Vol 5, Issue 5, Sep-Oct 2018 ISSN 2349-7041

Research Article

EVALUATION OF POLYHERBAL FORMULATION FOR ITS ANTI-DIABETIC ACTIVITY AGAINST STZ INDUCED DIABETES IN RAT

VISHAKHA JAGTAP[1], PRADNYA JAGTAP[1], MANOJ JAYABHAYE[1], GAJANAN BHAGWAT[2], ASHWIN PORWAL[2], VAISHALI UNDALE[1]

[1]Department of Pharmacology, Seth Govind Raghunath Sable College of Pharmacy, Saswad, [2]Healing Hand & Herbs, Tilak Road, Pune
Email: pnj1511@gmail.com

ABSTRACT

Objective: The present work was undertaken to validate the folk use of traditional Indian medicinal plant in the treatment of Diabetes Mellitus disorder by using scientific methods. Method: In the present work, anti-hyperglycemic activity of the polyherbal formulation in STZ induced diabetic rats was carried out. The blood glucose level in STZ induced diabetic rats significantly reduced by aq. extract. **Result:** The present study showed that treatment of the polyherbal formulation at 500mg/kg dose in streptozotocin induced rats showed antihyperglycemic effect. **Conclusion:** The polyherbal formulation showed potential in their role to reduce the blood glucose level. These herbs may lead to development of more potent anti-diabetic formulation.

Keywords: Polyherbal formulation, Anti-diabetic, Streptozotocin, Blood Sugar.

INTRODUCTION

Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases.[1] It is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin.[2]Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body system, in particular the blood vessels and nerves.[3]

As the numbers of people with diabetes multiply worldwide, the disease takes an ever increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years. Regions with greatest potential are Asia and Africa, where DM rates could rise to two to three-folds than the present rates. [3]It is currently growing at a rapid rate throughtout the world, and it is the 16th leading cause of global mortality. [4]

Plants are a rich source used for centuries to cure various diseases and disorders from natural products. Hence, it is necessary to find out new formulation from natural sources that have less expensive, least side effects, on a long term therapy which can provide better safety and efficacy.

Polyherbal combination contain different herbal plant, they have reported as different pharmacological activities, the plant like *Gymnema Sylvestre, Syzygium Cumini, Momordica Charantia, Tinospora Cordifolia, Cinnamomum Zeylanicum, Plumbago Zeylanica, Asphaltum*.[5-11]

The present investigation was designed to investigate Anti-hyperglycemic activity of poly herbal formulation in Wistar Rat.

MATERIALS AND METHODS

Animals

Healthy Wistar rats of either sex weighing between 150-180 g were used for study and they purchased from APT Testing and Research Pvt. Ltd. Pune. The rats were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination housed in well ventilated Polypropylene cages under the standard laboratory condition at 22± 3°C,relative humidity 50-60%.The animal were fed with pelletized feed and water was provided *at libitum.*

The study was approved by Animal Ethical Committee of APT Research Foundation centre (Approval no: RP25/17-18).

Chemical and Instrument

Straptozotocin: Research Lab, Glibenclamide: Pharma Ltd, Triglyceride: Coral clinical system, Cholesterol: Coral clinical system, HDL-Coral clinical system, SGOT, SGPT: Coral clinical system, Oral Glucose Tolerance Test, One Touch Horizon Glucometer. Instrument name: Biochemistry analyser.

Experimental Protocol

Initially 24 rats were given Streptozotocin at a dose of 30mg/kg s.c. to undergo induction of diabetes mellitus. The surviving rats showing more than 300mg/dl blood glucose were selected for further study and they were grouped as follows:

Sr. No.	Group
Group I	Normal control group (No treatment)
Group II	Disease control group (STZ 30mg/kg s.c.)
Group III	Standard group (STZ+ Glibenclamide 10mg/kg)
Group IV	Test group (STZ+ DS-O1 500mg/kg)

After induction of diabetes, test drug was administered for the duration of 28 days to evaluate its anti-diabetic activity. During the study, weekly Glucose and body weight was monitored. On the 28th day OGTT was done and finally at the end of the study, Biochemistry (Glucose, Total Protein, Urea, Creatinine, Cholesterol and ALP) was done before sacrifice. Major organs Kidney were preserved in 10% formalin for histopathology.

Toxicological study

Acute Oral Toxicity of Aqueous extract

Acute oral toxicity was carried out according to OECD guidelines 425. Female albino mice used for the study. The limit test at single dose administration of 2000 mg/kg and 5000 mg/kg of the test drug was conducted and the main was started at 175 mg/kg and observed for 14 days.

Evaluation Parameter:

The Oral Glucose Tolerance Test (OGTT) was done in accordance with the protocol. Rats were fasted overnight and the baseline blood glucose was determined next day with the help of an automated glucometer (Accucheck active). The rats were loaded with 2gm/kg of glucose 30 minutes after administration of drug at their respective doses. The blood glucose was monitored for 30, 60 and 120 minutes thereafter.

Biochemical estimation:

For Biochemical estimation blood was collected by retro-orbital sinus puncture, under mild ether anesthesia. The collected samples were centrifuged for 15 minutes at 2500rpm.Then serum samples were collected and analyzed for serum. All estimation was done by using respective commercially available kits.

Estimation of Urea and ALP

Urea and ALP were estimated by using commercially available kit according manufactures protocol.

Statistical Analysis

The results were analysed for statistical significance by one way ANOVA and were expressed as Mean ± SEM by using Graphpad prism 5 version.

RESULT AND DISCUSSION

Effect of DS-01 on Body weight

The animals in Diabetic control group were found to be reduction (Day 14th and 21st) of body weight as compared to the Normal control. As shown in Table 1.

Effect of DS-01 on Blood glucose

After streptozotocin induction, the animals have shown significant increase in the fasting blood glucose levels ($p < 0.001$) as compared to Normal Control animals. These animals were then randomly divided into three groups viz. Disease control, Standard and Test respectively. The Glucose levels were weekly monitored in all animals. On 28th day, the glucose levels were found to be significantly decreased ($p<0.05$) in Standard group as well as Test drug treated group as compared to Disease control. The oral glucose tolerance test was performed on Day 14 and Day 28. On 28th day there was significant reduction in the glucose levels of Test group ($p<0.05$) at 1st hr as compared to Disease control animals. Also there was significant reduction in Test group ($p<0.05$) and Standard drug treated group ($p<0.05$) as compared to Disease control animals. As shown in Table 2: Figure 1.1, 1.2, 1.3, 1.4, 1.5 respectively.

Effect on Creatinin, Total protein, ALP, Urea in streptozotocin-induced diabetic rats.

In Creatinine, Total Protein, ALP and Urea, there was significant change in the Creatinine, Total protein content, and Urea levels in all groups as compared to Disease control animals. The ALP levels were found to be significantly increased ($p<0.001$) in Disease control animals as compared to Normal Control group. In Standard drug treated group the ALP levels were significantly ($p<0.01$) decreased in comparison with Disease control animals. Although there was reduction in ALP levels of Test sample treated group, the difference was not statistically significant in comparison with Disease control animals. The relative organ weight data has also shown non-significant changes in the organ weights of animals in comparison with Disease control animals.

Histopathology of Kidney

In Normal control group, the pathological changes were no abnormality detected. In DC group, the pathological change was found to be moderate. In STD group, the pathological change was found to be no abnormality detected and In Test group, the pathological change was found to be minimal.

Table 1: Effect of DS-01on body weight (0-28th day)

Group	0 day	7 day	14 day	21 day	28 day
NC	199.3	204.2	207.5	216.8	236.8
	±19.7	±20.4	±20.2	±19.9	±25.1
DC	206.5	202.3	170.0	180.0**	209.6
	±24.6	±22.4	±36.7	±25.2	±39.1
STD	192.7	179.3	174.2	186.3**	207.2*
	±18.1	±32.3	±42.8	±37.8	±43.9
TEST	200.5	208.8	203.3	215.7*	214.3*
	±16.8	±18.7	±20.3	±25.9	±29.0

Table 2: Effect of DS-01 on blood glucose (0-28th day)

Group	0 day	7 day	14 day	21 day	28 day
NC	120.7	123.5	120.8	120.2	115.2
	±2.8	±3.3	±4.4	±3.4	±7.9
DC	357	351.8	341.7	296.4	354.6
	±23.0	±31.8***	±44.5***	±43.1***	±51.9**
STD	357.7	331.3	291.7	276	275.7
	±68.6	±58.0***	±48.1***	±66.9***	±48.4***
TEST	357.7	337.5	312	218.7	276.5
	±57.2	±52.3**	±53.9***	±47.6**	±42.8**

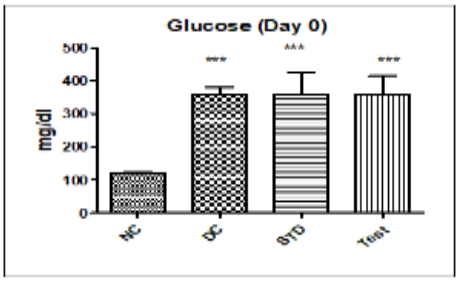

Fig 1.1 Effect of DS-01 on Blood glucose level on 0 day

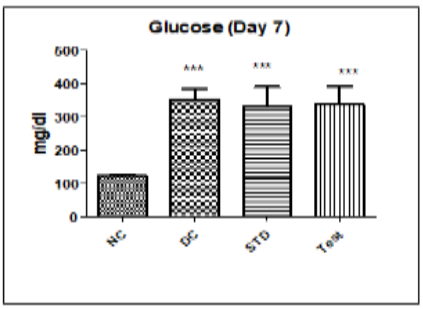

Fig1.2 Effect of DS-01 on Blood glucose level on 7th day

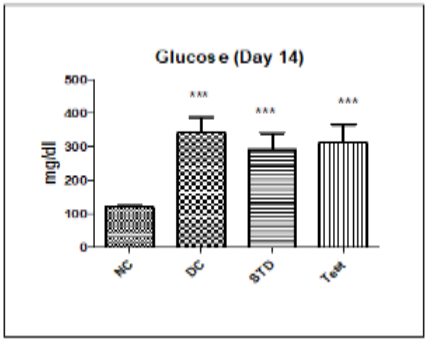

Fig 1.3 Effect of DS-01 on Blood glucose level on 14th day

Innoriginal International Journal of Sciences | Volume 5 | Issue 5 | Sep-Oct 2018 | 1-5

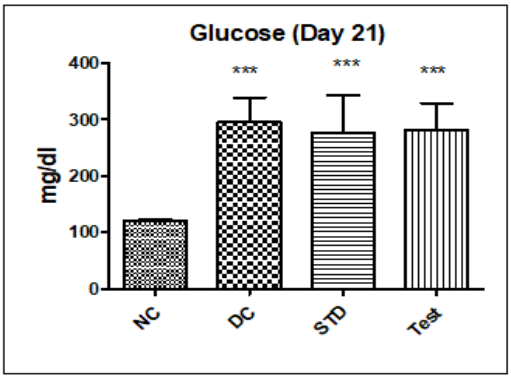

Fig 1.4 Effect of DS-01 on Blood Blood glucose level on 21st day

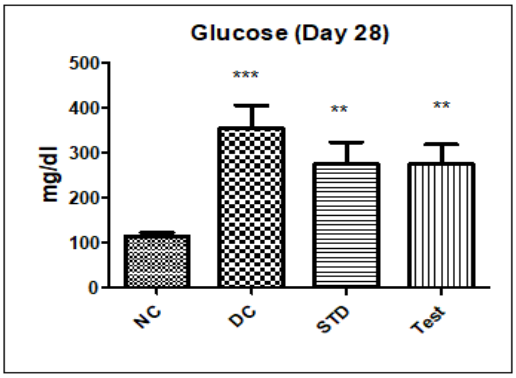

Fig 1.5 Effect of DS-01 on Blood glucose level on 28th day

Values are expressed as mean ± S.E.M. (n=6). *P<0.05 **P<0.01 ***P<0.001 when compared with diabetic group (ANOVA followed by Dunnet,s test). The glucose levels were found to be significantly decreased (P<0.05) in standard group as well as Test group as compared to Disease control.

Table 3: Effect on Creatinin, Total protein, ALP, Urea in streptozotocin-induced diabetic rats.

Group	Creatinin (mg/dl)	TP (g/L)	ALP (U/L)	Urea (mg/dl)
NC	1.1 ±0.3	6.9 ±0.6	465.8 ±122.0	38.5 ±3.7
DC	0.8 ±0.1*	7.6 ±0.4	131 ±296.4*	81.1 ±22.9*
STD	0.7 ±0.4	7.3 ±0.8	719 ±124.1	74.5 ±21.3
TEST	0.8 ±0.2*	334.7 ±67.9	7.1 ±0.7***	59.1 ±3.7**

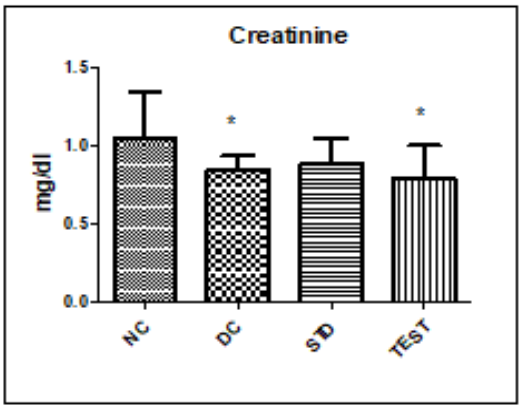

Fig 2.1 Effect of DS-01 on Creatinine

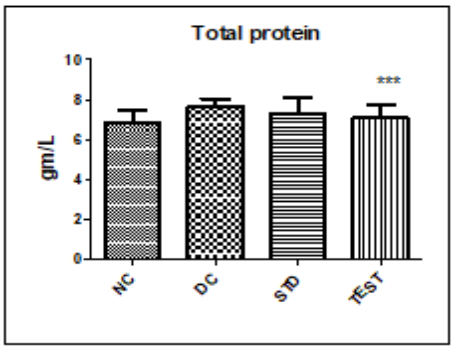

Fig 2.2 Effect of DS-01 on Total Protein

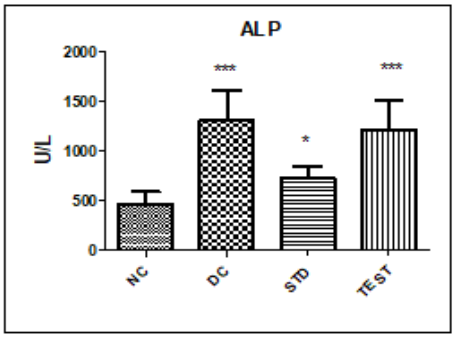

Fig 2.3 Effect of DS-01 on ALP

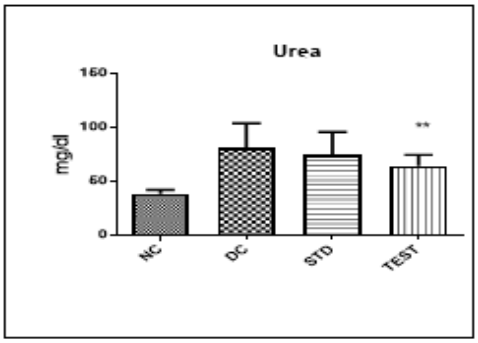

Fig 2.4 Effect of DS-01 on Urea

Values are expressed as mean ± S.E.M. (n=6). *P<0.005 **P<0.01 ***P<0.001 when compared with diabetic group (ANOVA followed by Dunnet,s test). The ALP levels were found to be significantly increased (p<0.001) in Disease control animals as compared to Normal Control group. In Standard drug treated group the ALP levels were significantly (p<0.01) decreased in comparison with Disease control animals. Although there was reduction in ALP levels of Test sample treated group, the difference was not statistically significant in comparison with Disease control animals.

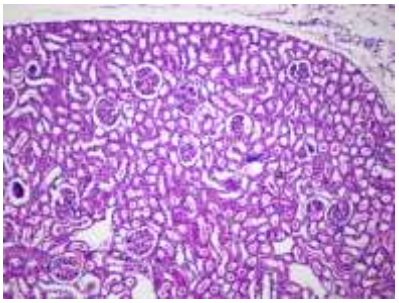

Fig 3.1: Group NC Pathological changes: NAD

Innoriginal International Journal of Sciences | Volume 5 | Issue 5 | Sep-Oct 2018 | 1-5

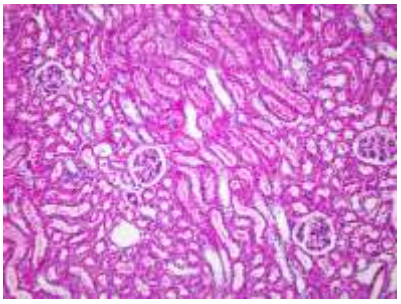

Fig 3.2 Group DC Pathological changes: Moderate (+++)

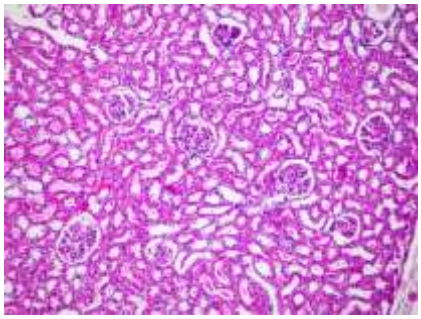

Fig 3.3: Group STD Pathological changes: NAD

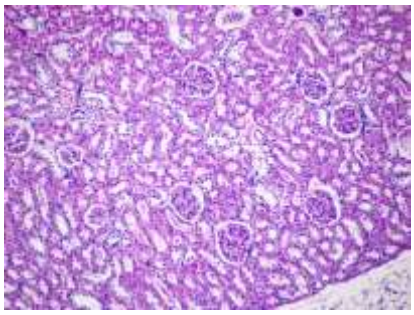

Fig 3.4 Group Test Pathological changes: Minimal (+)

Fig 4 Histopathology of Pancreas

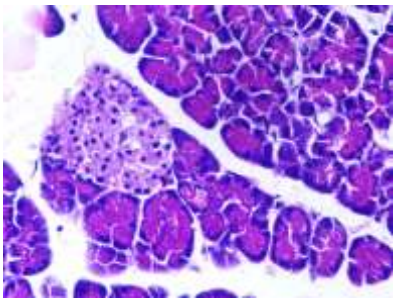

Fig 4.1 Group NC Pathological changes: NA

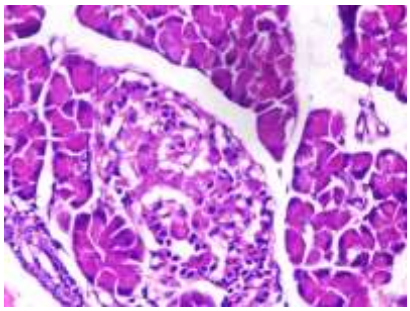

Fig 4.2 Group DC Pathological changes: Moderate (+++)

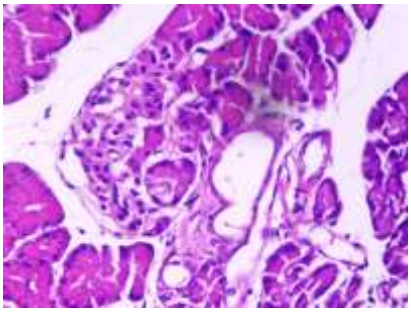

Fig 4.3 Group STD Pathological Changes: NAD

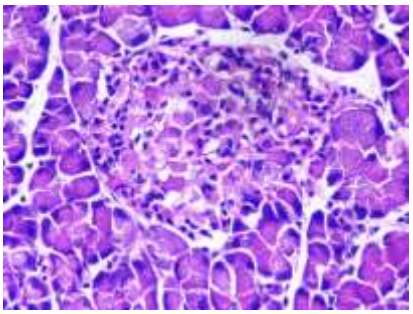

Fig 4.4 Group Test Pathological changes: Minimal (+)

NAD =No Abnormality Detected
Minimal changes (+1)
Moderate changes (+3)

The acute oral toxicity study was carried out according to the OECD guidelines 425. The limit test at single dose administration of 2000mg/kg and 5000mg/kg of the test drug was conducted. No death was found at 5000mg/kg, so we take 1/10th dose of 5000mg/kg that is 500mg/kg as a therapeutic dose.

In this present study, the animals in Diabetic control group were found to be reduction (Day 14th and 21st) of body weight as compared to the Normal control.

After streptozotocin induction, the animals have shown significant increase in the fasting blood glucose levels ($p< 0.001$) as compared to Normal Control animals. These animals were then randomly divided into three group's viz. Disease control, Standard and Test respectively. The Glucose levels were weekly monitored in all animals. On 28th day, the glucose levels were found to be significantly decreased ($p<0.05$) in Standard group as well as Test drug treated group as compared to Disease control. The oral glucose tolerance test was performed on Day 14 and Day 28. On 28th day there was significant reduction in the glucose levels of Test group ($p<0.05$) at 1st hr as compared to Disease control animals. Also there was significant reduction in Test group ($p<0.05$) and Standard drug treated group ($p<0.05$) as compared to Disease control animals.

The various parameters were evaluated like creatinine, total protein, ALP, and Urea. In Creatinine, Total Protein, and Urea, there was no significant change was found in all groups as compared to Disease control animals. The ALP levels were found to be significantly increased ($p<0.001$) in Disease control animals as compared to Normal Control group. In Standard drug treated group the ALP levels were significantly ($p<0.01$) decreased in comparison with Disease control animals. Although there was reduction in ALP levels of Test sample treated group, the difference was not statistically significant in comparison with Disease control animals. The relative organ weight data has also shown non-significant changes in the organ weights of animals in comparison with Disease control animals. At the end of the study the histopathology of kidney and pancreas was done. The damage to the organs was severe to moderate (+++) for diabetic group and in test and standard the pathological damage was mild (+).

CONCLUSION

Nowadays diabetes and obesity are leading health problems in India and worldwide.[12] Hyperlipidemia and Hyper-cholesterolemia are not only secondary metabolic deregulations associated with diabetes but also represent increased risk factors for development of diabetes.[13-15]

In this study the formulation contains various antidiabetic plants having vast therapeutic potential as per reports. The present study showed that treatment of the polyherbal formulation at 500mg/kg dose in streptozotocin induced rats showed antihyperglycemic effect. Based on the above results it can be concluded that the polyherbal formulation may act as anti-diabetic agent in experimental rats at the given dose. However, further studies are required to validate the hypothesis**.**

REFERENCES

1. Davis S. Insulin, Oral Hypoglycemic Agents and the pharmacology of the Endocrine Pancreas. Goodman and Gilmans the Pharmacological Basis of Therapeutics. 2006; 1613-1645.
2. Matsui T. Tanaka T.Tamura S. Toshima A. Alpha glucosidase inhibitory profile of catechins and theaflavins. J Agric Food Chem. 2007; 55:99-105
3. Nagappa A. Thakurdesai P. et al. Antidiabetic activity of Terminalia Catappa Linn fruits. Journal of Ethnopharmacology. 2003; 88: 45-50.
4. Murray C. Lopez A. Mortality by cause for eight regions of the world.global burden of disease study. Lancet 1997; 349:1269-76.
5. Mall G, Mishra P, Prakash V. Antidiabetic and hypolipidemic Activity of Gymnema Sylvestre in Alloxan induced Diabetic rats. Global Journal of Biotechnology and Biochemistry. 2009; 4 (1): 37-42.
6. Kumar A, Ilavarasan R, Jayachandran T, Deecaraman M, Aravindan P, Padmanabhan N and Krishan M. Anti-diabetic activity of Syzygium cumini and its isolated compound against streptozotocin-induced diabetic rats. Jouranl of Medicinal Plants Research. 2008; 2 (9): 246-249.
7. Panday D, Rauniar G. Momordica Charantia (Karela); an Antidiabetic. World Journal of Pharmacy and Pharmaceutical science. 2014; 4(1): 84-99.
8. Shobha K and Patil G. Antidiabetic Activity of Tinospora Cordifolia (FAM: Menispermaceae) in Alloxan Treated Albino Rats. Applied Research Journal. 2015; 1(5): 316-319.
9. Hassan S, Barthwal R, Nair M, Haque S. Aqueous Bark Extract of Cinnamomum Zeylanicum: A Potential Therapeutic Agent for Streptozotocin-Induced Type 1 Diabetes Mellitus (T1DM) Rats. Tropical Journal of Pharmaceutical Research. 2012; 11(3); 429-435.
10. Sharma A and Singh N. A Multifarious Potent Herb: Plumbago Zeylanica –A Mini Review. International Journal of Recent Scientific Research. 2015; 6(6): 4825-4829.
11. Trivedi N, Muzumdar B, Bhatt J, Hemavati K. Effect of shilajit on blood glucose and lipid profile in alloxan induced diabetic rats. 2004; 36(6): 373-376.
12. Ramachandran A. Diabetes and Obesity – the Indian angle. Indian J Med Res 2004; 120: 437- 449.
13. Still W, Martin J, Gregor W. The effect of alloxan diabetes on experimental atherosclerosis in the rat. Exp Mol Pathol 1964; 3: 141-7.
14. Miller R, Wilson R. Atherosclerosis and Myocardial ischemic lesions in alloxan diabetic rabbits fed a low cholesterol diet. Arteriosclerosis 1984; 4: 586-591.
15. Chattopadhyay R, Bandhopadhyay M. Effect of Azadirecta indica leaf extract on serum profile changes in normal and streptozotocin induced diabetic rats. African j Biomed Res 2005; 8: 101-4.