Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (11):114-120
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Effect of single and combinational herbal formulation in alloxan induced hyperglycemia

Bhagyabhumi Patel, Jigar Patel and Samir Shah*

Department of Pharmacology, Sardar Patel College of Pharmacy, Bakrol-388315, Dist-Anand, Gujarat, India

---

ABSTRACT

Diabetes mellitus can be defined as a group of metabolic diseases characterized by chronic hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. The present work was aimed to evaluate the effect of single and combinational herbal formulation containing leaf extract of Leea indica and fruit & leaf extract of Lagerstroemia speciosa for antihyperglycemic activity. Leaves of Leea indica, leaves & fruits of Lagerstroemia speciosa were collected, authentified and extracted with 70% methanol and coded as LIE (Leea indica extract) LSE (Lagerstomia speciose extract). A formulation containing both the extract was coded as LLF (Leea indica& Lagerstomia speciose Formulation). Extracts were subjected to preliminary phytochemical study. Wistar rats were injected with Alloxan 120 mg/kg intraperitoneally for the induction of diabetes. After confirming the Diabetes induction, rats were randomly divided into six groups: Normal control, diabetic control, STD (standard injected with NPH insulin 1 unit s.c.), LIE treated (200 mg/kg, p.o.), LSE treated (200 mg/kg, p.o.) and LLF treated (300 mg/kg, p.o.) for 28 days. Various physical parameters (Body weight, Food intake, Fluid intake, urine output) and biochemical parameters such as Serum glucose, Creatinine, Urea and Protein levels were measured. Statistical analysis was carried out by ANOVA followed by Dunnett's post hoc test.Preliminary phytochemical investigation revealed the presence of alkaloids, glycosides, carbohydrates, saponins and flavonoids in LIE & LSE. Rats treated with STD, LLE, LSE, LLF forbaded the decrease in body weight, showed significant ($p<0.05$) reduction in food & fluid intake, urine output, blood glucose, serum urea & creatinine levels and significant increase in serum protein level as compared to diabetic rats. Leea indica, Lagerstroemia speciosa plant extracts and herbal formulation containing Leea indica and Lagerstroemia speciosa (1:2) possesses anti-diabetic activity. These may be due to presence of triterpenoid especially corosolic acid in Lagerstroemia speciosa and triterpene acid especially ursolic acid in Leea indica.

Keywords: Diabetes mellitus, *Lagerstroemia speciosa*, *Leea indica*, Alloxan, Insulin, herbal formulation

---

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high blood glucose concentration - hyperglycemia (fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or 2–h plasma glucose≥ 11.1 mmol/l (200 mg/dl)) caused by insulin deficiency, often combined with insulin resistance [1].Diabetes mellitus can be defined as a group of metabolic diseases characterized by chronic hyperglycemia, resulting from defects in insulin secretion, insulin action, or both [2]. The chronic hyperglycemia and attendant metabolic dysregulation of diabetes mellitus may be associated with secondary damage in multiple organ systems, especially the kidneys, eyes, nerves, and blood vessels [3].

DM is the metabolic disorder with the highest rate of prevalence and mortality worldwide. The World Health Organization (WHO) has predicted that the worldwide number of patients with diabetes will double by the year 2025, from the current number of approximately 150 million to 300 million. Diabetes mellitus is a major health problem in developed and developing countries. Management options for the disease in developed countries include

diet and administration of insulin and/or hypoglycaemic agents. However, these methods may not be affordable for patients in developing countries due to socio-economic conditions [4]. Currently over 30 million have now been diagnosed with diabetes in India. The CPR (Crude prevalence rate) in the urban areas of India is thought to be 9%. In rural areas, the prevalence is approximately 3 % of the total population. The population of India is now more than 1000 million: this helps to give an idea of the scale of the problem. The estimate of the actual number of diabetics in India is around 40 million. This means that India actually has the highest number of diabetics of any one country in the entire world. IGT (Impaired Glucose Tolerance) is also a mounting problem in India [5].

Chronic Complications of DM are Microvascular complications such as Eye disease, Retinopathy, Macular edema, Neuropathy and Nephropathy and Macrovascular Complications such as Coronary artery disease, Peripheral vascular disease, Cerebrovascular disease, Genitourinary Dermatologic-Infectious, Cataracts and Glaucoma [6].

Currently, three methods of treatment are available for diabetic patients: Diet, oral hypoglycemic drugs such as Sulphonylureas, Biguanides, α-glucosidase inhibitors, Thiazolidinediones, Non-sulphonylureassecretogogues (Rapaglinide and Nateglinide) and direct insulin therapy [7].However, these therapies are known to be associated with various adverse effects like Obesity, Hypoglycemia, Lactic acidosis etc. Recent target for treatment of DM is DPP-4 inhibitor, GLP-1 agonist [8,9]. Recently, there is growing interest towards herbal remedies to explore some cost effectiveness and reducing the adverse effects of the synthetic drugs.

Many herbal plants are used for the treatment of diabetes. *Azadirachta indica*, *Eugenia jambola*[10], *Andrographis paniculata* [11] and other plants has been proved to be efficient in management of diabetes [12].

Lagerstroemia speciosa (L.) Pers. known as “Banaba” is traditionally used as a herbalmedicine in the Philippines. *Lagerstroemia speciosa* has been shown to produce hypoglycemic effects in some mice models of diabetes. It has been reported that ethanolic extract of *L. speciosa* leaves possesses hypoglycemic activity in rabbit [13, 14]. It is traditionally mentioned that fruits of *L. speciosa* can be used for diabetes [15]. But it scientifically not proved. It is known to exhibit antidiabetic, antiobesity [16], and glucose transport activities through mechanisms not well defined.

Leea indica (Burm. f.) Merr. is a large evergreen shrub or small tree 1-3 indigenous to tropical Asia, Australasia, and the Pacific and grown mostly in Bangladesh, India, China, Bhutan, and Malaysia. Plant commonly known as Bendykoot berry. Plant used for, diarrhea, dysentery, colic, ulcers, skin diseases, vertigo, and headache [17]. It is traditionally mentioned that arial plant of *Leea indica* is used in treatment for diabetes [18]. But it scientifically not proved.

Thus in light of all above findings aim of the present study was to evaluate anti-diabetic activity of a methanolic extract of *Leea indica* and *Lagerstroemia speciosa*in alloxan induced diabetic rats. To study the Combine effect of plants, herbal formulation containing extract of both the plants was prepared and its antidiabetic activity was evaluated.

MATERIALS AND METHODS

1.1. Plant collection and authentication:

The fresh leaves of *Leea indica*, fruits & leaves of *Lagerstroemia fruticosa* were collected from Dang, Saputara, India and were authenticated by Dr. D. B. Patel, Anand Agriculture University, Gujarat, India. (Herbarium specimen no: SPCP/Herbarium- 122440903002/2014).

*1.2. Extraction procedure:***[19]**

The leaves of *Leea indica*, fruits & leaves of *Lagerstroemia fruticosa* were shade dried, powdered and extracted with of 70% v/v methanol. The extract was concentrated under reduced pressure to get a dry extract and was coded as LIE (*Leea indica* extract) and LSE (*Lagerstroemia speciosa* extract)and stored in air tight container.

1.3. Preparation of herbal formulation

LIE and LSE were mixed in a ratio of (1:2) and was suspended in tragacanth to obtain a herbal formulation and was coded as LLF (*Leea indica* and *Lagerstroemia speciosa* herbal Formulation).

1.4. Phytochemical Screening:

In the present study, phytochemical screening of LIE and LSE was carried out using standard procedures [20].

1.5. Animals

Wistar rats of either sex 180-220 g were used as experimental animals for the study. The animals were housed in a group of 6 rats per cage under well-controlled conditions of temperature (22 ± 2°C), humidity (55 ± 5%) and 12hrs/12hrs light-dark cycle. Animals had free access to laboratory rat chow diet and R.O. water *ad libitum*. The protocol- SPCP/IAEC/RP-015/2013 of the experiment was approved by Institutional Animal Ethics Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

1.6. Experimental Procedure: [21]

Diabetes was induced in the overnight fasted rats by a single intraperitoneal (i.p) injection of 150 mg/kg body weight of Alloxan monohydrate dissolved in normal saline. Alloxan administered animals were given 5% w/v glucose in drinking water for 24h. Diabetes was confirmed by measuring the elevated plasma glucose levels after 72 hr of alloxan administered by Glucose oxidase- Peroxidase method (GOD-POD).

The animals with serum glucose values >200 mg/dl were considered diabetic and were separated and divided into six different groups of six rats each and treated orally once a day for 28 days as follows:

Group I (NC): Normal control received food and water ad libitum
Group II (DC): Diabetic rats received food and water ad libitum
Group III (STD I): Diabetic rats treated with NPH insulin 1 unit (s.c)
Group IV (LIE): Diabetic rats treated with *Leea indica* extract 200 mg/kg (p.o)
Group V (LSE): Diabetic rats treated with*Lagerstroemia speciosa* 400 mg/kg (p.o)
Group VI (LLF): Diabetic rats treated withherbal formulation 300 mg/kg (p.o).

Several physical parameters like Body weight was measured on 0, 7th, 14th, 21st and 28th day, food intake, fluid intake and urine output was measured on daily bases. Blood samples were withdrawn from retro orbital route at 0, 7th, 14th, 21st and 28th day [22]. Serum was separated and was used for the estimation of glucose [23]. Serum separated on 28th day was used to measure kidney parameters such as serum urea [24], serum creatinine [25]and serum protein [26].

1.7. Statistical analysis:

Results represented are mean ± SEM. Statistical difference between the means of the various groups were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test with p value <0.05. Statistical analysis was done using Graph Pad Prism software, version 6.1.

RESULTS

1.8. Preliminary phytochemical screening:

Results of preliminary phytochemical investigation of LIE & LSE revealed the presence of Saponins, Carbohydrates, Glycosides, Alkaloids, Flavanoids, Tannins and Terpenes in the extract.

Table I. Effect on changes in body weight in alloxan induced diabetic rats

Groups	0 DAY	7th DAY	14th DAY	21st DAY	28th DAY
NC	195.0± 2.23	197.0± 2.56	197.0± 3.39	195.0± 3.16	197.0± 2.55
DC	199.8± 2.56	183.4± 2.24*	179.0± 7.31*	177.6± 2.54*	176.0± 2.83*
STD I 1 UNIT	189.0± 2.91	191.0± 3.67	193.0± 3.74	200.4± 5.56$^{\#}$	204.2± 6.09$^{\#}$
LIE (200 mg/kg)	186.0± 1.87	187.0± 2.56	192.0± 2.55	199.6± 1.63$^{\#}$	201.2± 4.91$^{\#}$
LSE (400 mg/kg)	201.0± 1.87	200.0± 2.23$^{\#}$	204.0± 1.87$^{\#}$	208.8± 1.85$^{\#}$	210.4± 5.49$^{\#}$
LLF (300 mg/kg)	200.4± 1.63	199.8± 3.76$^{\#}$	200.0± 4.47$^{\#}$	207.8±2.26$^{\#}$	213.6± 5.56$^{\#}$

All values are expressed as Mean± SEM for each group. Statistical analysis was carried out by One way ANOVA followed by Dunnet's-Post hoc test.

* indicates significant difference from Normal control at P<0.05

$^{\#}$ indicates significant difference from Diabetic control at P<0.05

1.9. Effect of various treatments on physical parameters

Effect on change in Body weight of rats

The changes in body weight were measured on 0, 7th, 14th, 21st, and 28th day of the study. Body weight of diabetic rats in DC group was significantly ($p< 0.05$) decreased as compared to NC group. The diabetic rats treated with STD, LIE, LSE and LLF significantly ($p< 0.05$) prevented the fall in body weight from 21st day of treatment. (Table I).

Effect on Food intake, Fluid intake and Urine output of rats

Food intake, fluid intake and urine output were significantly ($p< 0.05$) increased in DC group as compared to NC group. Treatment with STD and LIE reduced the fluid intake and food intake compare to normal. Urine output also decreased in STD group compare to NC group. Whereas, diabetic rats treated with LSE and LLF significantly ($p< 0.05$) decreased the food intake compare to DC groups animal (Table II).

Table II. Effect on food Intake, fluid Intake and urine output in alloxan induced diabetic rats

Groups	Fluid intake (ml/animal/day)	Food intake (g/animal/day)	Urine output (ml/animal/day)
NC	13.25±1.49	12.75±2.13	8.86±1.85
DC	31.80±2.40*	28.25±2.95 *	15.00±1.52 *
STD I (1 Unit)	19.75±1.75#	16.25±0.85 #	10.25±0.62 #
LIE (200 mg/kg)	18.00±1.08#	23.75±1.18 #	11.25±0.85#
LSE (400 mg/kg)	29.00±3.02	21.00±0.70#	14.50±1.04
LLF (300 mg/kg)	27.05±1.04	20.50±1.55#	15.25±1.85

All values are expressed as Mean± SEM for each group. Statistical analysis was carried out by One way ANOVA followed by Dunnet's-Post hoc test.

** indicates significant difference from Normal control at P<0.05*

 # indicates significant difference from Diabetic control at P<0.05

1.10. Effect on blood glucose level:

A marked elevation in blood glucose level was observed in diabetic control rats as compared to normal control rats. Diabetic rats treated with STD, LSE and LLF exhibited a significant ($P< 0.05$) reduction in blood glucose levels on 7th, 14th, 21st& 28th day after treatment as compared to rats of DC group, while LIE treated rats showed significant decrease in blood glucose level on 14th, 21st& 28th day of the treatment (Figure I).

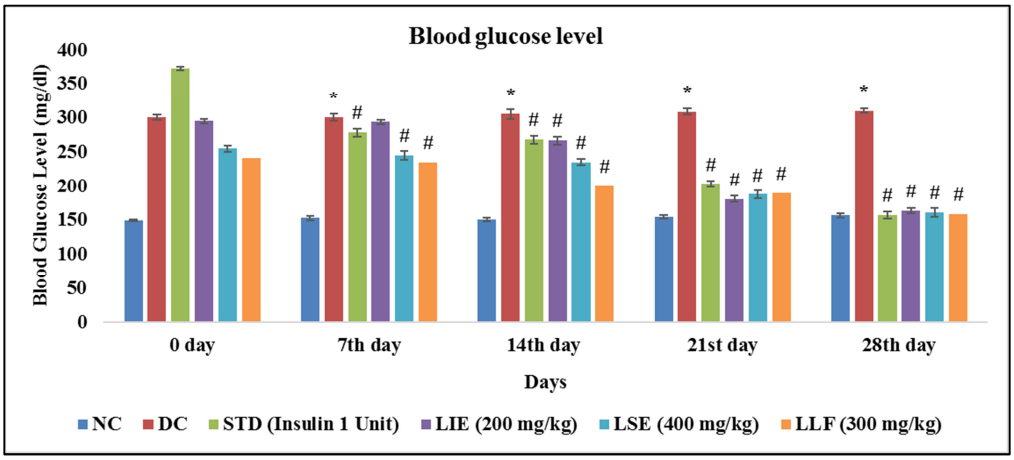

Figure I. Effect on Blood glucose level in alloxan induced diabetes in rats

All values are expressed as Mean± SEM for each group. Statistical analysis was carried out by One way ANOVA followed by Dunnet's-Post hoc test.

** indicates significant difference from Normal control at P<0.05*

 # indicates significant difference from Diabetic control at P<0.05

1.11. Effect of various treatments on kidney function parameters:

Serum protein was significantly ($P< 0.05$) decreased in DC group compared to NC group. Diabetic rat treated with STD, LIE, LSE & LLF significantly increased Serum protein as compared to DC group. Whereas, Serum creatinine & protein was significantly ($P< 0.05$) increased in DC group as compared to NC group. STD, LIE, LSE & LLF treated rats showed significant reduction in Serum creatinine & protein levels as compared to DC group (Table III).

Table III. Effect on kidney function parameters in alloxan induced diabetic rats:

GROUPS	SERUM PROTEIN (g/dl)	SERUM CREATININE (mg/dl)	SERUM UREA (mg/dl)
NC	6.33±0.46	0.86±0.08	30.05±1.15
DC	3.95±0.07 *	1.56±0.05 *	41.67±1.85 *
STD I (1 Unit)	6.60 ±0.32#	0.78±0.06 #	29.27±2.30#
LIE (200 mg/kg)	7.46±0.24#	0.67±0.04#	30.97±2.82#
LSE (400 mg/kg)	6.93±0.32#	0.94±0.02#	37.50±1.00
LLF (300 mg/kg)	6.86±0.31#	0.74±0.06#	33.83±0.60#

All values are expressed as Mean± SEM for each group. Statistical analysis was carried out by One way ANOVA followed by Dunnet's-Post hoc test.

** indicates significant difference from Normal control at P<0.05*

 # indicates significant difference from Diabetic control at P<0.05

DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted[27].
Diabetes mellitus causes failure to use glucose for energy that leads to increase utilization and decrease storage of protein responsible for reduction of body weight essentially by depletion of body proteins [28].

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing ß cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "alloxan diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic ß cells because it preferentially accumulates in ß cells through uptake via the GLUT2 glucose transporter [29].

Insulin enhances fat storage (lipogenesis) and prevents the metabolization of fat for energy, it decreases the breakdown of fatty acids to ketone bodies. In diabetes due to distruction of ß cell glycogenolysis, gluconeogenesis, lipolysis increases which leads to decrease in body weight [30]. Results of present study supported the fact and diabetic rats showed decrease in body weight. Oral administration of STD, LIE, LSE and LLF to the diabetic rats significantly (P<0.05) forbaded the fall in body weight. This indicates that the LIE, LSE, LLF prevented the muscle tissue damage in diabetic condition. An increase in body weight can be related to the improvement in insulin secretion and glycemic control. Similar kind of effect i.e. body weight gain was previously reported with other plants, such as*Aavaraiyathi churnam* well known for their antidiabetic activity[31].

Alloxan (150 mg/kg, i.p) in adult rats produced cardinal signs of type-1 diabetes i.e., loss of body weight, polyphagia, and polydipsia. The loss of body weight in diabetic animals could be related to insulin deficiency since insulin is major anabolic hormone in the body. Its deficiency not only affects the glucose metabolism but also affects the protein and fat metabolism [32] In present study, data suggested significant increase in fluid intake, food intake and urine output in alloxan induced diabetic rats as compared to normal control rats. Treatment with STD, LIE, LSE, LLF significantly reversed this condition. Thus, treatment with STD, LIE, LSE, LLF has beneficial effect in improving the polydipsia and polyphagia in diabetic rats.

Earlier studies also indicated that many herbal extract do possess the potential to regenerate the damaged pancreatic ß cells. e.g Oral administration of extracts of A*llium sativum* bulbs juice and oil of garlic enhanced the insulin secretion in diabetic animals [33, 34].These suggest the role of herb in present study may be regenretion of pancreatic ß cells. Polyherbal fomulation containing *Curcuma longa, Coscinium fenestratum, Strychnos potatorum, Tamarindus indica, Tribulus terrestris and Phyllanthus reticulants* decreased the blood sugar level in diabetic rats [35]. Similar effect on blood glucose level was observed in present study, diabetic rats treated with STD, LIE, LSE, LLF exhibit significant decreased in blood glucose level, depicting its antihyperglycemic activity.

Diabetes mellitus is an endocrine disorder in which metabolic dysregulation occur thus produce kidney dysfunction. Excessive catabolism of proteins and fats leads to decrease in protein levels and increase in serum urea and creatinine levels in diabetic condition. Gandhi et al. in 2012 reported that there was significant increase in serum creatinine and urea levels and significant decrease in protein levels indicated impaired renal function in diabetic animals [36]. Diabetic rats in the present study showed similar results. While STD, LIE, LSE, LLF treated rats showed significantly reduction in serum creatinine & urea level and elevation in serum protein level.

Plants contain various phytoconstituents like alkaloids, terpenoids, flavanoids, glycosides etc. that are frequently implicated as having antidiabetic effect. Such constituents were present in *Leea indica* and *Lagerstroemia speciosa* plant extract, thus its antidiabetic activity may be due to presence of such constituents.

CONCLUSION

The results of the present study indicated that single as well as combinational herbal formulation containing *Leea indica*and *Lagerstroemia speciosa* plant extracts possesses significant antidiabetic activity against alloxan induced diabetic rats. Thus justifies the traditional use of these plants in the treatment of diabetes mellitus. Further investigation can be carried out to establish the exact mechanism of action.

Acknowledgment
We are thankful to Sardar Patel College of Pharmacy for providing necessary facilities during research work. We would also like to thank our rats for sacrificing their lives for the betterment of society.

REFERENCES

[1] American Diabetes Association. Diagnosis and classification of diabetes mellitus, Diabetes Care **2012**;35(27): S72.
[2] M Muthulingam.*Int. J. Pharm. Biomed. Res.*,**2010,** 1(2), 28-34.
[3] V Kumar; AK Abbas, Fausto Nelson. Mitchell Richard. Robbins Basic Pathology, 8thed., New York;Elsevier Science Health Science div, **2007**.
[4] DR Whiting; L Guariguata; C Weil;J Shaw. *Diabetes Res. Clin. Pract.*, **2011**,94, 11-21.
[5] V Mohan; CS Shanthirani; M Deepa; R Deepa;RI Unnikrishnan; M Datta. J. Assoc. Physicians India, **2006**, 54, 113-7.
[6] W Roger, W Cate. Clinical pharmacy and therapeutics.4th ed.,Philadelphia, **2007.**
[7] HP Rang, MM Dale, JM Ritter, PK Moore PK. Pharmacology 5th ed., Elsevier Science Limited, New Dehli,**2003.**
[8] B Ahren B;M Landin-Olsson;PA Jansson.*J. Clin. Endocrinol.*Metab., 2004, 89, 2078-4.
[9] Standards of Medical Care in Diabetes—2012.March **2014**Available from: URL:http://care.diabetesjournals.org/content/35/Supplement_1/S11.
[10] JK Grover JK;V Vats;SS Rathi.*J. Ethnopharmacol*.,**2000**, 73, 461- 470.
[11] KR Nalamolu.*Iranian J. Pharmacol & therap.*,**2006**, 5(1), 47-50.
[12] PK Mukherjee;K Maiti; PJ Houghton. *J. Ethnopharmacol.*,**2006**, 106, 1-28.
[13] SM Shareef;I Sridhar;S Mishra;Y Venkata. *Int J Med Res Helth Sci*., **2012**, *2(2)*,217-222.
[14] V William V;A Judy.*Journal of Ethnopharmacology*,**2003**,*87*,115-117.
[15] CP Khare. Indian Medicinal Plants An Illustrated Dictionary; Springer Science, Business Media, New Delhi, **2007**, pp 358-367.
[16] G Klein; J Kim; K Himmeldirk; Y Cao; X Chen.*Evid Based Complement Alternat Med.*, **2009**, 4(4), 401-7.
[17] P Swati; S Dibyajyoti. *Asian J. Res. Pharm. Sci.*,**2012**, 2(4),137-139.
[18] T Pullaiah;KC Naidu.Antidiabetic Plants in India and Herbal Based Antidiabetic Research.Daya Publishing House, NewDelhi, **2003**.
[19] MO Raihan. *DrugDiscov Ther*.,**2011**, 5(4), 185-189.
[20] V Maithili;S Dhanabal;S Mahendran;R Vadivelan. *Indian J. Pharmaco.*, **2011**, 43(4), 455–459.
[21] S Jyotsna;K Poonam. *J. Ethnopharmacol.*,**2009**,123, 22–26.
[22] First report of the Joint Working Group on Refinement Removal of blood from laboratory mammals and birds. *Lab Anim*. **1993**, 27, 1-22
[23] P Trinder. *Ann. Clin Biochem*.,**1969**, *6*, 24-7.
[24] JK Fawcett; JE Scott.*J. Chim. Pathol*., **1960**, 13,156.
[25] RW Bones. *J. Bio Chem*.,**1945**, 158, 581.
[26] AG Gornall;CJ Bardawill;MM David. *J. Biol. Chem*.,**1949**, *177*, 751-66.
[27] S Vivek; Skumar;H Kumar. *International J Pharmaceutical science review & research*.,**2010**, *1(4)*, 18–22.
[28] G Tirtha;M Kumar;S Pinaki;B Anindya.*Iranian Journal of Pharmaceutical Res.*,**2008**, *7(1)*, 61-68.
[29] S Lenzen. *Diabetologia*, **2008**,*51(2)*, 216-226.
[30] S Leon;FS Paul;H Allan;N Larry. Comprehenshive pharmacy review; 7th ed., New delhi, wolters Kluwer, **2009**, pp1167-78.
[31] N Anbu; MD Musthafa; V Velpandian. *International Journal of Toxicological and Pharmacological Research*.**2012-13**,*4(4)*,77-80.
[32] B Kameswararao; M Kesavulu; C Apparao.*Fitoterpia*,**2003**, 74, 7.
[33] GR Kumar; KP Reddy;*Indian.J. Expt. Biol.*,**1999**, *37*, 662–666.

[34] F Fischbach; M Dunning. Manual of laboratory and diagnostic tests; 8th ed.,Lippincott Williams and Wilkins, **2009**,pp376.
[35] RC Jain RC;DB Konar.*Medikon*,**1977**, 6, 15–18.
[36] RG Gandhi; P Sasikumar. *Asian pac. J. Trop. Biomed.*,**2012**, 2, 281-86.