

Antidiabetic and Antihyperlipidemic Effect of *Alstonia scholaris* Linn Bark in Streptozotocin Induced Diabetic Rats

Deepti Bandawane^{1*}, Archana Juvekar², Manasi Juvekar³

Department of Pharmacology, P.E.Society's Modern College of Pharmacy, Nigdi, Pune 411 044.^{1*}

Department of Pharmacology, University Institute of Chemical Technology, Matunga, Mumbai - 400 019.²

Shri Bhagwan Homeopathic Medical College, CIDCO, Aurangabad – 431 003.³

ABSTRACT

Submitted: 31/5/2010

Revised: 02/8/2010

Accepted: 26/10/2010

The objective of the present work is to study the antidiabetic and antihyperlipidemic effect of aqueous extract of *Alstonia scholaris* Linn bark in streptozotocin (STZ) induced diabetes in rats. The diabetes was induced by single dose of STZ (65 mg/kg) in citrate buffer, while the normal control group was given the vehicle (citrate buffer) only. After three days of induction of diabetes, the diabetic animals were treated further four weeks with aqueous extract of *Alstonia scholaris* bark (150 mg/kg and 300 mg/kg) and glibenclamide (4 mg/kg). Blood glucose estimation was performed every week of the study. At the end of study period, animals were sacrificed for biochemical studies. STZ-induced diabetic rats showed marked hyperglycemia, hypertriglyceridemia and hypercholesterolemia at the end of study period. Body weight and liver glycogen levels were reduced and glycosylated haemoglobin levels were significantly increased in diabetic rats. The four week treatment with aqueous extract of *Alstonia scholaris* bark (150 mg/kg and 300 mg/kg) significantly ameliorated the alterations in fasting blood glucose, serum triglyceride, serum cholesterol, liver glycogen, glycosylated haemoglobin and body weight in diabetic rats. Thus the present study suggested the potential of *Alstonia scholaris* bark in diabetes as well as related cardiovascular complications due to its antidiabetic and antihyperlipidemic properties.

KEY WORDS: Streptozotocin, *Alstonia scholaris*, Antidiabetic, Antihyperlipidemic, Antiatherogenic.

INTRODUCTION

Diabetes mellitus is a common endocrine disorder caused due to either deficiency in insulin production or due to ineffectiveness of the insulin produced. Such a deficiency results in impaired metabolism of glucose and other energy-yielding fuels like lipids and proteins¹. The metabolic disturbances contributes massively to most neurological, cardiovascular, retinal and renal diabetic complications². The estimation that diabetes mellitus will affect more than 300 million people by the year 2025 shows the need for improvement in the treatment aspect of this chronic disorder³. Currently available pharmacotherapy for the treatment of diabetes mellitus include oral hypoglycemic agents and insulin. However these current drugs do not restore normal

glucose homeostasis and they are not free from side effects⁴. Moreover due to high cost of allopathic drugs it is difficult to provide modern medical healthcare especially in developing countries. It is therefore become necessary to make use of vast reserves of plant origins for medical purposes which will help to search effective as well as safer drug remedy for diabetes mellitus.

Plant *Alstonia scholaris* (Linn.) belonging to family apocynaceae is commonly known as Saptaparna. The plant is native to India and grows in deciduous and evergreen forests and also in plains. The bark is useful in malarial fevers, abdominal disorders, dyspepsia and in skin diseases⁵. The bark is bitter, astringent, digestive, laxative, anthelmintic, antipyretic, stomachic, cardiogenic and tonic⁶. The bark extract has been reported to possess antiplasmodial, immunostimulant, anticancer effect and is also hepatoprotective^{7,8}. In Ayurveda, it is reported that the bark of the plant when soaked in water overnight, can reduce the blood glucose level after oral administration⁹ however no

*Address for Correspondence:

Deepti Bandawane, Department of Pharmacology, P.E.S. Modern College of Pharmacy, Nigdi, Pune - 411 044.

E-mail: rspatil_pharma@yahoo.co.in

much characterization of this activity has been done on scientific basis. We therefore subjected the aqueous extract of bark of *Alstonia scholaris* L. to preliminary phytochemical investigation which showed presence of alkaloids, tannins, flavonoids, saponins, glycosides and triterpenoids. The phytochemicals are indicative of its potential in the treatment of diabetes mellitus hence we undertook the present work to study the chronic antidiabetic effect and antihyperlipidemic effect of the bark extract in healthy and streptozotocin diabetic rats with the objective to focus on mechanism underlying the activity.

MATERIALS AND METHODS

Plant material

Dried bark of *Alstonia scholaris* L. were procured from local market of Pune and were authenticated by Dr. A. M. Mujumdar, Agharkar Research Institute, Pune (Auth. 08-134).

Preparation of extract

The dried bark of *Alstonia scholaris* L. was subjected to size reduction to a coarse powder by using dry grinder. The powdered material was subjected to extraction by maceration using water as solvent. The extract was concentrated under vacuum to get residue. The residue was dried in vacuum desiccator and stored in air tight container in refrigerator. Various concentrations of the bark extracts were prepared freshly everyday using distilled water for the period of 28 days.

Chemicals

Streptozotocin (STZ) was purchased from Sigma chemicals, Germany. Glucose, triglycerides, total cholesterol and HDL-cholesterol kits were purchased from Span Diagnostics, Gujarat. All other chemicals used in the study were of analytical grade.

Animals

Male wistar rats (150-200gm) were obtained from Yash Pharma, Pune. Before and during the experiment rats were fed with standard diet (Amrut Foods Ltd.). After randomization into various groups and before initiation of the experiment, the rats were acclimatised for a period of 7 days under standard environmental conditions of temperature, relative humidity and dark/light cycle. Animals stated as fasting were deprived of food and water for 16 hrs. ad libitum. All the procedures were performed in accordance to Institutional Animal Ethical Committee.

Acute and short term toxicity study

The aqueous extract of *Alstonia scholaris* L. bark was tested for its acute and short term toxicity in mice. To determine acute toxicity of the drug, overnight fasted wistar albino mice were orally fed with extract in increasing dose levels of 100, 500, 1000, 3000 and 5000 mg/kg body weight. The mortality and general behavior of the animals were observed periodically for 48 h. The animals were observed continuously for the initial period of 4 h, intermittently for the next 6 h, then again at 24 h and 48 hrs following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex and convulsions^{10,11}.

Determination of test dose

During preliminary toxicity study, no adverse effects or mortality was observed in experimental animals with oral administration of bark extract up to a high dose of 5 gm/kg body weight observed for 24 hrs. Hence submaximal doses of 150 mg/kg and 300 mg/kg were selected as a test dose.

Experimental induction of diabetes

Diabetes was induced by using streptozotocin as diabetogenic agent. Streptozotocin (65 mg/kg) was dissolved in ice cold citrate buffer (pH 4.3) immediately before use. The solution was injected intraperitoneally in the dose of 65 mg/kg in rats¹². 5 % glucose solution was administered orally for 24 hrs. to prevent mortality due to initial hypoglycemia induced by streptozotocin. After 72 hrs. of STZ injection, fasting blood glucose levels were tested using glucose oxidase-peroxidase reactive strips (Accu-chek, Roche Diagnostics, USA). Rats showing fasting blood glucose more than 200 mg/kg were considered diabetic and used for further study.

Experimental Groups

Rats were divided in different groups as follows.

Group I: as normal control where rats received citrate buffer daily.

Group II: as diabetic control where diabetic rats received citrate buffer daily.

Group III: diabetic rats received 150 mg/kg aqueous extract of bark of *Alstonia scholaris*

Group IV: diabetic rats received 300 mg/kg aqueous extract of bark of *Alstonia scholaris*

Group V: diabetic rats received 4 mg/kg of glibenclamide, an oral hypoglycemic agent.

Group VI: normal rats received 150 mg/kg aqueous extract of bark of *Alstonia scholaris*

Group VII: normal rats received 300 mg/kg aqueous extract of bark of *Alstonia scholaris*

Experimental procedure

Blood glucose estimation

Fasting blood glucose levels were determined in all experimental rats initially to determine the diabetic status and thereafter every week during the 28 day study period. Blood was obtained by snipping tail of rat with the help of sharp razor and blood glucose levels were determined using glucometer (Ultra Touch Two, Johnson and Johnson). Each time the tail of the rat was sterilized with spirit.

Serum lipid profile estimation

At the end of 28 days, blood was collected from inferior vena cava, serum separated for determination of parameters like total cholesterol, HDL- cholesterol and triglycerides using commercially available kits (Span diagnostics). VLDL cholesterol and LDL-cholesterol were calculated using the Friedewald's formula¹³.

$$\text{VLDL} = \text{Triglycerides} / 5$$

$$\text{LDL} = \text{Total cholesterol} - (\text{HDL-CH} + \text{VLDL-CH})$$

Liver glycogen estimation

Liver of individual animal was homogenized in 5% w/v trichloroacetic acid and its glycogen content were determined by the method of Carrol¹⁴.

Glycosylated haemoglobin determination

At the end of 28 days, blood was collected from retro-orbital

plexus and subjected for the determination of glycosylated haemoglobin.

Statistical analysis

All results are expressed as the mean \pm SEM. The results were analysed for statistical significance by one way ANOVA followed by Dunnet's Multiple Test for comparison.

RESULTS

The effect of STZ and plant extracts on blood glucose level is shown in Table 1. On repeated administration of aqueous extract of *Alstonia scholaris* L. for 28 days, a sustained and significant ($p < 0.01$) decrease in blood glucose level of diabetic rats was observed in dose dependent manner as compared to diabetic control group. In diabetic rats blood glucose level was reduced by 30.3% and 45.79% at 150 and 300 mg/kg doses of the extract respectively. The standard oral hypoglycemic drug glibenclamide (4 mg/kg) showed more potent antidiabetic activity by reducing blood glucose level by 59.25% as compared to diabetic control group. However there was no significant effect of the extract on the blood glucose level of normoglycemic rats.

As shown in Table 2, STZ diabetic rats treated with extract showed significant ($p < 0.01$) reduction in the elevated levels of total cholesterol and triglycerides in diabetic rats. Chronic treatment of extract (300 mg/kg) and glibenclamide (4 mg/kg) reduced the LDL-cholesterol by 53.79% and 64.13% respectively as compared to diabetic control group. Also the extract significantly ($p < 0.01$) improved the HDL-cholesterol level at 300 mg/kg. In addition the bark extract in dose of 300 mg/kg showed significant ($p < 0.01$) reduction in atherogenic index as comparable to glibenclamide (4 mg/kg).

Table No. 1 - Effect of chronic administration (28 days) of aqueous extract of *Alstonia scholaris* L. bark on fasting blood glucose in STZ diabetic rats.

Experimental Groups	Fasting Blood Glucose (mg%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	78.16 \pm 4.96	71.16 \pm 3.06	74.16 \pm 3.11	70.33 \pm 3.07	73.5 \pm 3.47
Diabetic control	297.5 \pm 5.68	308.66 \pm 8.08	300.66 \pm 7.7	292.66 \pm 5.9	297.83 \pm 5.25
Diabetic + 150 mg/kg AEAS	304.5 \pm 8.42	267.5 \pm 10.11*	238.5 \pm 6.73**	222.16 \pm 7.12**	207.16 \pm 7.24**
Diabetic + 300 mg/kg AEAS	292.5 \pm 7.36	248.83 \pm 5.91**	223.5 \pm 6.21**	186.33 \pm 8.86**	161.16 \pm 8.6**
Diabetic + GL(4mg/kg)	295.16 \pm 6.63	219.16 \pm 6.11**	195.16 \pm 6.96**	149 \pm 7.88**	121.5 \pm 7.51**
Normal + 150 mg/kg AEAS	75.16 \pm 3.82	72.5 \pm 2.75	76.66 \pm 4.2	78.16 \pm 4.96	74.16 \pm 3.11
Normal + 300 mg/kg AEAS	74.16 \pm 3.47	76.33 \pm 4.43	75.33 \pm 3.66	72.33 \pm 3.42	75.66 \pm 4.52

n=6, * $p < 0.05$, ** $p < 0.01$. Values are mean \pm S.E.M., AEAS: Aqueous extract of *Alstonia scholaris* bark; GL: Glibenclamide, Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet 's Multiple Test for comparison.

Table No. 2 - Effect of chronic administration (28 days) of aqueous extract of *Alstonia scholaris* L. bark on lipid profile in STZ diabetic rats.

Experimental Groups	T-CH	TG	HDL	VLDL	LDL	Atherogenic index
Normal control	80.2±2.77	66.5±1.91	28.8±1.01	13.3±0.38	38±2.6	1.79±0.12
Diabetic control	198±2.5	169.2±2.33	18.8±0.7	33.8±0.47	145.3±2.79	9.96±0.45
Diabetic+150 mg/kg AEAS	143.3±3.42**	130.8±2.14**	22.2±1.58 ^{ns}	26.2±0.43**	95±2.71**	5.63±.039**
Diabetic+300 mg/kg AEAS	110.3±2.36**	90.6±1.2**	24.3±0.91**	18.1±0.29**	67.8±2.84**	3.56±0.22**
Diabetic+GL(4mg/kg)	93.3±1.23**	74.2±1.14**	26±0.85**	14.8±0.27**	52.4±2.22**	2.62±0.18**
Normal+150 mg/kg AEAS	78.2±1.64	70.6±1.45	30.3±1.28	14.1±0.3	33.7±1.56	1.59±0.09
Normal+300 mg/kg AEAS	82.3±2.06	65.5±2.17	28.2±2.12	13.1±0.43	41±1.11	1.97±0.13

n=6, **p<0.01, ns: non-significant. Values are mean ± S.E.M., AEAS: Aqueous extract of *Alstonia scholaris* bark, GL: Glibenclamide, T-CH: Total cholesterol, TG: Triglycerides, HDL- CH: High density lipoprotein cholesterol, LDL-CH: Low density lipoprotein cholesterol, VLDL- CH: Very low density lipoprotein cholesterol. Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnett 's Multiple Test for comparison.

Table No. 3 - Effect of chronic administration (28 days) of aqueous extract of *Alstonia scholaris* L. bark on liver glycogen and glycosylated haemoglobin in STZ diabetic rats.

Experimental Groups	Liver glycogen (g/100gm)	Glycosylated haemoglobin (%)
Normal control	3.5±0.1	5.38±0.21
Diabetic control	0.83±0.01	8.53±0.2
Diabetic + 150 mg AEAS	1.9±0.07**	7.86±0.11**
Diabetic + 300 mg AEAS	2.46±0.11**	6.91±0.21**
Diabetic + GL (4 mg/kg)	3.1±0.1**	6.13±0.25**
Normal + 150 mg/kg AEAS	3.6±0.14	5.73±0.25
Normal + 300 mg/kg AEAS	3.42±0.12	5.28±0.21

n=6, **p<0.01, ns: nonsignificant. Values are mean ± S.E.M., AEAS: Aqueous extract of *Alstonia scholaris* bark, GL: Glibenclamide, Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnett 's Multiple Test for comparison.

Table No. 4 - Effect of chronic administration (28 days) of aqueous extract of *Alstonia scholaris* L. bark on body weight in STZ diabetic rats.

Experimental Groups	Body Weight (G)	
	Initial	Final
Normal control	197.7±5.1	243.7±4.72
Diabetic control	203.2±5.24	158.7±4.16
Diabetic + 150 mg AEAS	202.7±3.74	170.8.58 ns
Diabetic + 300 mg AEAS	205.0±5.1	185.16±2.77**
Diabetic + GL (4 mg/kg)	207.7±6.37	210.3±6.88**
Normal + 150 mg/kg AEAS	205.5±4.28	250.7±5.81
Normal + 300 mg/kg AEAS	210.3±6.88	240.7±6.58

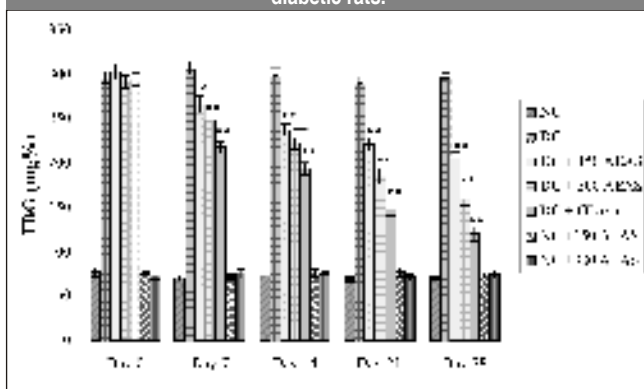
n=6, **p<0.01, ns: nonsignificant. Values are mean ± S.E.M., AEAS: Aqueous extract of *Alstonia scholaris* bark, GL: Glibenclamide, Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnett 's Multiple Test for comparison.

As shown in Table. 3, there was a significant elevation in the blood glycosylated haemoglobin and a decrease in liver glycogen levels in STZ diabetic rats as compared to normal rats. Oral administration of the extract (300 mg/kg) significantly (p<0.01) restored the increased glycosylated

haemoglobin and decreased liver glycogen level in streptozotocin-diabetic rats as comparable to glibenclamide.

As shown in Table 4, STZ diabetic rats showed significant (p<0.01) reduction in body weight from 203.2 g to 158.7 g as compared to normal group. Oral administration of aqueous

Fig. 1: Effect of chronic administration (28 days) of aqueous extract of *Alstonia scholaris* L bark on fasting blood glucose levels in STZ diabetic rats.



n=6, *p<0.05, **p<0.01. Values are mean \pm S.E.M., AEAS: Aqueous extract of *Alstonia Scholaris* bark. NC: Normal control; DC: Diabetic control; GL: Glibenclamide. Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Test for comparison.

extract of bark of *Alstonia scholaris* (300 mg/kg) significantly ($p<0.01$) and periodically improved the body weight after 28 days as compared to diabetic control.

DISCUSSION

Diabetes mellitus patients in India are increasing day by day probably due to change in lifestyle, change in food pattern i.e. from traditional fiber rich diet to sugary fast food diet and also because of genetic basis. The disorder being chronic in nature needs long term treatment to prevent the complications arising due to persistent high blood glucose level¹⁵. Pharmacotherapy available for the treatment of diabetes in modern healthcare system include insulin and oral hypoglycemic drugs¹⁶. However due to economic constraints, it is not possible for majority of the diabetic patients in developing countries like India to use these drugs on regular basis. Moreover these synthetic antidiabetic drugs are associated with large number of adverse effects. Hence there is increase in the trend to use traditional indigenous plants widely available in India for the treatment of diabetes mellitus. Over 150 plant extract and some of their active principles including flavonoids, tannins, alkaloids etc are used for the treatment of diabetes¹⁷. However very few of these plants have been screened pharmacologically¹⁸.

Streptozotocin induced diabetes in experimental animals is a valuable model for induction of diabetes mellitus. Diabetes mellitus induced by streptozotocin may be due to pancreatic beta cell destruction¹⁹. However the animals survived without insulin treatment and showed improvement by glibenclamide which act by stimulating residual beta cells of the pancreas indicate incomplete destruction of pancreatic beta cells of the diabetic rats in the present study. The model can be therefore

be considered as poorly controlled type I diabetic model showing symptoms like hyperglycemia, glycosuria, polyuria, loss of body weight inspite of polyphagia.

In the present study streptozotocin-diabetic rats exhibited significant increase in blood glucose level. Chronic treatment with aqueous extract of *Alstonia scholaris* L. bark reduced blood glucose level throughout the experimental period in duration dependent manner indicating its antihyperglycemic activity. However blood glucose levels were not altered in normoglycemic rats further strengthening the antidiabetogenic potential of the extract.

In diabetes mellitus, body cells are unable to utilize glucose as a source of energy due to which proteins are spared as energy source. This leads to decrease in protein storage which in turn reduces body weight²⁰. In the present study streptozotocin-diabetic rats show decrease in body weight throughout the experimental period. Oral treatment with aqueous extract of *Alstonia scholaris* L. bark significantly improved the body weight loss in diabetic rats as compared to diabetic control indicating possible role of the extract in restoration of protein metabolism.

In diabetic mellitus, due to persistent hyperglycemia, the excess blood glucose reacts with haemoglobin in a non-enzymatic process to form glycosylated haemoglobin. Since the glycation rate is directly proportional to blood glucose concentration, level of glycosylated haemoglobin indicates glycemic control in the diabetic state²¹. Estimation of haemoglobin is a well established parameter useful in the management and prognosis of the disorder²². In the present study, administration of aqueous extract of *Alstonia scholaris* L. bark significantly reduced the elevated glycosylated haemoglobin levels in streptozotocin-diabetic rats further substantiating its potential in long term glycemic control of diabetes mellitus.

In the present study, decrease in glycogen content in streptozotocin-diabetic rats support the findings of Grover et al.¹⁸. The decrease in glycogen content may result due to disturbances in glycogen synthetase system. Improvement in liver glycogen of diabetic rats after chronic treatment with aqueous extract of *Alstonia scholaris* L. bark indicates that possible way of antidiabetic of the extract may be by improvement of glycogenesis and / or suppression of glycogenolysis.

It has been reported that 75% of early deaths in diabetes mellitus are associated with coronary artery disease caused by abnormal lipid metabolism altering the lipid profile in

diabetic state²³. Metabolic disturbances of carbohydrates, lipids and proteins during diabetes mellitus causes insulin deficiency stimulating lipolysis in adipose tissues. Diabetes mellitus therefore leads to fatty liver, hypercholesterolemia and hypertriglyceridemia²⁴. Furthermore increased triglycerides result in increase in free fatty acid level and its oxidation which disturbs glucose metabolism as well as utilization and also impairs insulin action leading to development of hyperglycemia²⁵.

The present study showed increase in plasma triglycerides, total cholesterol and LDL cholesterol with decrease in HDL cholesterol supporting the findings of the other researchers^{23,25}. Potential of the extract to decrease cholesterol and triglyceride levels could be helpful in improving lipid metabolism in diabetics which in turn will help to prevent diabetic complications²⁶. LDL- cholesterol being involved in the transport of cholesterol from liver to peripheral tissues is the key factor in atherogenesis. Potential of the extract to reduce LDL-cholesterol thereby indicates its possible involvement in prevention of diabetes mellitus induced cardiovascular complications.

CONCLUSION

The present study showed that aqueous extract of *Alstonia scholaris* significantly reduced elevated blood glucose level in STZ diabetic rats without showing any hypoglycemic effect in normal rats. Since STZ effectively destroys pancreatic beta cells and causes persistent hyperglycemia, the mechanism of action of *Alstonia scholaris* might involve actions other than pancreatic beta cells insulin release or secretion. The antidiabetic effect of the extract could be due to increased utilization of glucose by peripheral tissues, improved sensitivity of target tissues for insulin or it may be due to improved metabolic regulation of glucose.

Our findings that *Alstonia scholaris* bark significantly reduced serum triglyceride levels in STZ diabetic rats support its long term use not only for better control of blood glucose but also for normalization of disturbances in lipid metabolism which may prevent further predisposition of the patients to cardiovascular complications.

Thus the present study showed that bark of *Alstonia scholaris* L. possesses antidiabetic and antihyperlipidemic effects in STZ diabetic rats. The antiatherogenic potential of the bark extract indicates its usefulness not only in diabetes mellitus but also in long term complications associated with diabetes mellitus. However comprehensive research is required to identify the active constituents responsible for this effect.

ACKNOWLEDGEMENTS

The authors are thankful to the Management and Principal, P.E.S. Modern College of Pharmacy, Nigdi, Pune for providing the facilities to carry out this study.

REFERENCES

- Gale EAM, Anderson JU. Diabetes mellitus and other disorders of metabolism. In: Kumar P, Clark M, editors. Clinical Medicine. 5th ed. London: WB Saunders; 2002. 1069-101.
- Mohanty P, Hamouda W, Garg R, Aljada A, Grahim H, Dandona P. Glucose challenge stimulates reactive oxygen species generation by leucocytes. J. Clin. Endocrinol. Metab. 2000; 85: 2970-3.
- King H, Aubert RE, Herman WH. Global burden of diabetes 1995-2025 prevalence, numerical estimates and projections. Diabetes Care 1998; 21:1414-31.
- Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's Pharmacology. 6th ed. Philadelphia: Churchill Livingstone Elsevier; 2007.
- Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol.- II. Dehradun: International Book Distributors; 1999.
- Nadkarni AK, Indian Materia Medica, Vol.-1. Mumbai: Bombay Popular Prakashan; 1976.
- Lin SC, Lin CC, Linn YH, Supriyatna S, Pal SL. The protective effect of *Alstonia scholaris* R.Br. on hepatotoxicity induced acute liver damage. Am. J. Clin. Med. 1996; 24: 153-64.
- Saraswathi V, Ramamoorthy N, Subramaniam S, Mathuram V, Gunaseharam P, Govindasamy S. Inhibition of glycolysis and respiration of sarcoma 180 cells by echitamine chloride. Chemotherapy 1998; 44: 198-205.
- Shah VK, Chauhan MD. Abhinav Madhume Vigyana, 1st ed. Varanasi: Chaukhamba Orientalia; 2003.
- Turner RA. Screening Methods in Pharmacology, 1st ed. New York: Academic Press; 1971.
- Arambewela LSR, Arawwawala LDAM, Ratnasooriya WD. An antidiabetic activity of aqueous and ethanolic extracts of *Piper betle* leaves in rats. J. Ethnopharmacol. 2005; 102: 239-45.
- Adaramoye OA, Adeyemi EO. Hypoglycemic and hypolipidemic effects of fractions from kolaviron, a biflavonoid complex from *Garcinia kola* in

- streptozotocin induced diabetes mellitus rats. *J. Pharma. Pharmacol.* 2006; 58:121-8.
13. Friedewald WT, Levy RI, Fradrickson DS. Estimation of concentration of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge. *Clinical Chemistry* 1972; 18: 499-502.
 14. Carrol VV, Longly RW, Joseph HR. Determination of glycogen in liver and muscle by use of anthrone reagent. *J. Biol. Chem.* 1956; 220: 583-93.
 15. Tiwari AK, Madhusudana RJ. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospectus. *Current Science* 2002; 83: 30-8.
 16. Tripathi KD. *Essentials of Medical Pharmacology*, 5th ed. New Delhi: Jaypee Publication; 2003.
 17. Erememisoglu A, Kelestimur F, Kokel AH, Utsun H, Tekol Y, Ustdal M. Hypoglycemic effect of *Zizyphus jujube* leaves. *J. Pharm. Pharmacol.* 1995; 47: 72-4.
 18. Grover JK, Vats U, Yadav S. Effect of feeding aqueous extract of *Petrocarpus mercupium* on glycogen content of the tissues and the key enzymes of carbohydrate metabolism. *Molecular Cellular Biochemistry* 2002; 241: 53-9.
 19. Kawalali G, Tuncel H, Goksel S, Hatemi HH. Hypoglycemic activity of *Urtica pilulifera* in streptozotocin diabetic rats. *J. Ethnopharmacol.* 2002; 84: 241-5.
 20. Guyton A.C., Hall J.E. *Textbook of Medical Physiology*, 9th ed. Philadelphia; WB Saunders; 1996.
 21. Monnier VK. Non enzymatic glycosylation and browning in diabetes and aging. *Diabetes* 1982; 31: 57-66.
 22. Chang AT, Nobel J. Estimation of HbA1c like glycosylated proteins in kidneys of streptozotocin diabetes and controlled rats. *Diabetes* 1979; 28: 408-415.
 23. Tattersalt R. Targets of therapy for NIDDM. *Diabetes Res. Clin. Pract.* 28 (Suppl.), 1995: 49-55.
 24. Davis SN, Granner DK. Insulin, Oral Hypoglycemic Agents and the Pharmacology of the Endocrine Pancreas. In: Hardman JG., Limberd LE, editors. *Goodman and Gillman's The Pharmacological Basis of Therapeutics*, 10th ed. USA: McGrew Hill; 2001: 1679-1714.
 25. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty acid cycle, its role in insulin sensitivity and metadisturbances in diabetes mellitus. *Lancet* 1963; 1: 785-789.
 26. Sivajyothi V, Dey A, Jaykar B, Raj Kapoor B. Antihyperglycemic, antihyperlipidemic and antioxidant effect of *Phyllanthus rheedii* on streptozotocin induced diabetic rats. *Iranian Journal of Pharmaceutical Research* 2008; 7(1): 53-9.