International Journal of Pharmacological Research
ISSN: 2277-3312
www.ssjournals.com
Journal DOI:10.7439/ijpr

Protective Effect of Flower buds of *Lonicera Japonica* Extract on Diabetes Mellitus Type 2 and Associated Vascular Complications in STZ-HFD Treated Rats

Chirag Prajapati*[1] and Falguni Majmudar[2]

[1]*Hemchandracharya North Gujarat University, Patan, Gujarat, India.*
[2]*Smt. N.H.L. Municipal Medical College, Ahmedabad, Gujarat, India.*

Corresponding author*
Chirag Prajapati
Research Scholar,
Hemchandracharya North Gujarat University, Patan, Gujarat, India
E-mail: chirag11_pharma@yahoo.co.in

Abstract
Objective: To study the protective effect of *Lonicera Japonica* Alcoholic Extract (LJALE) on Diabetes Mellitus II (DM) and associated vascular complications.
Methods: Induction of Diabetes Mellitus II with 35 mg / kg STZ-HFD leads to excessive level of 11β-HSD1 enzyme. It has been found that flower buds of *Lonicera Japonica* (LJ) has also been traditionally indicated for treatment of diabetes. Chlorogenic Acid (CA) is a major bioactive component in the flower buds of LJ has received more attention because of its 11β-HSD1 inhibitory action.11β-HSD1 regulates glucocorticoid action at the pre-receptor stage by converting cortisone to cortisol. Elevated glucocorticoids are a key risk factor for metabolic diseases. LJALE was prepared with help of Microwave Assisted Extraction (MAE) method. STZ-HFD-induced diabetic rats were treated with LJALE (200 mg/kg, 500 mg/kg) and Metformin (50 mg/kg). It was given for 4 weeks.
Result: LJALE treated Diabetic rats showed significant decreased in blood glucose levels, plasma insulin, serum cortisol as well significant increased hepatic glycogen, 11β-HSD1 index compare to diabetic control rats. LJALE (500 mg/kg) treatment showed the return of islets close to good vascular pattern. LJALE treatment restored endothelium dysfunction.
Conclusions: From all results it is reasonable to conclude that LJALE (500 mg/kg) may be used in treatment of diabetes and diabetes associated vascular dysfunction.
Keywords: Diabetes Mellitus, 11β-HSD1, CA, *Lonicera Japonica*

1. Introduction

According to WHO, Diabetes mellitus has been defined as "a metabolic disorder of multiple etiology, characterized by chronic hyperglycemia with disturbances of carbohydrates, fats & proteins metabolism resulting from defects in insulin secretion, insulin action or both. The effects of diabetes mellitus include long term damage, dysfunction & failure of various organs."[1] India is the diabetic capital of the world. Presently approximately 387 million people worldwide, or 8.3%, in the age group 20-79, suffered from diabetes. By 2035, some 592 million people, or 11 % of the adult population, are projected to have diabetes.[2]

It has been found that flower buds of LJ has also been traditionally indicated for treatment of diabetes, hepatic disorders, antiinflamatory disorder anti cancer activity [3,4] *Lonicera Japonica* Thunb. (Caprifoliaceae), a widely used traditional Chinese medicine, was known as Jin Yin Hua (Chinese), Ren Dong and Japanese honeysuckle [5]. Since 1995, *Lonicera Japonica* has been listed in the "Pharmacopoeia of the People's Republic of China" and more than 500 prescriptions containing LJ have been used to treat various diseases in China [6].

CA and Rutin, a major bioactive component in the flower buds of LJ [**7]**, have received more and more attention because of its antioxidant [8], 11β HSD 1 inhibitory activity [9], anticancer [10] and anti-inflammatory [11], Hepatoprotective [12] and anti lipase activities [13]. In current Chinese Pharmacopoeia (Committee for the Pharmacopoeia of PR China, 2010), Rutin improved Ischemia reperfusion induced myocardial contractile function and reduced infarct size due to its antiapoptotic effect [14]. Some flavonoids and triterpenoids of LJ also revealed antioxidant property. CAs, rutin, flavonoids and triterpenoids have been officially used as the indicator compounds to characterize the quality of LJ.

CA mostly found in abundant amount in LJ. CA is an ester of caffeic with quinic acids. CA is a catecholic acid that is found in such plants and coffee drinks.[15,16] CA having ability to scavenge free radicals and to inhibit lipid peroxidation prevents STZ induced oxidative stress and protects b-cells, resulting in increased insulin secretion and decreased plasma glucose levels.[17,18] CA is a novel insulin sensitizer that potentiates insulin action similar to the

therapeutic action of metformin.[19] CA reduces blood glucose level by virtue of its ability to inhibit glucose-6-phosphatase activity, with the related effects on hepatic glycogenolysis[20]. CA increased the expression of PPAR-γ of adipose tissue so it also increases insulin sensitivity.[21] CA is a specific Glucose-6-Phosphate translocase inhibitor. Glucose-6-Phosphate enzyme plays a very important role in regulation of 11β- HSD 1 activity. CA caused a 96% decrease in G6P-stimulated 11β-HSD1 activity. It may conclude that CA is an indirectly 11β-HSD1 inhibitor.[22] Recent studies have shown that CA can decrease oxidation of low density lipoprotein (LDL) and exhibits antioxidative properties in several cell types, including smooth muscle cells, endothelial cells and messangial cells.[23]

Considering the importance of plants as a source of medicines and the data available on the biological activity of this plant LJ has been selected. So the present investigation was aimed to study the protective effect of LJ with reference to diabetes mellitus.

2. Materials & Methods

2.1 Animals

Healthy young adult nulliparous and non-pregnant female or male Spargue-Dawley (SD) rats, weighing 200-250 g at the beginning of the experiment and were procured from animal house of A.P.M.C College of Pharmaceutical Education and Research, Himatnagar.

2.2 Chemicals

STZ was obtained from Divya Chemicals (Delhi), all other chemical used were of analytical grade. Flowerbuds of LJ powder was obtained from USA. Other chemical like Metfomin and Sodium pyruvate were obtained from local dealer. Diagnostic kit for determination of glucose. Cholesterol, triglyceride, HDL, cortisol were obtained from Nicholas Piramal limited.

2.3 Induction of diabetes in rats

Healthy Spargue-Dawley (SD) rats showing normal plasma glucose level in the range of 80-120 mg/dl were used. Animals were fed with high fat diet for two weeks prior to Streptozotocin injection. A single dose of streptozotocin (35mg/ kg, i.p.) was administered for induction of diabetes. Blood glucose level was measured after 48 hours of streptozotocin treatment. Those animals showing plasma blood glucose more than or equal to 300mg/dl were considered as diabetic and was used for further studies. Diabetic animals were also fed with the high fat diet till the practical termination. Plasma glucose was measured again at the end of every week to confirm consistent hyperglycemia. Blood glucose was measured with glucometer at first day of treatment and the last day of experimental termination.[24]

2.4 Ethanolic Extraction of flowerbuds of LJ

A household microwave oven was modified in our laboratory with the addition of a magnetic stirrer, water condenser, temperature measurement and time controlling for automatic Microwave Assisted Extraction (MAE). With ice water running through the condensation pipe of the MAE system, 10 gm of dried powder of LJ was mixed with 100 ml mixtures of ethanol and water, and then the suspensions were irradiated automatically with microwave at a power of 700W in a pre-setting procedure in order to keep a desired extraction temperature 60°c. Heat-reflux extraction using a water-bath was performed with 10 gm dried plant samples and 100 ml of mixtures of ethanol and water in a 250 ml flask with a mechanical stirrer and the extraction temperature was kept at 60°c. All samples were centrifuged at 4250×g for 5 min, and filtered through 0.45μm membrane before analysis by high performance liquid chromatography (HPLC).[25]

2.5 Study design

All animals are age matched. The animals were divided in 5 groups:

Experimental design and drug treatment: Earlier it has been reported that Diabetic vascular complication develops within 6 weeks after the induction of diabetes. In this study, development of diabetic vascular complication was ensured before starting the treatment by measuring blood glucose, lipid profile and cortisol. Diabetes was induced with help of 35 mg/kg streptozotocin and initial therapy of high fat fed diet. Animals were divided in five groups viz normal control, Disease Control, LJALE) (200 mg/kg) treated diabetic rat, LJALE (500 mg/kg) treated diabetic rat, Standard Metformin (50 mg/kg) treated diabetic rat. Six weeks after the diabetic vascular complication development, therapeutic treatment with (LJALE) (200 mg/kg, p.o. daily), (LJALE) (500 mg/kg, p.o. daily) and Metformin (50 mg/kg, p.o. daily) was given for weeks (4 weeks). Whereas the animals of normal control and disease control group received saline and HFD diet respectively until the end of the study. All animals were sacrificed after 10th week. All parameters viz blood glucose, plasma insulin, hepatic glycogen, serum cortisol, histopathological study of Pancreas, Invivo pyruvate load test, Vascular reactivity study, exvivo 11β –HSD1 inhibition assay study were evaluated on completion treatment.

2.6 Parameters:

- Blood Glucose
- Serum Insulin
- Serum Glycogen

- 11β-HSD1 Index
- Serum Cortisol
- *In vivo* Pyruvate Load test
- Histopathological Study of Pancreas
- Vascular reactivity study

3. Results

3.1 Blood Glucose:

Blood glucose level was measured using one touch horizon blood glucose monitoring system.

Table 1.Comparison of Blood Glucose between Normal, Diabetic & treated diabetic Animals.

Blood Glucose (mg/dl)																									
Normal					**Diabetic**					**LJALE (200 mg/kg)**					**LJALE (500 mg/kg)**					**Metformin (50 mg/kg)**				
Animal			Mea		Animal			Mea	SE	Animal			Mea	SE	Animal			Mea	SE	Animal			Mea	SE
I	II	III			I	II	III			I	II	III			I	II	III			I	II	III		
90	111	119	107	9	330	341	356	342	8	200	185	175	187	7	155	170	150	158	6	150	140	141	144	3

3.2 Serum Insulin

Serum Insulin was assayed by ELISA between Normal, Diabetic and various treatment groups as shown in figure 1.

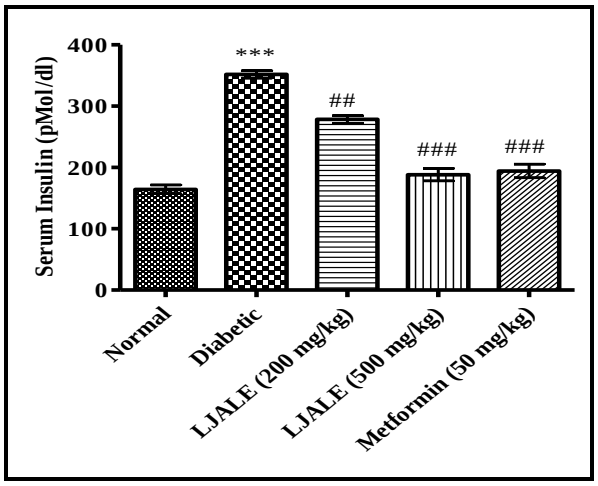

Figure 1: Showing the comparison of serum insulin in Normal rats, diabetic rats, LJALE (200 mg/kg) , LJALE (500 mg/kg) and standard Metformin (50 mg/kg) treated diabetic rats. Values are expressed in mean ± S.E.M. n = 4-6. *** p < 0.001 Vs Normal Control and ## p< 0.01, ### p < 0.001 Vs Diabetic control.

3.3 Hepatic Glycogen

Glycogen can be converting to glucose by glycogenolysis process. Here, hepatic glycogen was estimated by colorimetric method.

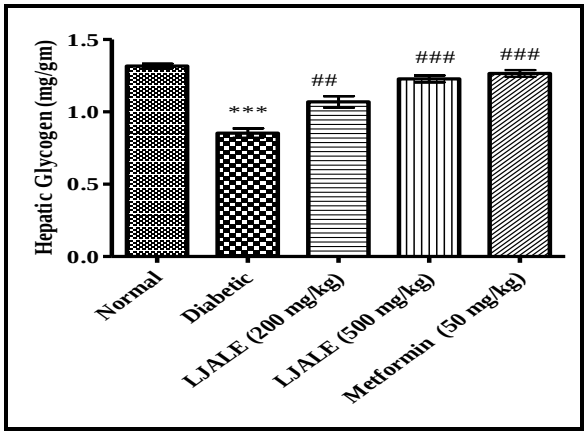

Figure 2: Showing the comparison of hepatic glycogen in Normal Rats, Diabetic rats, LJALE (200 mg/kg) , LJALE (500 mg/kg) and standard Metformin (50 mg/kg) treated diabetic rats. Values are expressed in mean ± S.E.M. n = 4-6. *** p < 0.001 Vs Normal Control and ## p< 0.01, # # # p < 0.001 Vs Diabetic control.

3.4 11β-HSD1 Index

11β-HSD 1 Index is a ratio between cortisone to cortisol.11β-HSD1 Index showed expression of 11β-HSD1 activity. CA from LJALE is an inhibitor of 11β-HSD 1. 11β-HSD1 was estimated by LC-MS/MS analysis between Normal, Diabetic and various treatment groups as shown in table 2.

Table 2: Comparison of between 11β-HSD 1 Index between Normal, Diabetic control & treated diabetic animals

11β- HSD 1 Index (%)																								
Normal					**Diabetic**					**LJALE (200 mg/kg)**					**LJALE (500 mg/kg)**					**Metformin (50 mg/kg)**				
Animal			Mean	SEM	Animal			Mean	SEM	Animal			Mean	SEM	Animal			Mean	SEM	Animal			Mean	SEM
I	II	III			I	II	III			I	II	III			I	II	III			I	II	III		
11	14	10	12	1	91	83	85	86	2	54	49	50	51	2	20	23	23	22	1	75	72	77	73	2

3.5 Serum Cortisol

Serum cortisol is an indicator of 11β-HSD1 activity. Here, serum cortisol was measured by Chemiluminescent assay between Normal, Diabetic and various treatment groups as shown in figure 3.

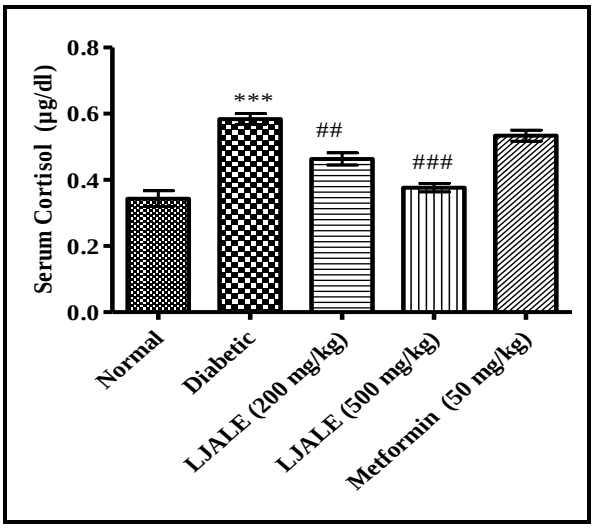

Figure 3 showing the comparison of serum cortisol in Normal rats, Diabetic rats, LJALE (200 mg/kg), LJALE (500 mg/kg) and standard Metformin (50 mg/kg) treated diabetic rats. Values are expressed in mean ± S.E.M. n = 4-6. *** p < 0.001 Vs Normal Control and ## p< 0.01, # # # p < 0.001 Vs Diabetic control.

3.5 *In vivo* Pyruvate Load test

Table 3: Blood Glucose Level after Invivo Pyruvate Load Test

Groups	Glucose (mg/ dl) Time: 0 min.	Glucose (mg/ dl) Time: 30 min.	Glucose (mg/ dl) Time: 60 min.	Glucose (mg/ dl) Time: 90 min.
Normal	111	116	124	119
Diabetic	376	395	408	414***
LJALE(200 mg/kg)	181	192	203	209#
LJALE (500 mg/kg)	171	179	185	185##
Metformin (50 mg/kg)	160	164	171	172##

Table 3 showing the comparison of blood glucose in Normal rats, Diabetic rats, LJALE (200 mg/kg) , LJALE (500 mg/kg) and standard Metformin (50 mg/kg) treated diabetic rats.. Values are expressed in mean ± S.E.M. n = 4-6. *** p < 0.001 Vs Normal Control and ## p< 0.01, # # # p < 0.001 Vs Diabetic control.

3.6 Histopathological Study of Pancreas:

On the 30th day, after 3 hrs of last dose of respective treatments, the animals were anaesthetised under light ether anaesthesia. Blood was withdrawn through retro orbital. The animals were sacrificed by overdose of ether and autopsied. Pancreas was removed, washed with saline and pancreas stored in 10% formalin for histopathological studies.

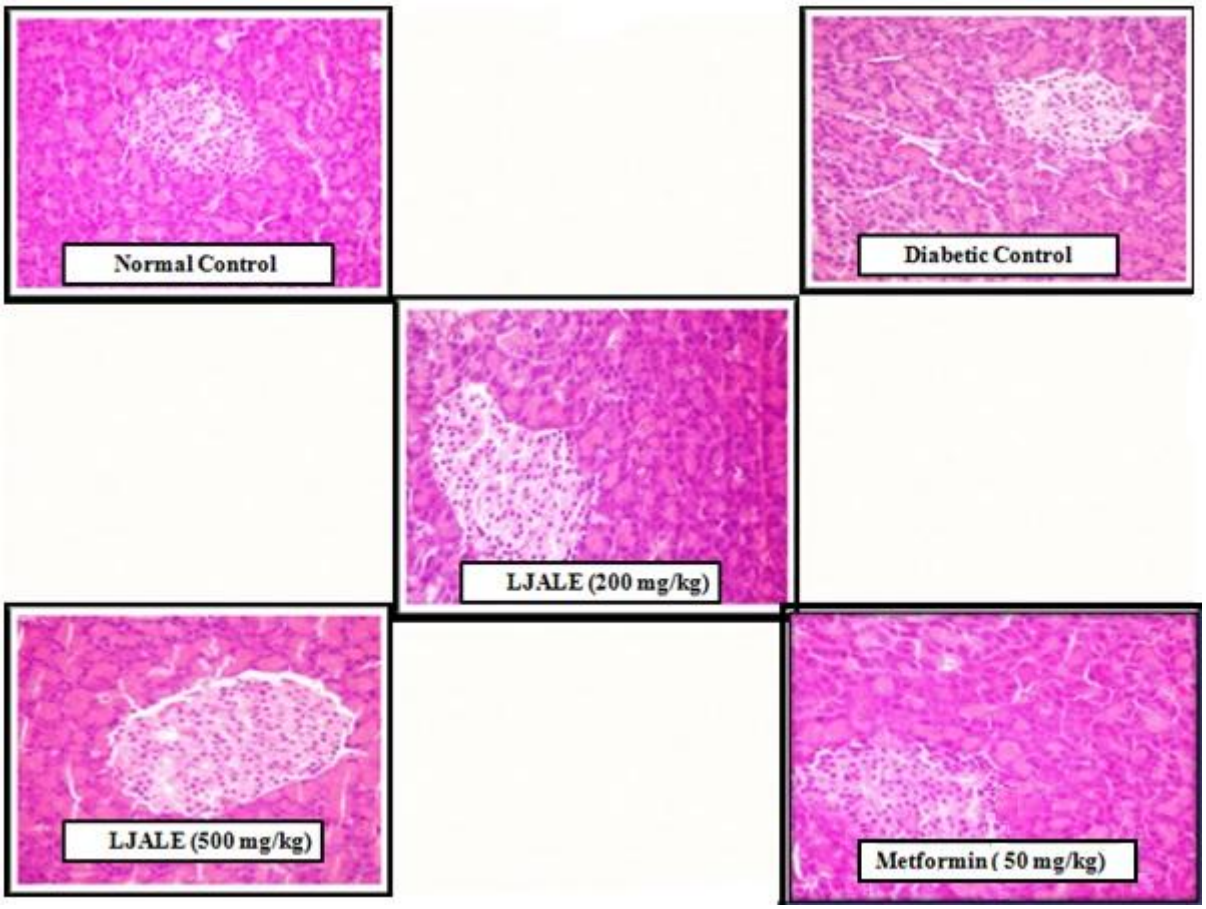

Figure 4: Histopathological Study of Pancreas

3.7 *In Vivo* Vascular Reactivity Study

Effect of LJALE (200 mg/kg), LJALE (500 mg/kg) and standard Metformin (50 mg/kg) treatment on Acetylcholine induced relaxation in STZ-HFD diabetic rat.

Vascular reactivity study was performed by taking Acetylcholine induced relaxation response in the Phenylepherine induced precontractile Diabetic and treated rat thoracic aorta.

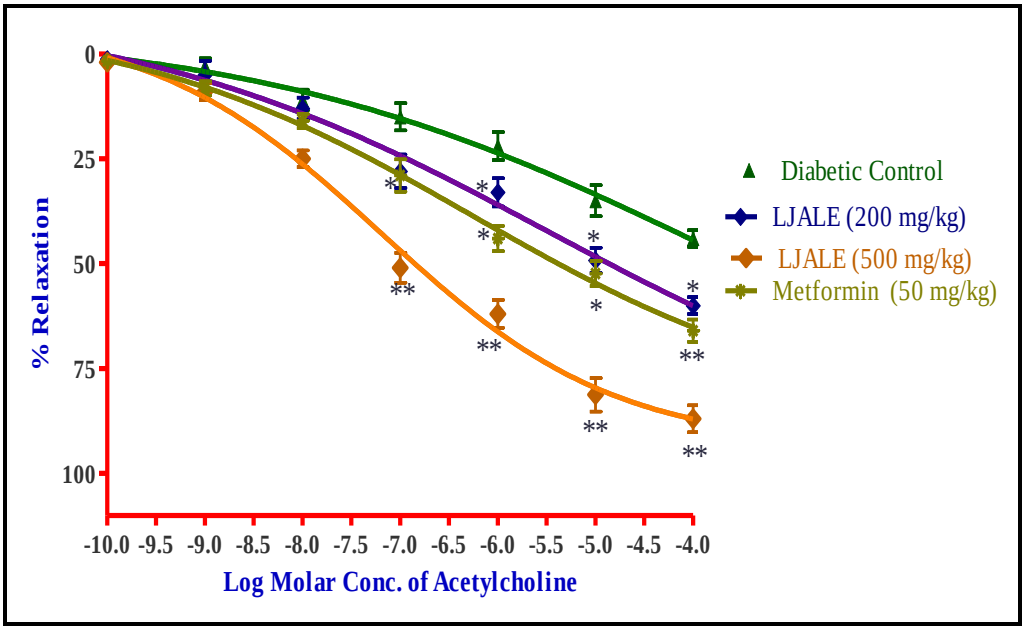

Figure 5 showing CRCs of Acetylcholine in endothelium intact aortic spiral preparations obtained from STZ-HFD diabetic rats and 4 weeks treated diabetic rats with LJALE (200 mg/kg), LJALE (500 mg/kg) and Metformin (50 mg/kg). Values are expressed as mean ± SEM n = 4 - 6. For unpaired t test *p < 0.05, **p <0.001 Vs diabetic Control.

Table 4: pD2 and R max (%) of Ach. in Diabetic Control & treated rat thoracic aorta

Groups	Acetycholine	
	pD_2 values	**R max %**
Diabetic	6.13 ± 0.026	43.29 ± 1.99
LJALE (200 mg/kg)	6.49± 0.042*	60.05 ± 2.0*
LJALE (500 mg/kg)	7.18 ± 0.032**	87.02 ± 3.21*
Metformin (50 mg/kg)	6.65 ±0.063**	65.17 ± 2.6**

4. Discussion

Primary goal of this experiment was to study the potential role of LJALE on Diabetes Mellitus and associated vascular dysfunction in rat. Diabetes Mellitus represents a spectrum of metabolic disorders, which has become a major health challenge worldwide. Schrauwen (2007) has reported that the HFD feeding rats develop insulin resistance. At the same time, STZ has been known to selectively target and destroy the pancreatic β cell by necrosis. Therefore, the rat model by high-fat diet following low-dose STZ, which closely mimic the natural history and the metabolic characteristics of the type 2 diabetes in humans. STZ-HFD treatment in rats leads to excessive or abnormal level of 11β-HSD1 contributes metabolic abnormalities.[26]

A significant rise in blood glucose level was seen in the STZ-HFD rats as compared to normal rats ($p<0.001$). The mechanism by which STZ-HFD brings about its diabetic state include selective destruction of pancreatic insulin secreting β cells, which make cells less active and lead to poor glucose utilization by tissues. STZ induces over-expression of 11β-HSD1 leads to excessive generation of cortisol from inactive cortisol which may be responsible for hyperglycemia and associated vascular complication.

Treatment of LJALE (200 mg/kg), LJALE (500 mg/kg) and Metformin (50 mg/kg) were started after the 4^{th} week of STZ injection and carried out for next 4 weeks. Administration of LJALE (200 mg/kg), LJALE (500 mg/kg) and Metformin (50 mg/kg) tended to bring blood glucose levels down near normal levels. LJALE and Metformin treated animals showed significant decreased ($p < 0.001$) in blood glucose levels compare to diabetic control rats particularly LJALE (500 mg/kg) (Table 1). LJ have been found to possess active chemical constituents like CA, Flavanoids, Coumarins, Saponins in the LJALE which may be responsible for antidiabetic activity. CA is a physiological inhibitor of 11β-HSD1. So it may be CA from LJALE has restored STZ-HFD induced hyperglycemia.

Insulin resistance is a specific characteristic of type 2 diabetes mellitus.[27] Insulin level was significant elevated in diabetic rats, while flowerbuds of LJALE showed dose dependent reduction in insulin. Figure 1 represent that LJALE (500 mg/kg) significant ($p < 0.001$) decrease insulin level as compared to LJALE (200 mg/kg) ($p < 0.01$).

The quantity of glycogen in liver was observed to decrease significantly in diabetic rat as STZ selectively destroy β cells of Islets of Langerhans and thereby inhibiting glycogen deposition. This is similar to our observation in this experiment. LJALE (500 mg/kg) significantly ($p < 0.001$) increases the glycogen content in liver of STZ-HFD diabetic rats as compared to LJALE (200 mg/kg) ($p < 0.01$). LJALE and Metformin treatment resulted in a significant recovery with respect to this effect and this may be due to either stimulation in insulin release from β cells noted in this study or due to insulinomimetic activity.

11β-HSD 1 is mainly found in the tissues such as liver, blood vessels, adipose, skeletal muscle, brain and immune system, over expression or increased activity of 11β-HSD1 leads to increase in cortisol levels in the body; It is also been postulated that, prolonged hyperglycemia itself will activate the 11β-HSD1 activity and ultimately leads to obesity, diabetes and other health problems. STZ-HFD treated diabetic animals showed significantly ($p < 0.001$) increased in expression of 11β-HSD1 enzyme compared to normal control animals. LJALE treated diabetic animals showed significant dose dependent inhibition of 11β-HSD 1 activity in liver in ex vivo conditions. Furthermore, at higher dose LJALE (500 mg/kg) have offered maximum inhibition ($p < 0.001$) of 11β-HSD1 in liver compared to LJALE (200 mg/kg) ($p<0.01$). LJALE (500 mg/kg) was found more potent than LJALE (200 mg/kg). CA is a physiological inhibitor of 11β-HSD1. From these findings CA isolated from LJALE showed dose dependent inhibition of 11β-HSD1 activity and hence thought to be useful in the treatment of diabetes.

Abnormal cortisol synthesis is major factor contributing to postprandrial hyperglycemia in patients with diabetes mellitus. Cortisone/Cortisol ratio is an indicator of 11β-HSD1 activity. Cortisol has been shown to destroy β cells in the islets of HFD rats. Biochemically this leads to elevate the blood glucose level.

In present study STZ-HFD diabetic rats showed significant ($p<0.001$) increased cortisol level compared to normal control rats. Significant decreased in Cortisol level was found in diabetic animals treated with LJALE (200 mg/kg) ($p < 0.01$) and LJALE (500 mg/kg) ($p < 0.001$) compared to STZ-HFD diabetic animals (Figure 3). Decreased in cortisol level in LJALE treated animals is due to 11β-HSD1 inhibitor activity. CA may inhibit the physiological activities of 11β-HSD1.

The restoration of cortisol was found significant in animals treated with LJALE, particularly with LJALE (500 mg/kg) treatment. So it may conclude that 11β-HSD1 enzyme plays a key role in hypercortisolism associated with Diabetes Mellitus.

11β-HSD1 enzyme plays a very key role in the gluconeogenesis pathway via (Phosphoenolpyruvate Carboxykinase) PEPCK induction. In the gluconeogenesis pathway, oxaloacetate is converted into phosphoenolpyruvate with help of PEPCK enzyme and finally increase hepatic glucose output.[28] Sodium pyruvate is the main source of hepatic glucose production after a long fasting period in the gluconeogenesis pathway. So in regards with above fact pyruvate load test was performed to find out role of 11β-HSD1 in gluconeogenesis. In this study after sodium pyruvate administration at different time interval glucose level were significantly ($p < 0.001$) higher in diabetic group compare to normal control group. In 90 min. increased glucose level came back to initial value in normal control rats while in diabetic animals it did not come back to initial values, which indicate the stimulation of gluconeogenesis pathway in STZ-HFD diabetic rats. Diabetic animals treated with LJALE (200 mg/kg) ($p < 0.5$), LJALE (500 mg/kg) ($p < 0.01$) showed significant reduction in increased glucose level compared to diabetic control which indicates the inhibition of gluconeogenesis pathway in treated animals. These results show the role of 11β-HSD1 in gluconeogenesis in diabetic animals. However Metformin also showed significant ($p < 0.01$) inhibition of gluconeogenesis.

STZ is a DNA alkylating agent, which leads to necrosis of pancreatic beta cells and thus to a state of insulin – dependent diabetes mellitus. The action of STZ could also be due to alterations in membrane fatty acid content, which may affect Na+, K+- ATPase activity, membrane fluidity, and fatty acid content. The islet cells were shrunken and lytic cellular changes were observed in disease control animals. LJALE (200 mg/kg) Low doses had reduced the degenerative changes but LJALE (500 mg/kg) high doses of respective treatment showed the return of islets close to normal cytoarchitecture. Metformin treated islets were large cells were clearly observed with good vascular pattern.

Macrovascular and microvascular diseases are currently the principal causes of morbidity and mortality in patients with diabetes. In present study endothelial dysfunction was accessed by relaxation of acetylcholine in phenylephrine induced precontractiled response in rat thoracic aorta. In diabetic rat thoracic aorta, Ach induced relaxation responses were impaired as compared to normal control. There was significant ($p < 0.001$) decrease in pD_2 and R_{max} of acetylcholine were found in diabetic control group compared to normal control group. It indicates the endothelium dysfunction in STZ-HFD diabetic rats. These results suggest that the decrease in release of nitric oxide may be responsible for vascular complications in STZ-HFD diabetic rat. In invivo vascular reactivity study, both LJALE & Metformin treated group showed improvement in relaxation response of acetylcholine (10^{-10} M to 10^{-4} M) in phenylephrine induced contraction in diabetic rat thoracic aorta. Significant increased ($p < 0.01$) in pD_2 and R_{max} of acetylcholine were found in LJALE and Metformin ($p < 0.01$) treated group compared to STZ-HFD diabetic rats particularly LJALE (500 mg/kg) ($p < 0.01$). These results indicate that LJALE attenuate the STZ-HFD induced endothelium dysfunction in rats.

5. Conclusion

High fat fed with low dose of STZ leads to the prolong hyperglycemia and vascular complications. Diabetic animals showed increased level of cortisol compared to normal control animals. 11β-HSD1 catalyses the interconversion from inactive cortisol to active cortisol. So it indicates that overexpression of 11β-HSD1 in STZ-HFD diabetic rats. Results from our laboratory point that CA from LJ as particularly effective chemical constituents that can alter 11β-HSD1 function and at the same time restore Diabetes and associated vascular complication. From the results it is confirmed that LJALE have anti-hyperglycemic. It was clearly demonstrated from our in-vivo study that 11β-HSD1 inhibition restores endothelial dysfunction in STZ-HFD diabetic rats. From all results it is reasonable to conclude that LJALE may be used in treatment of diabetes and diabetes associated vascular dysfunction.

References

[1] Ibolya C, Miklós C, József M, Angelo B, István K, G, G6PT-H6PDH-11βHSD1 triad in the liver and its implication in the pathomechanism of the metabolic syndrome. *World J Hepatol* 2012; 4(4): 129-138.

[2] Neeraj K, Bikram S, Pamita B, Ajai PG, Sanjay KU, Vijay KK, Biflavonoids from *Lonicera japonica*. *Phytochemistry* 2005; 66: 2740–2744.

[3] Eun JL, Ju SK, Hyun PK, Je HL, Sam SK, Phenolic constituents from the flower buds of *Lonicera japonica* and their 5-lipoxygenase inhibitory activities. *Food Chemistry* 2010; 120: 134–139.

[4] Xiaofei S, Hu P, Maoxing L , Xiaolou M, Hong D, "*Lonicera japonica* Thunb.: Ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine", *Journal of Ethnopharmacology* 2011; 138: 1– 21.

[5] http://www.zysj.com.cn

[6] Zhang B, Yang RY, Chun Z, "Microwave-assisted extraction of CA from flower buds of *Lonicera japonica* Thunb", *Separation and Purification Technology* 2008; 62 : 480–483.
[7] Clifford MN. CAs and other cinnamatesd nature, occurrence and dietary burden. J Sci Food Agric 1999; 79:362-372.
[8] Un HJ, Young L, Sung KK, June KK, Won HP, Jong GK, A phenolic compound, 5-caffeoylquinic acid (CA), is a new type and strong matrix metalloproteinase-9 inhibitor: Isolation and identification from methanol extract of Euonymus alatus. *Life Sciences* 2005; 77; 2760–2769.
[9] Changhai S, Yang T, Guangzhi L, Saburo Y, Junko Yokota, Metabonomics study of the protective effects of *Lonicera japonica* extract on acute liver injury in dimethylnitrosamine treated rats. *Journal of Pharmaceutical and Biomedical Analysis* 2010; 53: 98–102.
[10] Yip EC, Chan AS, Pang H, Tam YK, Wong YH, Protocatechuic acid induces cell death in HepG2 hepatocellular carcinoma cells through a c-Jun Nterminal kinase-dependent mechanism. *Cell Biol. Toxicol* 2006; 22: 293–302.
[11] Sharma N, Sharma VK, Seo SY, Screening of some medicinal plants for anti-lipase activity. *Journal of Ethnopharmacology* 2005; 97: 453–456.
[12] Jae JJ, Yu MH, Yong CJ, Eun J L, Ju SK, Hye J K, Rutin from *Lonicera japonica* inhibits myocardial ischemia/reperfusion-induced apoptosis in vivo and protects H9c2 cells against hydrogen peroxide-mediated injury via ERK1/2 and PI3K/Akt signals *in vitro*. *Food and Chemical Toxicology* 2009; 47: 1569–1576.
[13] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*. 2005; 112:2735–52.
[14] World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complication. Report of a WHO consultation; 1999.
[15] Alberti KG, Zimmet P, Shaw J. IDF Epidemiology Task Force Consensus Group: the metabolic syndrome – a new worldwide definition. *Lancet* 2005; 366:1059–62.
[16] Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA* 2001; 285:2486-2497.
[17] Mancia G, De Backer G, Dominiczak A. Guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2007; 25:1105-1187.
[18] Roger VL, Turner MB. Executive summary: heart disease and stroke statistics–2011 update. *Circulation.* 2011; 123:459-463.
[19] Erwin RB. Prevalence of metabolic syndrome among adults 20 years of age and over by sex, age, race and ethnicity, and body mass index: United States 2003-2006. National Health Statistics Reports; No.13.
[20] Lavie CJ, Milani RV, O'Keefe JH. Dyslipidemia intervention in metabolic syndrome: emphasis on improving lipids and clinical event reduction. *Am J Med Sci.* 2011; 341(5):388-393.
[21] Joslin EP. The prevention of diabetes mellitus. JAMA 1921; 76:79-84.
[22] Kylin E. Studies of the hypertension-hyperglycemia-hyperuricemia syndrome (German). *Zentralbl Inn Med* 1923; 44: 105-27.
[23] Phillips GB. "Sex hormones, risk factors and cardiovascular disease". *The American Journal of Medicine* 1978; 65 (1): 7–11.
[24] Reaven. "Role of insulin resistance in human disease". Diabetes1988; 37 (12): 1595–607.
[25] Bin Z, Ruiyuan Y, Chun ZL. Microwave-assisted extraction of CA from flower buds of *Lonicera japonica* Thunb. *Separation and Purification Technology* 2008;62 : 480–483
[26] Saredi S, Patte MC, Melcangib RC. Effect of streptozotocin-induced diabetes on the gene expression and biological activity of 3β-hydroxysteroid dehydrogenase in the rat spinal cord. *neuroscience 2005*; 135: 869–877.
[27] Andrews RC. Effects of the 11β-hydroxysteroid dehydrogenase inhibitor compound 544 on insulin sensitivity in men with type 2 diabetes. *J. Clin. Endocrinol. Metab* 2003; 88: 285–291.
[28] Friedman JE. Phosphoenolpyruvate carboxykinase (PEPCK) gene transcription and hyperglycemia are regulated by glucocorticoids in genetically obese db/db transgenic mice. *J. Biol. Chem*1997; 272: 475–481.