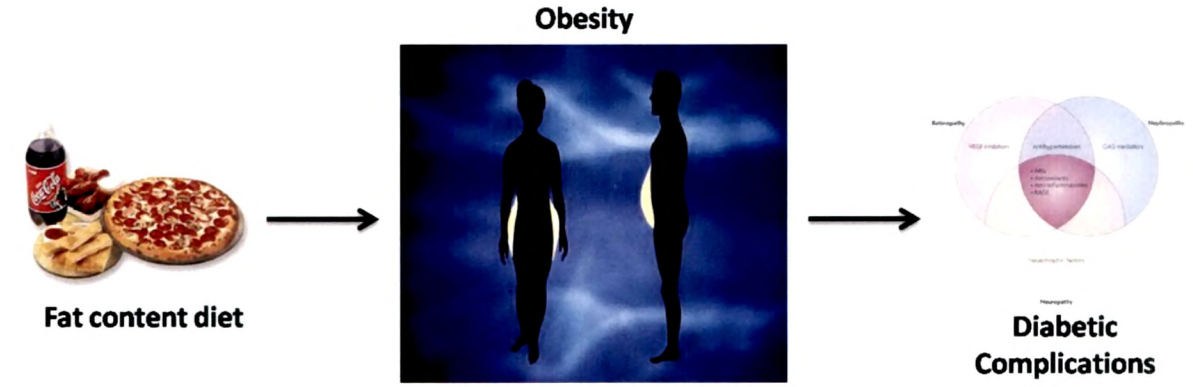
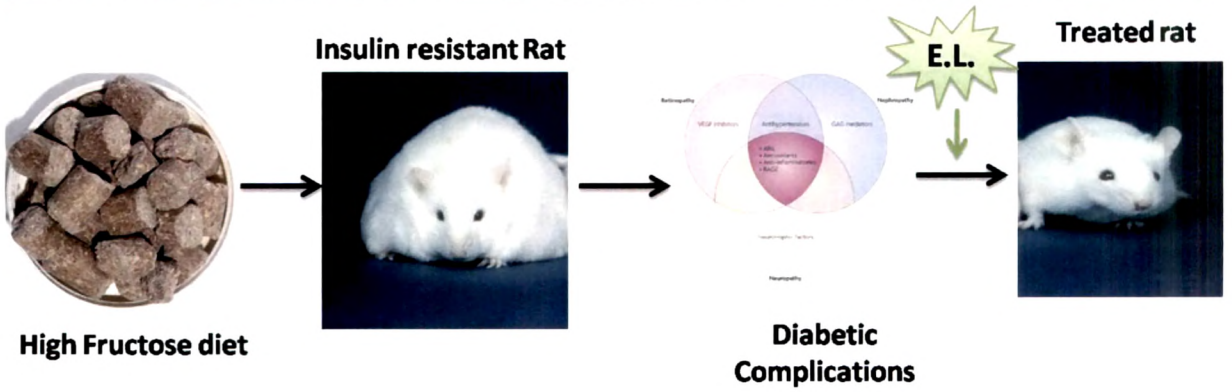
Chapter 3:

Evaluation of efficacy of *Encostemma littorale* aqueous extract in diet induced insulin resistance rat model.





Chapter 3. Evaluation of efficacy of *E. littorale* aqueous extract in diet induced insulin resistance rat model.

3.1. Review of literature

USA and developing countries like china, India have high epidemic rates of obesity and type2 diabetes. Global figures are predicted to rise from 150 millions cases in 2000 to 221 million in 2010 (Zimmet et al., 2001). The rising prevalence of obesity in childhood and adolescence in North America has been paralleled by the emergence of type2 diabetes in the adolescent age group. Surveys of the prevalence of type2 diabetes at the adolescent diabetes clinic at the Auckland Diabetes Centre were undertaken in 1996 and 2002. The prevalence of type 2 diabetes was 1.8% (2/110) in 1996, and 11.0% (18/163) in 2002 ($P = 0.008$). Type 2 diabetes accounted for 12.5% (6/48) of incident cases of diabetes in the years 1997–1999, and 35.7% (10/28) of cases in the years 2000–2001, indicating a sharp rise in the incidence ($P = 0.017$) between two periods (Hotu et al., 2004).

Risk factors for cardiovascular disease were common in the subjects with type 2 diabetes: 85% had dyslipidaemia, 58% had increased albumin excretion rates and 28% had systolic hypertension (Hotu et al., 2004). The Chennai Urban Population Study (CUPS) and CURES provided valuable data from India on the complications related to diabetes. The prevalence of coronary artery disease (CAD) was 21.4 per cent among diabetic subjects compared to 9.1 per cent in subjects with normal glucose tolerance (Mohan et.al., 2001).

This epidemic of type 2 diabetes is complicated by the fact that it is a multi-factorial disease, frequently associated with a cluster of pathologies including obesity, hypertriglyceridemia, impaired glucose tolerance, and insulin resistance collectively referred as metabolic syndrome (syndrome X). The main driving forces for the increased prevalence of insulin resistance are modern Westernized diets and patterns of eating associated with the dramatic rises in obesity (Feskens et al., 1995; Hill et al., 1992; Kromhout et al., 1995; Romieu et al., 1988). A high intake of refined carbohydrates may also increase the risk of insulin resistance (Liu et al., 2001; Jenkins et al., 1988).

Obesity may be the root cause of or precursor to other disease such as insulin resistance, abnormal lipid profile and hypertension. There was a significant correlation in the prevalence of diabetes with fat, carbohydrate, corn syrup, and total energy intakes. Most striking was the fact that when total energy intake was accounted for, corn syrup was positively associated with type 2 diabetes, while protein and fat were not (Gross et al., 2004). High fructose corn syrups (HFCS) are quite commonly incorporated into many convenient pre-packaged breakfast cereals and baked goods. Fructose consumptions thus largely increased over the past few decades likely as a result of this increased use of HFCS contains 90% fructose (Bray et al., 2004). From 1965 to 1996, a food consumption study involving 11 to 18 year olds revealed that total energy and fat intakes were increasing. There were significant decreases in milk consumption but large increases in the consumption of soft drinks and non-citrus juices (Cavadini et al., 2000). This becomes a major problem because while these high-calorie beverages are being consumed, calories from the rest of the diet are not subsequently reduced. Data indicate that energy from beverages generally does not displace or decrease from other foods consumed, leading to energy imbalances (Wharton and Hampl, 2004).

3.1.1. Diet and insulin resistance:

In our present society, we have a variety of foods that are high in saturated fat, simple sugars, and salt. Many of these foods are inexpensive and highly accessible, and tasty. Hence there is excess consumption, leading to disease and most likely early death. High fructose corn syrup (HFCS) is so sweet and inexpensive, hence used in many processed foods, which humans eat. As we have learned over the past few decades, an increased intake of refined carbohydrates, such as HFCS and the disaccharide sucrose (which is composed of fructose + glucose), is associated with increased weight gain, elevated circulating TG levels, and insulin resistance (IR) in humans and animal models (Daly et al, 1997).

For thousands of years humans consumed fructose amounting to 16–20 grams per day, largely from fresh fruits. Westernization of diets has resulted in significant increases in added fructose, leading to typical daily consumptions amounting to 85–100 grams of fructose per day. It seems that the fructose component of sucrose is largely responsible for the hypertriglyceridemia and IR produced by high sucrose diets (Sleder et al., 1980). Unless fed for a prolonged period of time, these high fructose/sucrose diets do not appear to lead to excessive weight gain (Chicco et al., 2003).

3.1.2. How fructose induces insulin resistance?

Fructose is readily absorbed and rapidly metabolized by human liver. The exposure of the liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and TG accumulation, which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance. Intake of nutritive sweetener above 25% of total energy consumed will cause hypertriglycridemia and gastrointestinal symptoms. The long-term effect of high fructose diet can include changes in digestion, absorption, appetite and hepatic metabolism leading to development of diabetes, obesity and cardiovascular disease (Mehnert, 1976; Moore et al., 2000).

In the liver, fructose is metabolized into glyceraldehydes and dihydroxyacetone phosphate. These particular fructose end products can then readily converge with the glycolytic pathway. The key importance is the ability of fructose to by-pass the main regulatory step of glycolysis, the conversion of glucose-6-phosphate to fructose 1,6-bisphosphate, controlled by phosphofructokinase (Fig.3.1) (Basciano et al., 2005). Thus, while glucose metabolism is negatively regulated by phosphofructokinase, fructose can continuously enter the glycolytic pathway. Therefore, fructose can uncontrollably produce glucose, glycogen, lactate, and pyruvate, providing both the glycerol and acyl portions of acyl-glycerol molecules. These particular substrates, and the

resultant excess energy flux due to unregulated fructose metabolism, will promote the overproduction of TG (Mayes, 1993).

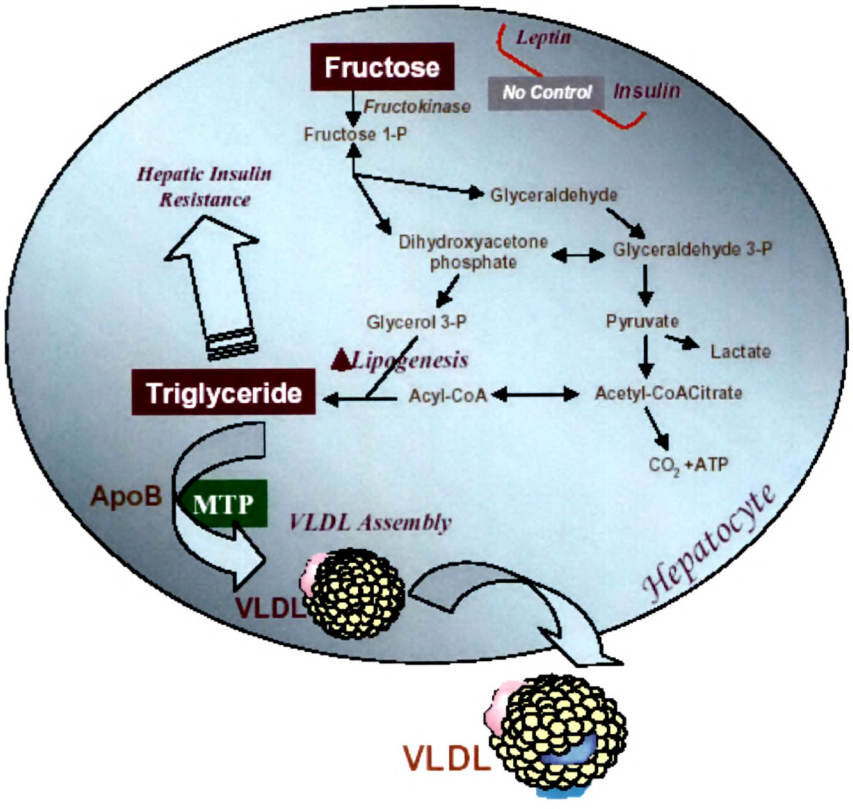


Figure 3.1: Hepatic fructose metabolism: A highly lipogenic pathway.

The costs of treating the MS (metabolic syndrome) are clearly growing, and therefore the research community is seeking animal models that mimic the human phenotype so that potential therapies can be tested (Alexander Tenenbaum et.al, 2003).

High fat diets were used for diet induced obesity model; diet high in saturated fat and cholesterol for hypercholesterolemia and atherosclerosis. High fructose /sucrose diets for hypertriglyceridemia and insulin resistance in rats and rodents, diet high in sodium (and fructose) for hypertension. The Sprague-

Dawley and Wistar rat are established models of sucrose-induced IR and hypertriglyceridemia (Pagliassotti et al., 1996).Treatment with 10% fructose in drinking water (equivalent to a diet containing 48-57% fructose) for one week or longer is appropriate for the rapid production of fructose-induced hypertension in Wistar rats, which is associated with elevated levels of plasma insulin, glucose, and triglycerides (Dai and McNeil, 1995).

Medicinal plants are rich source of various phytochemicals and used for treatment of various disease. Several plants demonstrate hypoglycemic potential and recommended for treatment of diabetes. *Gymnema Sylvestre* assists the pancreas in the production of insulin in Type 2 diabetes. It also improves the ability of insulin to lower blood sugar in both Type 1 and Type2 diabetes (Persaud et al., 1999). It decreases cravings for sweet. This herb can be an excellent substitute for oral blood sugar-lowering drugs in Type 2 diabetes. Some people take 500 mg per day of gymnema extract. *Allium cepa and Allium sativum* (Onion and Garlic). They have significant blood sugar lowering action (Facts and comparisons, 1999). The principal active ingredients are believed to be allyl propyl disulphide (APDS) and diallyl disulphide oxide (allicin), although other constitutents such as flavonoids may play a role as well. Experimental and clinical evidence suggests that APDS lowers glucose levels by competing with insulin for insulin-inactivating sites in the liver. This results in an increase of free insulin. Onion extract was found to reduce blood sugar levels during oral and intravenous glucose tolerance. Onions affect the hepatic metabolism of glucose and/or increase the release of insulin, and/or prevent insulin's destruction. The additional benefit of the use of garlic and onions are their beneficial cardiovascular effects. They are found to lower lipid levels, inhibit platelet aggregation and are antihypertensive. So, liberal use of onion and garlic are recommended for diabetic patients. Asian ginseng is commonly used in traditional Chinese medicine to treat diabetes. It has been shown to enhance the release of insulin from the pancreas and to increase the number of insulin receptors (Jellin et al., 1999). It also has a direct blood sugar-lowering effect. A

recent study found that 200 mg of ginseng extract per day improved blood sugar. Trigonella foenum graceum (fenugreek) has been studied, particularly in India, for treatment of diabetes. The fenugreek treated group exhibited a 54 % decrease in 24-hour urinary excretion of glucose, as well as reduction in total cholesterol, LDL, VLDL and triglycerides (Sharma et al., 1990). Animal study has also demonstrated the hypoglycemic and hypolipidemic effect of fenugreek (Khosla et al., 1995). Purified methanolic extract of Salacia reticulata Weight ameliorates insulin resistance and metabolic alterations in rats fed high fructose diet.

Long-term administration of *Eucommia ulmoides* Oliv. leaf extract ameliorates pre-diabetic state of insulin resistance and abnormal perivascular innervation in the hyperinsulinemic state (Xin et al., 2010). Purified methanolic extract of *Salacia reticulate* Wight. ameliorates insulin resistance and metabolic alterations in rat fed high fructose diet. Grape seed extract prevents high-fructose diet-induced insulin resistance and oxidative stress in rats (Wannaporn et al., 2010). *Brassica juncea* (Rai) *and Tinospora cordifolia* significantly prevented the development of insulin resistance in rats fed fructose-enriched diet (Yadav et al., 2004; Sreenivasa Reddy et al., 2009).

Ethanolic extract of *Solanum torvum* could prevent the development of high blood pressure induced by a diet rich in fructose by reversing the metabolic alterations induced by fructose (Mohan et al., 2009). Two different extracts of bitter melon on insulin resistance in rats fed a high-fructose diet thereby producing evidence of the role of changes in expression of PPARγ and GLUT4 (Shih et al., 2009).

3.2. Experimental design

Male Charles Foster rats (body weight 200–250 g) were used for the study. They were allowed *ad libitum* access to water and food. During the study, rats were maintained in cages with a 12-h light/dark cycle. All the animal studies

were approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

3.2.1. Fructose enriched diet (FRU):

A special diet was prepared in such that, the fructose content provided 60% of total calories in the diet (Hwang *et al.. 1983;*N andbini *et al..* 2005). The diet was prepared in laboratory with the following composition (g kg^{-1}) Casein. High protein 207; DL-methionine 3; fructose 700; animal fat (bovine) 50.0; cellulose 79.81; mineral mix 50.0; zinc carbonate 0.04 and vitamin mixture 10.

3.2.2. Composition of salt mixture (g):

Calcium carbonate 16.67, Calcium phosphate 47.3, Copper sulphate 0.017. Ferric citrate 0.333, Magnesium sulphate 5.0, Manganese sulphate 0.417, Potassium chloride 11.67, Potassium iodide 0.017, Sodium chloride 6.67, Sodium phosphate (dibasic) 11.67 and Zinc carbonate 0.217.

3.2.3. The vitamin mixture has following composition (g):

Vitamin A acetate 1.8, VitaminD, 0.125, DL-α-tocopherol 22; Ascorbic acid 45; Inositol 5.0; Choline chloride 75; Menadione 2.25; Niacin 4.25; Pyridoxine HCl 1.00; Thiamine 1.00; Calcium pantothemte 3.00; Biotin 0.02 and Glucose q.s. to 1 kg.

Rats were divided into four groups of six rats each as follows:

Group-I : Control (C), Fed lab standard rat chaw and administered intragastrically (i.g) with normal saline.

Group-II : High fructose (HFD, 70%) fed rats in diet, administered saline by i.g. route.

Group-III : HFD + EL treated. EL was given i.g at a dose of 1.5g dry plant Equivalent extract/100gm Body wt./day (Maroo et al., 2003).

Group-IV : HFD + Rosiglitazone treated given i.g at a dose of 10mg/kg body wt/day.

Rats were kept on 12-16 hrs of fasting prior to fructose feeding, which increases their affinity for fructose diet (new diet as compared to regular lab chaw). The onset of insulin resistance was observed after fifteen days of HFD feeding by by oral glucose tolerance test and fasting glucose levels. Following the experimental regime as discussed previously in section 4.2, rats were sacrificed by decapitation on 45th after the treatment. Blood from orbital sinus was collected just prior to decapitation, in clean, dry eppendorfs containing anticoagulant. The clear plasma was removed after centrifugation at 1500×*g* for 15min at 4°C and the assay of enzymes LDH, CK-MB, SGOT and SGPT were carried out. Plasma samples were also assayed for blood glucose, insulin, testosterone, glycosylated hemoglobin and lipid profile measurements. For platelet aggregation study, plasma reach in platelet and plasma poor in platelet were prepared as per the procedure mentioned in chapter 2. Blood coagulation parameters like PT and APTT were also evaluated from the plasma samples. Testis were excised out and processed for 17- β HSD and 3-β HSD activity.

In one sent of experimental animals blood was withdrawn from the orbital sinus and used for evaluation of antioxidant parameters. These animals were also analyzed for vascular reactivity towards different drugs to evaluate its functionality, with the help of physiograph. Treatment with 10% fructose in drinking water (equivalent to a diet containing 48-57% fructose) for one week or longer is appropriate for the rapid production of fructose-induced hypertension in Wistar rats, which is associated with elevated levels of plasma insulin, glucose, and triglycerides (Verma et al., 1999).

3.3. Results

3.3.1. Changes in Body weight, fasting glucose, fasting insulin and Fasting insulin resistance index.

Rats were given 70% fructose in diet for 45 days, rather than in a water to avoid seasonal variation in water intake. Final body weights of animals were

recorded (Table 3.1). Weight gain was less in EL and R treated animals as compared to fructose fed rats. Fasting insulin and fasting glucose were significantly high in fructose-fed untreated rats as compared to control rats. The degree of insulin resistance as calculated by FIRI was higher in fructose-fed rats as compared to rats in control group. Fructose fed rats treated with EL aqueous extract and R significantly reduces fasting insulin, fasting glucose levels, as well as fasting insulin resistance index (Table 3.1).

Table:3.1 Effect of EL treatment on body weight, fasting glucose, fasting insulin and fasting insulin resistance index in fructose-induced insulin resistant rat model.

	Body Weight (gms) 45th Day	Fasting Glucose (mg/dl)	Fasting Insulin (µU/ml)	FIRI
Control	329 ± 5.85	75 ± 2.96	13 ± 0.72	0.01 ± 0.01
Fructose	307 ± 5.68^{ns}	107 ± 5.99^{aaa}	41 ± 2.57^{aaa}	0.65 ± 0.10^{aaa}
EL aqueous extract	271 ± 5.91^{bb}	86 ± 1.45^{bb}	22 ± 1.23^{bbb}	0.23 ± 0.03^{bbb}
Rosiglitazone	260 ± 4.96^{bbb}	84 ± 2.46^{bb}	22 ± 0.72^{bbb}	0.23 ± 0.02^{bbb}

Values are expressed as mean ± SEM (n=6 in each group).

aaa,P<0.001 vs. C, bb, P<0.01, bbb, P<0.001 vs. F. ns vs. C

3.3.2. Oral Glucose Tolerance Test

The fasting blood glucose levels were higher in all the experimental groups as compared to rat fed lab chow. Significant elevation in the blood glucose levels after the oral glucose load was noted in fructose-fed rats at all time points as compared to control rats. EL and std drug treated rats showed significant decrease in glucose concentration at all time points compared to fructose fed rats (Fig. 3.2).

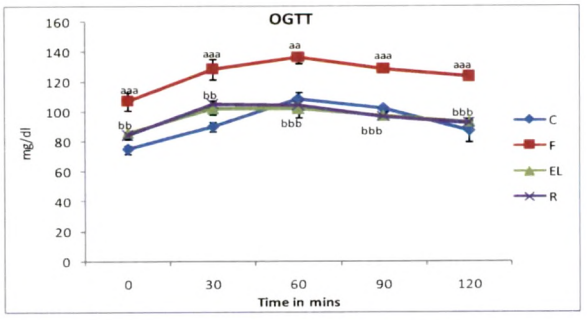


Figure 3.2: Effect of EL treatment on oral glucose tolerance test in fructose-induced insulin resistant rat model.

Values are expressed as mean ± SEM (n=6-8 in each group). aa, P<0.01, aaa, P<0.001 vs. C, bb, P<0.01, bbb, P<0.001 vs. F.

3.3.3. Hypolipidaemic parameters

Fructose fed rats were hypertriglyceridemic, which was clearly seen by the increased triglyceride levels by 107%. These rats also showed increase in serum VLDL levels by 57%. LDL, HDL and total cholesterol levels were not significantly changed among these rats. Both extract and standard drug Rosigiltazone (R) treated fructose fed rats, showed significant amelioration in serum triglyceride levels and VLDL levels as compared to untreated fructose fed rats. *E. littorale* treated fructose fed rats showed a decrease of 43% and 54% in serum triglycerides and VLDL cholesterol in fructose fed rats, while the R treated fructose fed rats showed a decrease of 50% and 62% in serum triglycerides and VLDL cholesterol (Fig. 3.3A & B). Lipid lowering effect of EL was comparable with alloxan-induced diabetic dyslipidemia (chapter 5).

3.3.4. Serum CK-MB, LDH, SGOT and SGPT activity

Serum CK-MB, LDH and SGOT activity increased by 164%, 107% and 53% in the untreated fructose fed rats as compared to the control (C) (Fig. 3.4, 3.5, 3.6 & 3.19). EL treatment attenuated the increase in serum CK-MB, LDH and SGOT activity by 54%, 52% and 74% respectively. Similarly, R treatment attenuated the increase in CK-MB, LDH and SGOT by 52% and 51% and 86% respectively.

Figure: 3.3 Effect of EL treatment on lipid profile of fructose-induced insulin resistant rat model.

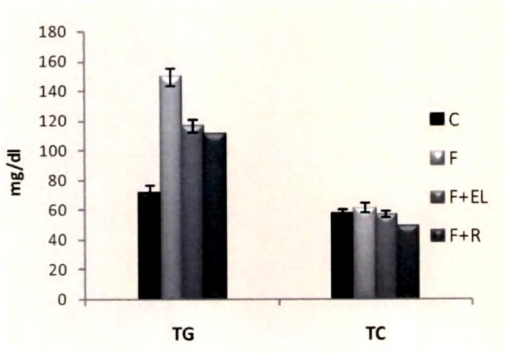


Figure: 3.3A Effect of EL treatment on lipid profile of fructose-induced insulin resistant rat model.

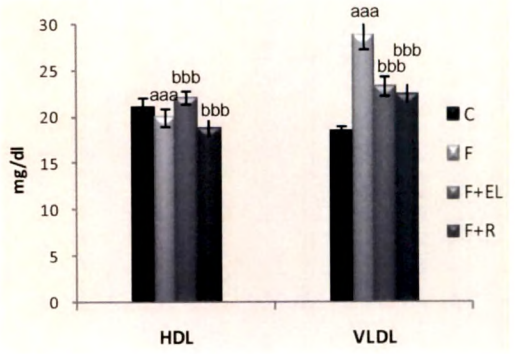


Figure: 3.4 Effect of EL treatment on serum **CK-MB** activity in fructose-induced insulin resistant rat model

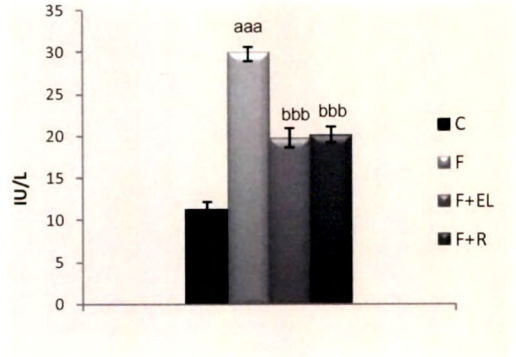


Figure: 3.5 Effect of EL treatment on **serum LDH** activity of fructose-induced insulin resistant rat model

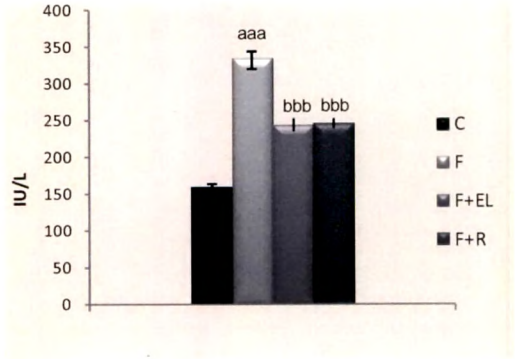


Figure: 3.6 Effect of EL treatment on *serum SGOT* in fructose-induced insulin resistant rat model.

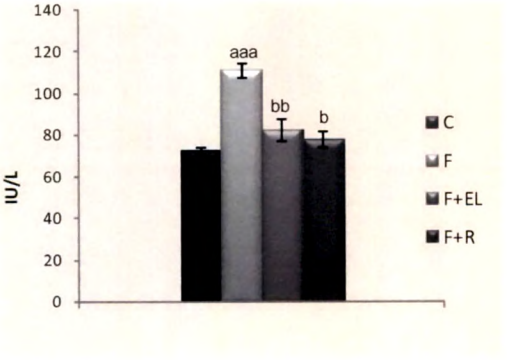
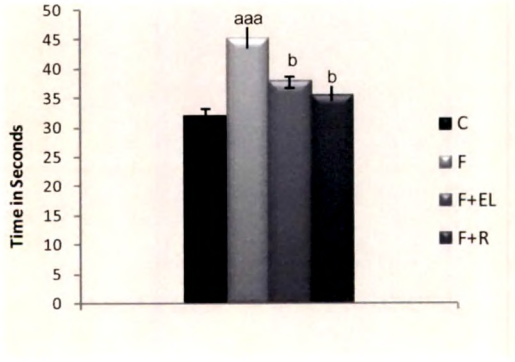


Figure 3.7 Effect of EL treatment on platelet aggregation in fructose-induced insulin resistant rat model.



Values are expressed as mean ± SEM (n=6 in each group). a, $p<0.05$, aa, $p<0.01$, aaa, $p<0.001$ vs. C, b,$p<0.05$, bb $p<0.01$, bbb, $p<0.001$ vs. F.

Serum SGPT activity, which is a marker of liver injury, increased by 98% while EL and R treatment reduces the activity by 58% and 67%.

3.3.5. Platelet aggregation, Platelet count and Blood clotting time (PT & APTT)

Untreated fructose fed rats showed 40% increase in platelet aggregation time, while 14% and 21% decrease in prothrobin time (PT) and activated partial thromboplastin time (APTT) (Fig. 3.7, 3.8). Treatment with EL to fructose fed rats for 45 days ameliorates platelet hyperaggregability by 58% and increases PT, APTT both by 107%. Fructose fed rats treated with R for 45 days also showed 73% reduction in platelet hyperaggregability, 87% both in PT and APTT. Fructose fed animals also showed increase in platelet count by 12% (Fig. 3.9). Platelet count decreases with EL and R treatment by 65% and 71% respectively.

3.3.6. Systolic blood pressure and vascular reactivity

Untreated fructose fed rats showed increase in systolic blood pressure by 80% as compared to control rats. EL treatment for 45 days, bring down systolic B.P by 44%, while R treatment decreases it by 43%. Both the treatments individually showed comparable effect in reducing systolic B.P (Fig. 3.10). Vascular responses to drugs phenylephrine and adrenaline (Fig. 3.17) increased significantly and decreased significantly when challenged with isoprenaline or acetylcholine in fructose fed rats (Fig. 3.18) at the end of 6 weeks all these are not seen in the figure. EL treated group showed significant decrease in vasoconstrictor responses when challenged with phenylephrine and adrenaline and were found to be having better effect than rosiglitazone group. Treatement with EL and rosiglitazone, to fructose fed rats displayed significant increase in vascular responses to acetylcholine and isoprenaline.

3.3.7. Serum Testosterone and 3β-HSD and 17 β-HSD activity

Fructose fed rats showed increase in serum testosterone levels by 226%. EL and R treated groups of rats showed decrease in serum testosterone levels by 64% and 73% (Fig. 12). Testicular 3β-HSD and 17 β-HSD activity were found

Figure: 3.8 Effect of EL treatment on plasma **PT & APTT** in fructose-induced insulin resistant rat model.

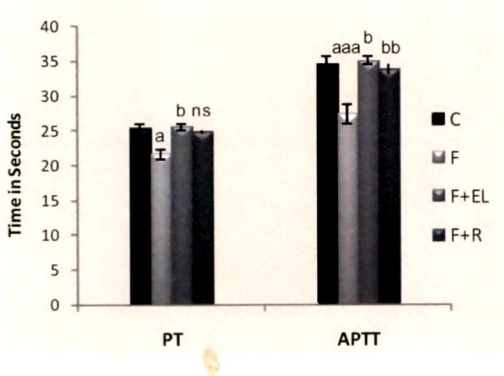


Figure: 3.9 Effect of EL treatment on platelet count in fructose-induced insulin resistant rat model.

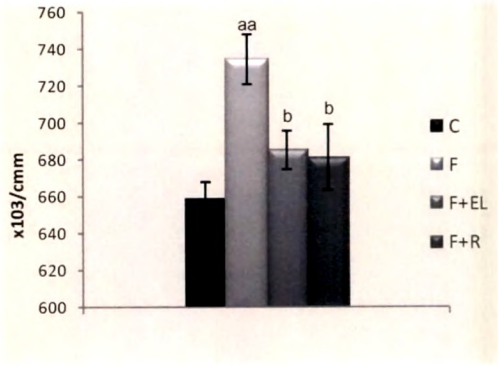


Figure 3.10: Effect of EL treatment on **systolic blood pressure** in fructose-induced insulin resistant rat model.

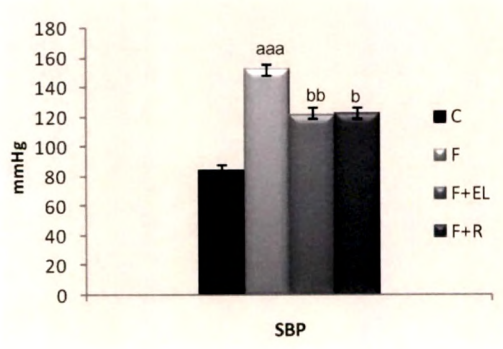


Figure 3.11: Effect of EL treatment on blood **LPO** levels in fructose-induced insulin resistant rat model.

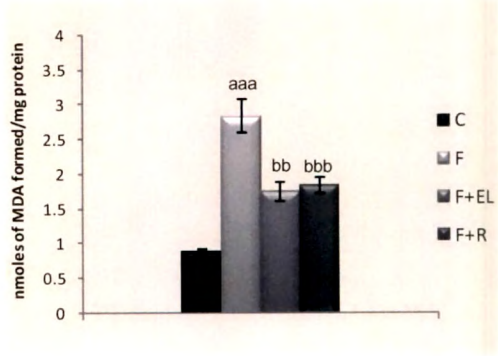
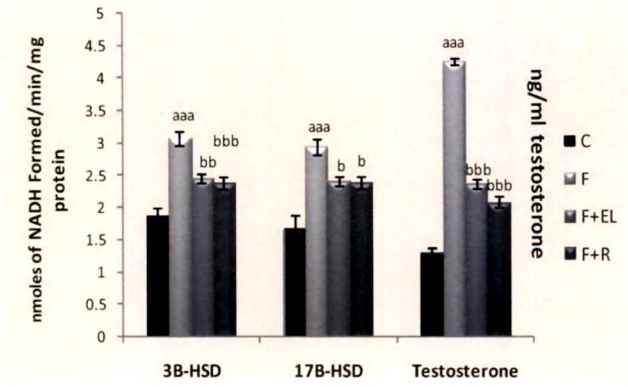


Figure 3.12: Effect of EL treatment on activity of testicular enzyme 3 **β-HSD, 17 β-HSD** and **Testosterone** in fructose-induced insulin resistant



lues are expressed as mean ± SEM (n=6 in each group). a, p<0.05, aa, p<0.01, aaa,p<0.001 vs. C, b,p<0.05, bb P<0.01, bbb, P<0.001 vs. F.

Figure 3.13: Effect of EL treatment on blood **GSH** levels in fructose-induced insulin resistant rat model.

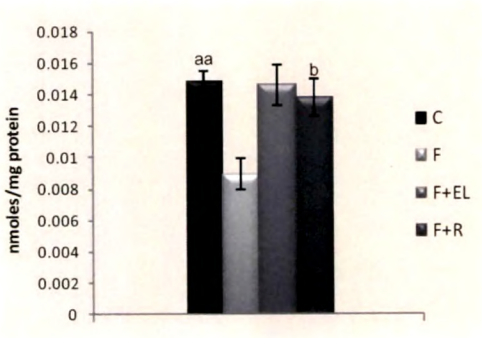


Figure 3.14: Effect of EL treatment on **blood SOD** activity in fructose-induced insulin resistant rat model.

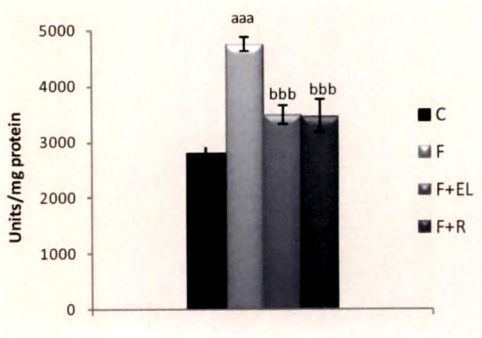


Figure 3.15: Effect of EL treatment on blood **glutathione peroxidase** activity in fructose-induced insulin resistant rat model.

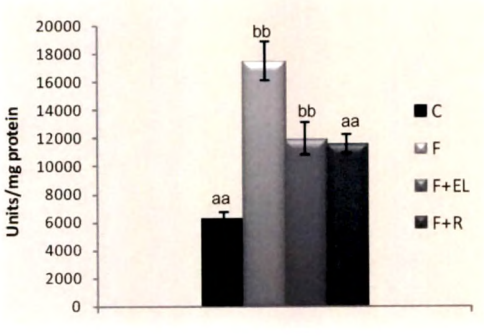


Figure 3.16: Effect of EL treatment on blood **catalase** activity in fructose-induced insulin resistant rat model.

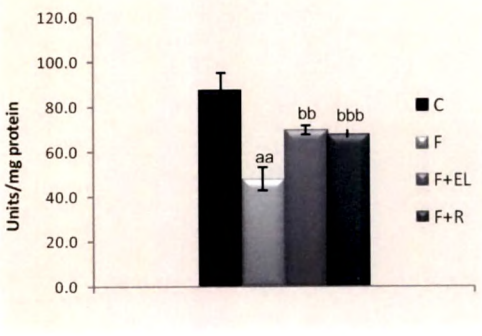


Figure 3.17: Effect of EL treatment on **vascular response to phenylephinre and adrenaline** in fructose-induced insulin resistant rat model.

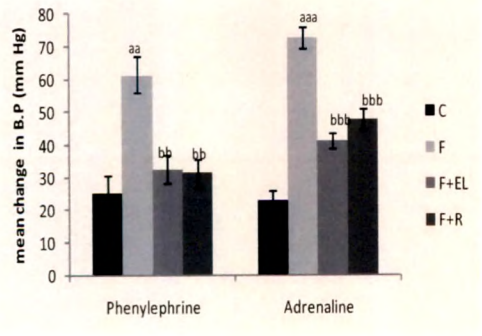
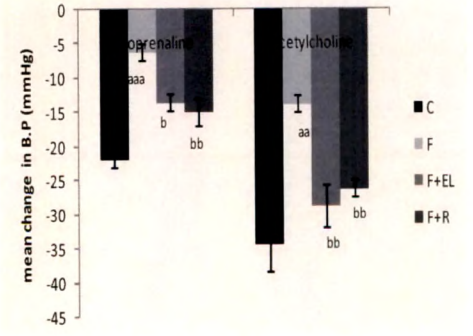
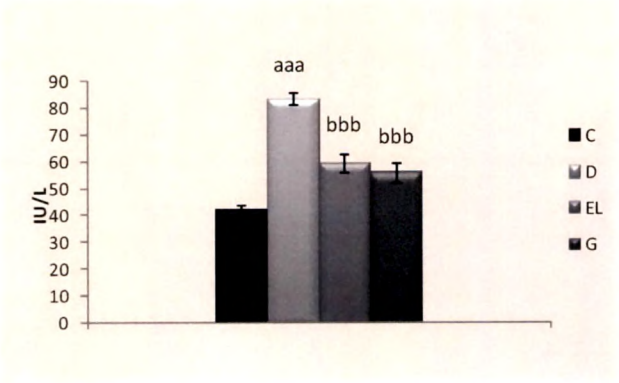


Figure 3.18: Effect of EL treatment on **vascular response to isoprenaline and ACH** in fructose-induced insulin resistant rat model.



Values are expressed as mean ± SEM (n=4 in each group). a, P<0.05, aa, P<0.01, aaa,P<0.001 vs. C, b,P<0.05, bb P<0.01, bbb, P<0.001 vs. F.

Figure 3.19: Effect of EL treatment on serum **SGOT** activity in fructose-induced insulin resistant rat model.



Values are expressed as mean ± SEM (n=4 in each group). a, P<0.05, aa, P<0.01, aaa,P<0.001 vs. C, b,P<0.05, bb P<0.01, bbb, P<0.001 vs. F.

3.3.8. LPO, GSH and Antioxidant enzymes activity in blood

Fructose fed rats showed 220% increase in MDA level and 39% decrease in GSH level in blood (Fig. 3.11, Fig. 3.13). This indicates generation of oxidative stress in systemic circulation. SOD and GPx activity increased by 41% and 182% in these rats (Fig. 3.14, Fig. 3.15). Fructose fed rats treated with EL decreases MDA content by 56%, while increases GSH content by 97%. It decreases SOD, GPx activity by 39% and 49% respectively (Fig. 3.14). Similarly, R treated diabetic rats showed decrease in MDA level by 51%, while increase GSH level by 83%. SOD, GPx activities, in these rats were improved by 40% and 52% respectively. CAT activity decreases by 45% in fructose fed rats which is improved upon EL and R treatment by 55% and 51% respectively (Fig. 3.16).

3.4. Discussion

The present study showed that 70% fructose feeding in diet for 45 days leads to elevation in fasting blood glucose, fasting insulin levels and triglyceride levels in rats. An abnormal response to oral glucose load was also observed in fructose-fed rats. Fructose-fed rats showed higher fasting insulin resistance index. All these results signify insulin resistance condition had been developed in fructose-

fed rats. The studies carried out by others indicated similar results (Bezerra et al., 2001). The rats fed with high fructose diet induced a non obese model of hyperlipidaemia, insulin resistance and hyperinsulinaemia, which might be causative factor for impaired glucose tolerance (Basciano et al., 2005). An adverse effect of fructose on insulin sensitivity in rats is well established. This phenomenon is believed to be related to the hypertriglyceridaemic effect of fructose (Lee et al., 1994). Fructose feeding stimulates the hepatic production of triglycerides, both by promoting the reesterification of circulating non-esterified fatty acids and by stimulating de novo fatty acids synthesis (Southgate et al, 1995). Insulin resistance may occur due to defect in insulin binding caused by decreased receptor number or affinity, or defect at the level of effector molecules such as glucose transporters and enzymes involved in glucose metabolism (Sachi et al., 1997; Paternostro et al., 1995; Kim et al., 2000).

The administration of EL to the fructose-fed rats mitigated the adverse effect of fructose. Glucose tolerance was improved and TG was brought to normal levels as compared to fructose rats. Previous studies from our lab showed that aqueous extract of EL is having hypolipidemic and antioxidant activity in cholesterol-fed rats (Vasu et al., 2005). These finding demonstrated that, enhancement of the sensitivity of target tissue to circulating insulin, might be due to lipid lowering effect of EL.

Further antihypertensive effect of EL was evaluated. The mechanism of fructose-induced hypertension is still not clear. Recent studies have shown that high fructose diet is associated with increased blood pressure in rats (Bunnag et al., 1997; Hese et al., 1998; Fang and Huang, 1998; Dimo et al., 2001a,b) several studies have demonstrated that chronic fructose feeding leads to insulin resistance, glucose intolerance, hyperinsulinemia, hyperglycemia and hypertryglyceridemia in a relatively short time in normal rats (Zavaroni et al, 1980; Hwang et al., 1987; Reaven et al., 1991; Limura et al., 1995; Erlich and Rosenthal., 1996) and these metabolic changes lead to essential hypertension (Madar et al., 1997; Rosen et al., 1997). Hyperinsulinemia could activate the

sympathetic system, sodium retention, and vascular smooth muscle cell proliferation, which in turn could elevate the BP (Hwang et al., 1987). Richey et al., 1998 reported an impaired response to endothelium-dependent vasodilators in fructose fed rats.

Several mechanisms have been proposed to mediate the link between insulin resistance/hyperinsulinemia and hypertension, including continued activation of the sympathetic nervous system (Rosen et al., 1997; Penicaud et al., 1998; Verma et al., 1999), increased production and/or activity of vasoconstrictors, such as ET-1 (Juan et al., 1998; Verma et al., 1997; Verma et al., 1995; Cosenzi et al., 1999), Ang II (Iyer and Katovich, 1994; Navarro-Cid et al., 1995; Iimura et al., 1995; Kobayashi et al., 1993) and thromboxane A2 (TxA2) (Galipeau et al., 2001), and impaired endothelium-dependent relaxation (Takagawa et al., 2001; Katakam et al., 1998; Miller et al., 1999; Verma et al., 1997). Each of the proposed mechanisms can contribute to an increase in vascular tone, which can mediate the development of endothelial dysfunction with an eventual increase in blood pressure.

The present study clearly demonstrate significantly increased systolic blood pressure and heightened vasopressor responses to adrenaline and phenylepinephrine and lesser vasodilator responses to acetylcholine and isoprenaline in fructose fed rats. Apparently, both a sympathetic activation as well as parasympathetic inhibition can be seen. EL extract could prevent essential hypertension developing due to a fructose rich diet and these rats showed normal systolic blood pressure and complete absence of exaggerated responses to sympathetic agonist and the jolted responses to parasympathetic agonists. These observations suggest that preventive effect of EL extract in fructose-induced hypertension similar to standard drug rosiglitazone.

The liver helps maintain normal blood glucose concentration in the fasting and postprandial states. Loss of insulin effect on the liver leads to glycogenolysis

and an increase in hepatic glucose production. Abnormalities of triglyceride storage and lipolysis in insulin-sensitive tissues such as the liver are an early manifestation of conditions characterized by insulin resistance and are detectable earlier than fasting hyperglycemia (Lewis et al., 2002). In animal models, chronic hyperinsulinemia is found to predispose the liver to relative resistance to insulin. Elevated transaminase is a marker for hepatocyte injury. It is also hypothesized that elevation in ALT, a gluconeogenic enzyme whose gene transcription is suppressed by insulin, could indicate an impairment in insulin signaling rather than purely hepatocyte injury (O'Brien and Granner, 1991). Our result suggests, fructose fed rats had increased serum ALT level and demonstrate the development of hepatic insulin resistance in these rats. Thus indicate that rat fed with high fructose diet is a suitable model for human non alcoholic fatty liver disease (NAFLD) (Ackerman et al., 2005). Liver cirrhosis is one of the responsible factors for the high bleeding tendency.

Insulin resistance is a uniform finding in type 2 diabetes, as are abnormalities in the microvascular and macrovascular circulations. These complications are associated with dysfunction of platelets and the neurovascular unit. Platelets are essential for hemostasis, and knowledge of their function is basic to understanding the pathophysiology of ascular disease in diabetes. Increased platelet numbers and platelet aggregation is high in type 2 diabetes in human as well as in animal models of type 2 diabetes (Queen et al., 2003). The prothrombotic state is a more recently recognized component of the metabolic syndrome; people with the metabolic syndrome exhibit a pattern of coagulation factors that promote thrombosis or retard thrombolysis (Grundy, 1998; Nolan and Vinik, 1996). The prothrombotic state is characterized by increased fibrinogen levels (Imperatore et al., 1998), increased plasminogen activator inhibitor (PAI)-1 (Byberg et al., 1998) and different abnormalities in platelet function (Nolan and Vinik, 1996, Trovati et al., 1988). Our results also demonstrated that PT and APTT decreases in fructose fed rats while EL and R treated animals showed improvement in these blood clotting parameters.

In our study we found enhanced lipid peroxidation in blood of fructose-fed rats which could be associated with high circulating glucose. Hyperglycemia is well known to increase reactive oxygen species generation and subsequent lipid peroxidation. Hypertriglyceridemia is another factor that could enhance the formation of lipid peroxides. It has been reported that lipid peroxide levels correlates with hypertriglyceridemia in diabetic patients (Stringer et al., 1989). In addition, fructose itself enhances the reactive oxygen formation *in vitro* (Sakai et al., 2002). Our result demonstrates that antioxidant enzymes SOD and GPx increases in fructose-fed rats. Increase in GPx activity leads to depletion of GSH level which is a co-substrate of this enzyme in fructose-fed rats. Treatment with EL brings the activity of SOD and GPx to normal levels. Treatment with EL increases GSH concentration compared to control rats.

Rosiglitazone is a potent peroxisome proliferator-activated recpetor gamma (PPARγ) agonist used in therapy of type 2 diabetes and other insulin resistance states (Olefsky et al., 2000). Their efficacy in improving glycemic control is attributed to enhancement of insulin-stimulated glucose disposal in liver, adipose and muscle tissues. However, the underlying mechanism for TZD action remains unclear (Cullen and Lorkowski, 2002). PPARγ activation, by reducing oxidative stress, increases NO bioavailability in coronary arterioles of mice with Type 2 diabetes (Bagi et al., 2004). The administration of Standard drug, rosiglitazone to the fructose-fed rats ameliorate the adverse effect of fructose. Glucose tolerance was improved and TG was brought to normal levels as compared to fructose rats. It also ameliorates antioxidant defense system. Proposed activity of rosiglitazone in fructose-fed rats is due to its PPARγ agonist activity and also on NF-κB.

All above results indicates that *"Enicostemma littorale"* and standard drug "Rosiglitazone" are showing almost similar insulin sensitizing, hypolipidaemic and antioxidant activity.

3.5. Summary

The metabolic syndrome is an important public health concern that predisposes individuals to the development of cardiovascular disease and/or Type 2 diabetes. Present study shows that consumption of high fructose diet leads to increase in fasting glucose, insulin levels as well as in insulin resistance index. High fructose fed rats also showed tryglyceridemia. High fructose diet induces liver stetosis condition marked by elevated serum ALT levels. Above all metabolic alteration in high fructose fed rats indicate the establishment of insulin resistant rat model. These rats had developed atherosclerotic and thrombotic conditions. These vascular changes led to the cardiovascular complication manifested by increase in serum cardiac specific markers. These rats also showed elevated systolic blood pressure, altered vascular reactivity towards vasodilator as well as vasoconstrictor drugs and indicates involvement of sympathetic and parasympathetic nervous system. Heightened systolic blood pressure is correlated with serum testosterone levels. Treatment with aqueous extract of EL decreases serum triglyceride level, which is responsible for the development of insulin resistance condition. It also improves alteration in Oral glucose tolerance test and other glycemic parameters and thus improves insulin sensitivity. It protects liver from damage caused by triglyceride accumulation and decreased insulin resistance. It ameliorates thrombotic and atherosclerotic phenomenon accompanied by reduced serum testosterone levels and thus protects heart. Systemic oxidative stress is also reduced upon EL treatment and helps in improvement of insulin sensitivity. Rosiglitazone treatment also showed similar effect as shown by EL extract because of its insulin sensitizing as well as antioxidant effect. In conclusion aqueous extract of EL ameliorates insulin resistance condition and seems to be a promising drug candidate for the treatment of metabolic syndrome or insulin resistance condition.

3.6. References

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