Effect of Hibiscus *rosa sinensis L.* basic leaf extract fraction on type-1 diabetic animal model

Fahmi S. Moqbel and Prakash R. Naik
Endocrinology Laboratory, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore,, Karnataka, India

Abstract
Type 1 diabetes mellitus (T1DM) results when pancreatic β-cells are destroyed or absent, leading to decrease in insulin production and secretion. The objective of the present investigation is to screen one of the isolated fractions of Hibiscus rosa sinensis on type-1 diabetic animal model for antidiabetic activity. Experiment was conducted by dissolving basic leaf extract fraction in tween-80 (1%) with two concentrations of 100 and 200 mg/kg body weight and feed orally to Non Obese Diabetic (NOD) mice. After oral feeding of basic leaf fraction there was a significant reduction in serum glucose, glycosylated haemoglobin, triglycerides, cholesterol, blood urea in the diabetic animal model. There was enhancement in HDL and liver glycogen compared to control diabetic animal. Basic leaf fraction has demonstrated significant antidiabetic properties on type-1 animal model by enhancing insulin secretion.

Key words: *Cholesterol, Glycosylated haemoglobin, Insulin, Liver glycogen, NOD mice*

Introduction

Diabetes mellitus is affecting nearly 170 million people and is expected to double by 2030.[1] The two major types of diabetes (type 1 and type 2) are characterized by chronic hyperglycemia due to loss of insulin secretion and/or defective insulin action. Type 1 diabetes mellitus (T1DM) is an autoimmune disorder resulting from lymphocyte-mediated destruction of insulin producing β cells, whereas type 2 diabetes is a complex metabolic disorder caused by combination of insulin resistance and concomitant impairment in insulin secretion.[2] Type 1 diabetes affects more than 5.3 million people worldwide.[3]

Several medicinal plants are having hypoglycemic effects.[4] The WHO has recommended that the assessment of traditional plant treatments for diabetes mellitus needs further investigation.[5] Several herbal products were described for the care of diabetes mellitus in ancient literature of Ayurveda in India. Many of the plants used as diet were reported to have antidiabetic property.[6] Ethanopharmacology surveys indicate that more than 1200 plants are used worldwide in traditional medicine for their alleged hypoglycemic activity.[7]

Hibiscus rosa sinensis is a shrub widely cultivated in tropics as an ornamental plant. It presents several forms with varying flower colors. Different parts of the plant are used in the preparation of variety of foods.[8] The leaves are used as a laxative, while the root is used in cough treatment. The flowers are considered to be aphrodisiac, emollient and emmenagogic and are used in bronchial catarrh, diarrhea and fertility control.[9] Recently few reviews are published on medicinal plants useful for treatment of diabetes.[10] Isolation of plant extracts for antiviral, antidiabetic and antioxidant properties have been reported.[11] Sachdewa and Khemani[12] and Sachdewa *et al.*[13] have reported on hypoglycemic activity of *Hibiscus rosa sinensis* in diabetes induced rats. Vimala *et al.*[14] published the insulin secreting activity of *H. rosa sinensis* in diabetes induced wistar rat.

The non-obese-diabetic (NOD) mouse has served as one of the primary models for type 1 diabetes in which new approaches for immunotherapy have been investigated.[15] The NOD mouse spontaneously develops type 1 diabetes that has many immunological and pathophysiological similarities to human insulin dependent diabetes mellitus (IDDM). The autoimmune nature of diseases is suggested by lymphocytic infiltration of islets of Langerhans which precedes the destruction of insulin producing β-cell. Many studies on diabetes use animal models by injecting streptozotocin or alloxan to induce diabetes whereas the objective of the present investigation is to screen one of the isolated fractions of *H. rosa sinensis* on NOD mouse for antidiabetic activity.

Materials and Methods

Extraction and Fractionation

Healthy disease free leaves of *H. rosa sinensis* were collected from the garden of our University campus. The authenticity of the plant was confirmed by a taxonomist of the Department of Botany University of Mysore, Mysore,

Received on: 12 October, 2014
Accepted on: 18 March 2015

Correspondence to: Prof. Prakash R. Naik, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore-570 006, Karnataka, India.
Email: prakashrnaik@yahoo.co.in

and preserved in the form of herbarium. Collected leaves were shade dried and ground in warring blender. The ground powder was extracted in soxhlet apparatus using ethanol until the final drop of the extract became colorless. The extract was dried using evaporator. Details of extraction and fractionation of *H. rosa sinensis* is published elsewhere.[16] After fractionation the five fractions were obtained namely ethyl acetate soluble fraction (F_1), ethyl acetate insoluble fraction (F_2), chloroform fraction (F_3), the basic fraction (F_4) and neutral fraction (F_5).

Animals

Animals-NOD mice were originally obtained from Center for Cellular and Molecular Biology (CCMB, CSIR), Hyderabad and maintained under standard environmental conditions of 12:12 h L: D cycle. Mice were fed with standard diet supplied by Ambruth Feeds Pvt. Ltd. Bangalore and water *ad libitum*. Healthy adult (30 weeks old) NOD mice of either sex weighing 25-30 g were selected for the study from the Central Animal Facility of the Department. All the selected animals showed the fasting blood glucose values of ≥ 242 mg/100ml indicating hyperglycemia, the diabetic status. Experimental protocols were approved from Institutional Animal Ethics Committee (IAEC).

Experimental Protocols

The pilot experiment was conducted to test the hypoglycemic properties of the five fractions obtained. Since F_2 did not dissolve in tween-80 (1%) remaining fractions F_1, F_3, F_4 and F_5 were dissolved in tween- 80 (1%) and fed orally with two concentration of 100 and 200 mg/kg body weight to NOD mice. F_1 did not show hypoglycemia whereas fractions F_3, F_4 and F_5 showed hypoglycemia at the termination of experiment (unpublished data) Since fraction-4 was not obtained in sufficient quantity, detailed investigation for antidiabetic properties of fractions F_3 and F_5 were published earlier.[16] Subsequently extraction procedure was repeated to obtain F_4 in adequate quantity and then it is subjected for detailed investigation for antidiabetic activity on NOD mice in the present investigation. The experiment was conducted by dissolving F_4 in tween- 80 (1%) and fed orally with two concentrations of 100 and 200 mg/kg body weight to NOD mice. NOD mice were randomly divided in to following groups with eight animals in each.

Group- I Control- NOD mice treated with vehicle alone.
Group- II NOD mice treated with insulin (1 ml of Biphasic isophane insulin purchased from pharmaceutical company dissolved in 100 ml saline and 0.1 ml/mouse / day was injected (intra peritoneal).
Group- III NOD mice fed orally with 100 mg/kg body weight of F4.
Group- IV NOD mice fed orally with 200 mg/kg body weight of F_4.

Since diabetes is a chronic disorder requiring long term therapy, there is a need to assess the effect of different extracts for antidiabetic activity for a longer duration. Hence the experiment was conducted for four weeks.

Biochemical analysis

Serum glucose level was estimated using a glucometer (EZ Omnitest) every week to ascertain the states of diabetes in different groups of mice; similarly body weight was recorded once in a week in every group. After 30 days animals were allowed to fast overnight with a free access to water and autopsied under light either anesthesia. The blood was collected from the carotid artery at the time of autopsy[17] and centrifuged at 4^{o}C, at 10000 rpm for 10 min; the separated serum was used for bio-chemical analysis.

Serum glucose level was estimated by Trinder's method[18] using GOD/POD Enzymatic kit. Glycosylated hemoglobin was determined according to the ion exchange resin method.[19] Triglycerides were measured by enzyme-colorimetric method.[20] HDL- cholesterol was assayed by the method of Burstein *et al*.[21] LDL-cholesterol and VLDL-cholesterol were measured by using the formula of Friedweld *et al*.[22] Blood urea was estimated by urea-glutamate dehydrogenase (GLDH) method. Plasma insulin levels were determined in duplicate using insulin RIA Kit (Linco, St. Charles MO) with rat insulin as a standard.[23]

To measure the liver glycogen 200 mg of the liver sample was finely ground with 20 ml of 5% TCA in a homogenizer. The precipitate of proteins is filtered off and the clear filtrate was used for measuring glycogen content by Anthrone method.[24]

All above parameter were recorded at the termination of experiment.

Statistical analysis

Data were statically evaluated by using one way ANOVA. Wherever the ANOVA values were found to be significant Duncan's new multiple range test (DMRT) was applied (SPSS computer software). The values were considered significant when $p < 0.05$.

Results

Bodyweight

Table 1 shows the body weight of experimental animals. Body weight of group I animals remained constant without any gain whereas there was a significant increase in body weight of the animals of group II - IV at the termination of the experiment. Table 2 shows the effect of F_4 on different parameter at the termination of the experiment.

Serum glucose level in group I (NOD mice) exhibited hyperglycemia on the day of commencement of experiments (287 mg/dl) and remained in the diabetic state throughout the experiment (Figure 1). Group II animals exhibited hyperglycemia (242.4 mg/100 ml) on the day of commencement of experiment but after intrapertonial daily injection of insulin-resulted in bringing the blood sugar level to non-diabetic status (103.4 mg/100 ml). Animals in groups III and IV exhibited hyperglycemia with the fasting blood glucose values of 258.22 and 264.48 mg/100 ml respectively on the day of commencement of experiment. After oral feeding of F_4 (100 and 200 mg/kg body weight) serum glucose level was brought to 119.01 and 108.58 experiment (Figure 1).

Table 1: Effect of *H. rosa sinensis* (HLE) basic leaf extract fraction (F_4) on body weight of experimental groups.

Parameters	Initial Body weight	Final. Body weight
Group I (NOD mice control)	27.8±0.254[a]	27.1±0.187[a]
Group II (Insulin injected NOD Mice)	30.14±0.403[a]	31.74±0.402[b]
Group III (HLE.F_4 100 mg/kg bw)	29.26±0.290[a]	30.92±0.086[b]
Group IV (HLE.F_4 200 mg/kg bw)	29.36±0.395[a]	31.14±0.33[b]

All values of find body weight are significant at P<0.05 except in Group I vs initial body weigh. Values expressed in g are mean ±SE from 8 animals in each group.

Table 2: Effects of H. rosa sinensis basic leaf extract fraction (F4) on glycosylated haemoglobin, Insulin, triglyceride, cholesterol, Liver glycogen and blood urea levels of control and experimental groups

Parameters/ group	*Glycosylated Haemoglobin (HbAlc%)*	*Insulin (μu/l)*	*Triglycerides (mg/dl)*	*Cholesterol (mg/dl)*	*Liver glycogen (mg/g)*	*Blood urea (mg/dl)*
Group I NOD mice control	9.54± 0.18[a]	0.14±0.51[a]	80.8±0.37[a]	78.2±0.583[a]	3.67±0.17[a]	61.4±0.01[a]
Group II Insulin injected NOD mice	6.44±0.12[b]	0.336±1.00[b]	98.4±2.57[b]	76.8±2.03[a]	5.34±0.16[b]	38±0.01[b]
Group III HLE.F4 100 mg/kg bw	5.38±0.14[c]	0.256±1.39[c]	60.18±0.78[c]	56.75±0.74[b]	12.69±0.13[c]	44.2±0.01[c]
Group IV HLE.F4 200 mg/kg bw	5.64±0.25[c]	0.274±1.14[c]	55.22±0.81[d]	46.36±0.48[b]	23.36±0.15[d]	36±0.01[b]
F value df (3,16)	110 sig p<0.05	41.74 sig p<0.05	194.43 sig p<0.05	184.44 sig p<0.05	3315.93 sig p<0.05	118.39 sig p<0.05

Values are mean ±SE from 8 animals in each group. All values are significant at P<0.05 vs diabetic control (Gr. I)

Table 2 shows the levels of different biochemical parameters at the termination of experiment.

Blood glycosylated haemoglobin (HbAlc) level

Control group showed maximum levels of fasting glycosylated haemoglobin whereas there was significant decrease in fasting glycosylated haemoglobin in insulin injected group. Oral administration of F_4 for 30 days at two different doses (100 and 200 mg/kg body weight) resulted in significant decrease on fasting glycosylated haemoglobin in the groups III - IV. (Table 2)

Serum insulin

Serum insulin level remained minimum in control group. Insulin level was enhanced significantly in the remaing groups. However the insulin level was not on par with group II, in groups III and IV animals (Table 2).

Blood triglycerides

The triglycerides levels significantly decreased in groups III - IV compared to group I animals (Table 2). Interestingly the levels were enhanced significantly in insulin treated mice compared to that of control group.

Cholesterol

There was no significant variation in cholesterol in control group and insulin injected group. After oral treatment with F_4 at two different doses (100 and 200 mg/kg body weight) the cholesterol level of group III - IV showed significant reduction compared to that of control group (Table 2)

Liver glycogen

Liver glycogen in different groups of experimental animals is shown in Table 2. Liver glycogen increased to (145.5%) of the control value in group II and the glycogen increased to (345.8%) and (629.7%) in groups III and IV respectively.

Blood urea

The blood urea in control group showed higher values where as it remained significantly lower in all the remaining groups of animals (Table 2).

LDL value remained higher in group I animals. LDL value decreased significantly in group II and IV animals whereas there was no significant variation in group III animals (Figure 2). The levels of VLDL in different groups of experimental animals at the termination of experiment are shown in Figure 2. The VLDL value was enhanced in group- II animals whereas there was no significant variation in group III and IV animals compared to that of control animals. Figure 2 also shows the levels of HDL in different groups of experimental animals at the termination of the experiment. HDL level remained higher significantly in all the groups compared to that of group I animals.

Discussion

It is evident from Figure 1 that basic leaf extract fraction of *H. rosa sinensis* reduced the serum glucose level significantly Loss of body weight due to defect in glucose metabolism and excessive breakdown of tissue protein is a

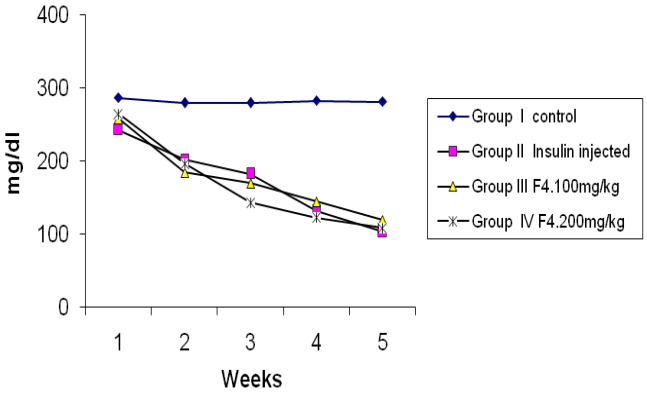

Figure 1: Effect of H. rosa sinensis (HLE) basic leaf extract fraction (F4) on serum glucose level (mg/dl).

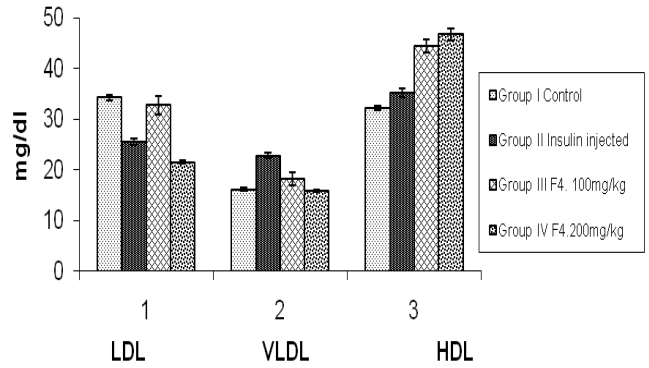

Figure 2: Effect of H. rosa sinensis (HLE) basic leaf extract fraction (F4) on LDL, VLDL, HDL (mg/dl)

characteristic condition in type 1 diabetes.[25] In the present investigation there was no body weight gain in control NOD mice. There was a significant body weight gain in insulin injected NOD mice whereas after oral feeding with F_4 with different concentrations there was also significant increase in body weight and this was associated with reduced serum glucose level throughout the experiment (Figure 1).

Patients with type 1 diabetes have to take exogenous insulin for survival.[26] In the present investigation group II animals were injected with insulin bringing the serum glucose level to non-diabetic status (Figure 1). Critically examining Table 2 it is obvious insulin failed to reduce triglycerides and cholesterol level comparing to control NOD mice. The cholesterol level in fact was enhanced in insulin treated group. F_4 with two different concentrations reduced the glycosylated hemoglobin, triglycerides cholesterol better than the insulin injected group. There was also enhancement of liver glycogen with different concentration of F_4 compared to insulin injected group which is a positive effect in reducing serum glucose level. Blood urea was also reduced significantly after treatment with F_4. Though exogenous insulin reduces the serum glucose level it has a side effect. There was enhancement in the insulin secretion from pancreatic islets after treatment with F_4 compared control NOD mice (Table 2). Though the quantity of insulin secreted after treatment with F_4 was not on par with exogenous insulin supplied to group II animals, the insulin secreted within had a better effect in regulating glycosylated hemoglobin, triglycerides, cholesterol and liver glycogen level to non-diabetic status than exogenous insulin supplied. Several investigators have recommended that glycosylated hemoglobin be used as an indicator of metabolic control of diabetes since glycohemoglobin levels approach normal values in diabetic after metabolic control.[27] Though the F_4 had an encouraging effect on above parameters F_4 could reduce LDL and VLDL only at 200mg/kg body weight (Figure 2) but could enhance HDL comparing to control NOD mice and insulin injected group. There are number of publication in recent years on isolation of plant extracts with different solvents to screen antidiabetic activity.[28,29,30,31] Babu *et al*.[28] published on antidiabetic activity of ethanol extract of *Cassia kleinii* leaf on diabetic rats. The antihyperglycemic activity was found predominantly in the chloroform fraction of alcohol extract. Hypoglycemic effect of *Gentiana olivieri* flowering herbs were studied in diabetes induced rats.[29] Methanolic extract was subjected for successive solvent extraction with chloroform, ethyl acetate and n-butanol. Hypoglycemic activity of each extract was studied. Ethyl acetate and n-butanol extracts showed significant hypoglycemic activity in diabetes rats. Further ethyl acetate extract was subjected for column chromatographic separation and identified the compound as isoorientin. They concluded that isoorientin at the dose of 15 mg/kg body weight for 15 days had a remarkable effect on blood glucose cholesterol and triglyceride concentration in diabetes induced rats. It did not enhance insulin secretion.

Prajapati *et al*.[30] demonstrated that ethanol and chloroform extracts of *Actinodaphne hookeri* leaf extracts were found to have significant lowering effect on blood glucose, total cholesterol, triglycerides, LDL and VLDL and enhanced HDL in diabetic rats. Jarald *et al*.[31] reported on aqueous extract and non-polysaccharide fraction of *Cynodon dactylon* of whole plant. Treatment of diabetic rats with these fractions decreased the elevated levels of blood glucose, urea, creatinine, serum cholesterol, serum triglyceride, LDL and glycosylated hemoglobin significantly in diabetic model.

Many studies on type 1 diabetes use animal models by injecting animals with streptozotocin or alloxan to induce diabetes[14,32] whereas the present investigation reports on NOD mice which spontaneously develop type 1 diabetes and has similar features to those of human type-1 diabetes. However none of the above cited studies reported on insulin secretion after treatment with different fractions. Hypoglycemic plants act through a variety of mechanisms by improving insulin sensitivity or augmenting glucose dependent insulin secretion or decreasing insulin resistance. In the present investigation there is a direct effect on insulin secretion.

Conclusions

In our previous report,[16] it was demonstrated that F_3 and F_5 have insulinotropic effect and also protective effect in NOD mice. F_4 also has insulinotropic effect similar to F_3 and F_5. Isolation of active component/s from these fractions will

reveal whether these fractions have similar compounds or different compounds with different potency. Isolation of active component/s is in progress.

Acknowledgements
The first author thanks Thamar University, Republic of Yemen for awarding fellowship and University of Mysore for the permission and facilities to do the research work. Both authors are thankful to UGC- New Delhi for partial financial assistance from SAP-programme.

References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-1053.
2. Hadjiyanni I, Drucker DJ. Glucagon-Like Peptide 1 and Type 1 Diabetes: NOD Ready for Prime Time? Endocrinology 2007; 148: 5133-5135.
3. Hauben E, Roncarolo MG, Nevo U, Schwartz M. Beneficial autoimmunity in type-1 diabetes mellitus. TRENDS in Immunology 2005; 26: 248-253.
4. Yeh GY, Eisenberg DM, Kaptchuk TJ, Phillips RS. Systematic review of herbs and dietary supplements for glycemic control in Diabetes. Diabetes Care 2003; 26: 1277-1294.
5. Yanardag R, Bolkent S, Oguz AT, Sacan, OO. Effects of Petroselinum crispum extract on pancreatic β-cells and blood glucose of streptozotocin induced diabetic rats. Biol Pharma Bulle 2003; 26: 1206-1210.
6. Kumari K, Augusti, KT. Antidiabetic and antioxidant effects of S-methyl cysteine sulfoxide isolated from onions (Allium cepa Linn) as compared to standard drugs in alloxan diabetic rats. Ind J Exp Biol 2002; 40: 1005-1009.
7. El-Hilaly J, Adil T, Zafar HI, Badiaa L. Hypolipidemic effects of acute and sub-chronic administration of an aqueous extract of Ajuga iva L.Whole plant in normal and diabetic rats. J Ethnopharmacol 2006; 105: 441-448.
8. Gilani AH, Bashir S, Janbaz KH, Shah AJ. Presence of cholinergic and calcium channel blocking activities explains the traditional use of Hibiscus rosa sinensis in constipation and diarrhea. J Ethnopharmacol 2005; 102: 289-294.
9. Kasture VS, Chopde CT, Deshmukh VK. Anticonvulsive activity of Albizzia lebbeck, Hibiscus rosa sinesis and Butea monosperma in experimental animals. J Ethnopharmacol 2000; 71: 65-75.
10. Modak M, Dixit P, Londhe J, Ghaskadbi S, Paul AT. Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr 2007; 40: 163-173.
11. Mendiole JA, Jaime L, Santoyo S, Reglero G, Cifuentes A, Ibanez E, Senorans FJ. Screening of functional compound in supercritical fluid extract from Spirulina platensis. Food Chem 2007; 102: 1357-1367.
12. Sachdewa A, Khemani LD. Effect of Hibiscus rosa sinensis Linn. Ethanol flower extract on blood glucose and lipid profile in streptozotocin induced diabetes in rats. J Ethanopharmacol 2003; 89: 61-66.
13. Sachdewa A, Nigam R, Khemani LD. Hypoglycemic effect of Hibiscus rosa sinensis L. leaf extract in glucose and streptozotocin induced hyperglycemic rats. Ind J Exp Biol 2001; 39: 284-286.
14. Vimala H, Naik PR, Chandavar VR. Insulin-secreting activity of Hibiscus rosa sinensis Linn. Leaf extract in diabetes-induced Wistar rat. The Bioscan 2008; 3: 293-297.
15. Zang ZJ, Davidson L, Eisenbarth G, Weiner HL. The non-obese diabetic (NOD) mouse has served as one of the primary models for type-1 diabetes in which new approaches for immunotherapy have been investigated. Proc Natl Acad Sci USA 1991; 88: 10252-10256.
16. Moqbel FS, Naik PR, Najma HM, Selvaraj S. Antidiabetic properties of Hibiscus rosa sinensis L. leaf extract fractions on non-obese diabetic (NOD) mouse. Ind J Exp Biol 2011; 49: 24-29.
17. Chandavar VR, Naik PR. Variation in plasma glucose and pancreatic β-cells in the turtle Lissemys punctata (order: Chelonia; family: Trionychidae). Acta Zool 2004; 85: 113-118.
18. Trinder P. Determination of blood glucose using an Oxidase-Peroxidase system with a noncarcinogenic chromogen, J Clin Pathol 1969; 22: 158-161.
19. Willey DG, Roseenthal MA, Caldwell S. Glycosylated hemoglobin and plasma glycoprotein assay by affinity chromatography. Diabetologia 1984; 27: 56-58.
20. Fossati P, Lorenzo P. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982; 28: 2077-2080.
21. Burstein M, Scholnick HR, Morgin R. Rapid method for the isolation of lipoprotein from human serum by precipitation with polyanion. J Lipid Res 1970; 11: 583-595.
22. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.
23. Jeppesen S, Gregersen KK, Alstrup, Hermansen K. Stevioside induce antihyperglycaemic, insulinotropic and glucagonostatic effects in vivo: studies in the diabetic Goto-Kakizaki (GK) rats. Phtomedicine 2002; 9: 9-14.
24. Vies JVD. Two methods for the determination of glycogen in liver. J Biochem 1954; 57: 410-416.
25. Andallu B, Varadacharyulu NC. Antioxidant role of mulberry (Morus indica L. cv. Anantha) leaves in streptozotocindiabetic rats. Clinica Chemica Acta 2003; 338: 3-10.
26. Bhattacharya S, Dey D, Roy SS. Molecular mechanism of insulin resistance, J Biosci 2007; 32: 405-413.
27. Mohammadi J, Naik PR. Anti-diabetic effects of Morus alba in experimentally induced type 2 diabetes in wistar rat. Biomedcine 2008; 28: 112-116.

28. Babu V, Gangadevi T, Subramoniam A. Antidiabetic activity of ethanol extract of Cassia kleinii leaf in streptozotocin induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. Indian J Pharmacol 2003; 35: 290-296.
29. Sezik E, Aslan M, Yesilada E, Ito S. Hypoglycemic activity of Gentiana olivieri and isolation of an active constituent through bioassay direct fractionation techniques. Life Sci 2005; 76: 1223-1238.
30. Prajapati DD, Patel NM, Savadi RV, Akki KS, Mruthunjaya K. Alleviation of alloxan-induced diabetes and its complications in rats by Actinodaphne hookeri leaf extract. Bangladesh J Pharmacol 2008; 3: 102-106.
31. Jarald EE, Joshi SB, Jain, DC. Antidiabetic activity of aqueous extract and non-polysaccharide fraction of Cynodon dactylon pers. Ind J Exp Biol 2008; 46: 661-667.
32. Mohammadi J, Naik PR. Evaluation of hypoglycemic effect of Morus alba in an animal model. Indian J Pharmacol 2008; 40: 15-18.