaminases through damaged membrane and their by resulting in the elevation of transaminases in plasma/serum (Rechnagel 1977). Many compounds exhibit hepatoprotective activity against CCl$_4$ either by decreasing the production of CCl$_3$ free radical or by impairment of CCl$_4$ induced lipid peroxidation (Mailing 1974). The rise in serum levels of AST, ALT and ALP following CCL$_4$ administration could also be attributed to the damaged structural integrity of the liver cell membrane (Zimmerman 1970) causing leakage of the cellular enzymes into the blood. Inhibition of CCL$_4$ bioactivation could reduce this toxic effect of CCl$_4$. It is possible that aqueous extract of *E. littorale* produces reduction in the levels of AST, ALT and ALP by preserving the structural integrity of the liver cell membrane. We found that aqueous extract of *E. littorale* not only reduces the levels of various marker enzymes of liver but also preserves the structural integrity of the hepatocellular membrane as revealed from histological studies. There was no change in serum protein levels in control, CCl$_4$ alone and drug treated animals. This observation is in accordance with the observation that proteins in general and albumin level in particular remain unchanged in acute liver damage (Edmondson 1985).

It has been established that since barbiturates are metabolized exclusively in the liver, the sleeping time after a given dose is a measure of hepatic metabolism. If there is any pre-existing liver damage, in this case by CCL$_4$-toxicity, the sleeping time after a given dose of the barbiturate will be prolonged because the amount of the hypnotic broken down per unit time will be less (Fujimoto 1960). We found that aqueous extract of
E. littorale reduces the CCL4-induced prolongation of the pentobarbitone sleeping time in mice. This further supports the antihepatotoxic potential of the aqueous extract of E. littorale.

Phytochemical studies of this plant showed that it contains triterpenoids, flavonoids, alkaloids and coumarins. Some flavonoids have been reported to inhibit drug metabolism. It is also possible that the active component in the aqueous extract of E. littorale may owe its antihepatotoxic effect to the inhibition of the biotransformation of CCl$_4$ into the active free radical CCl$_3$. Flavonoids are also known to scavenge free radicals (Middleton 2000).

Based on the results of the present study, it can be suggested that the aqueous extract of E. littorale prevents changes in plasma enzyme concentration and other metabolic concentrations as well as diminish the destruction of liver cell architecture initiated by administration of CCL$_4$. Further studies with isolated active principles of the plant may throw more light on the use of E. littorale for hepatoprotective activity.

In conclusion, our data suggest that aqueous extract of E. littorale and its ethyl acetate and n-butanol fractions possess potential antidiabetic activity in both the models of diabetes. Ethyl acetate and n-butanol fraction were found to be more active as compared to aqueous extract. Swertiamarin was found to be one of the major compounds in the aqueous extract and its ethyl acetate and n-butanol fractions. Swertiamarin was also found to possess potential antidiabetic activity in type I rats. The anti-diabetic activity and beneficial effect of E. littorale appears to be mainly due to its insulin sensitizing effects, involving increased GLUT-4 expression and antioxidant activity. E. littorale did not exert any toxic effects in STZ-induced impaired kidney and liver functions. It was rather found to be improving kidney and liver functions. Aqueous extract of E. littorale also showed anti-hyperlipidaemic in high cholesterol fed animals and also hepatoprotective effects in CCL$_4$-induced hepatic injury model. Active constituents of E. littorale responsible for hepatoprotective and anti-hyperlipidaemic activities requires further to be investigated.