

Ameliorative effect of hydro-methanolic extract from roots of *Rauwolfia serpentina* on some biochemical parameters of type 1 diabetic mice

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Abstract: Present work seeks to investigate the biochemical parameters in terms of hypoglycemic and hypolipidemic effects of hydro-methanolic roots extract (HyMREt) of *Rauwolfia serpentina* in type 1 (alloxan induced) diabetic mice. Animals were divided into seven groups, four control groups, and three were test groups (HyMREt at 50, 100, & 150mg/kg). Each treatment was repeated for 14 days regularly in all seven respective groups and afterwards the body weights, fasting blood glucose (FBG), insulin, and serum lipid levels were determined. Total body weights of diabetic mice treated with HyMREt extract were dose dependently ($p \leq 0.05$) improved. FBG of test groups were significantly ($p \leq 0.0001$) reduced in comparison with diabetic controls which displayed elevated fasting blood glucose level. The insulin levels of HyMREt treated groups were significantly ($p \leq 0.0001$) higher than those of diabetic controls. Lower triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) whereas elevated level of high density lipoprotein cholesterol (HDL-c) were observed in test dose treated groups. In comparison with diabetic controls, the converse levels of serum lipid were observed. Significant improvement in cardio-protective indices and coronary risk index was also observed. Findings of present study support the hypoglycemic and hypolipidemic potential of HyMREt of *R. serpentina*.

Keywords: Alloxan, glucose, lipid, *Rauwolfia serpentina*.

INTRODUCTION

Among other endocrine disorders, diabetes mellitus (DM) is considered as the most prevalent metabolic case all over the globe (Azmi *et al.*, 2017; Zhou *et al.*, 2016). Diabetes is characterized by elevated amount of blood glucose due to defective insulin synthesis or its action or both with consequent disturbances of carbohydrate, fat and protein metabolism (Kolluru *et al.*, 2012). It has both acute and chronic complications with dysfunction, damage, and failure of various body organs were also its long term complications (Marshall and Flyvbjerg, 2006; Young *et al.*, 2008). Statistics from published researches indicated that over 300 million people are affected by diabetes and these digits are predicted to rise to more than 400 million by the end of 2025 in which more than 200 million will be from Asian Continents (Shaw *et al.*, 2010; Shaikh *et al.*, 2011; Zhou *et al.*, 2016). More than 80% of diabetic patient belongs to low socioeconomic countries and the annual health costs of diabetes complications account for 6-12% of all health-care expenditures worldwide (Azmi *et al.*, 2017; Young *et al.*, 2008). Therapeutic strategies for the management of diabetes includes glycemic control, multi-factorial risk reduction, patient self-management and education with dietary and

exercised based reduction in calories are considered for lowering the magnitude of complications' risk (Rang *et al.*, 2003; Davis, 2006).

Plants have played a significant contribution in the introduction of new therapeutic derivatives (Mukhopadhyay *et al.*, 2012) which gained focus as a source of pharmacologically and biochemically active substances with prominent hypoglycemic and hypolipidemic potential (Malviya *et al.*, 2010; Alagumanivasagam and Veeramani, 2015). In this manner, scientists have focused on phytochemicals for developing new therapeutic agents because they are natural products which have minimal side effects. Similarly, invention of metformin as an antidiabetic agent from plant of *Galega officinalis* was a great success story of this subject (Bailey and Day, 2004). Researchers reported that medicinal attributes of plant product is due to presence of bioactive ingredients like alkaloids, flavonoids, phenolic derivatives and other compounds (Seifu *et al.*, 2012).

Rauwolfia serpentina Benth (family *Apocynaceae*) is recognized phytochemical for its potential effectiveness in the cure from venom (snake sources), gastric and intestinal disorders, cancer of mammary cells, skin (dermis) problems, etc (Qureshi *et al.*, 2009; Azmi and Qureshi 2012a). From last decades, its antidiabetic

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potential has been reported in animal models of DM (Qureshi *et al.*, 2009; Azmi and Qureshi 2012a; Azmi *et al.*, 2012b). It was also reported to improve the cardioprotective indices as well as reducing the oxidative stress and maintaining the haematinic potential in its pure methanolic form (Azmi *et al.*, 2012b; Azmi and Qureshi, 2013; Azmi *et al.*, 2015). In this study the effects of HyMREt of *R. serpentina* on biochemical parameters of glucose, lipids and coronary risk index in experimentally induced type 1 diabetic mice model.

MATERIALS AND METHODS

Collection of plant material and preparation of hydro-methanolic extract

The roots of *R. serpentina* were procured from Hamdard Dawakhana Sardar, Karachi-Pakistan in 2010. The plant specimen was verified and authenticated from Specialist of Botany Department, University of Karachi, Pakistan, before use. The extract used for this study was prepared according to the method described earlier by Azmi and Qureshi, 2014.

Experimental mice and ethical approval

Male albino mice (weight between 25-35g) were purchased from breeding house of Dow University of Health Sciences (DUHS), Karachi, Pakistan. The mice were acclimatized and maintained individually in cages in an air conditioned room of 12 hours light/ dark cycles with temperature 23±2°C (Relative humidity 55%) for one week prior to the experiment in the conventional animal house of the same university. They were given standard laboratory diet with free access to water *ad libitum* and no physical stress was provided. During the entire experimental period, the care and handling of these mice were in accordance with internationally accepted standard guidelines. The experimental protocol was approved by the Institutional Ethical Review Board (IERB) of Dow University of Health Sciences, Karachi (Letter Ref. No: IRB-186/DUHS-10).

Chemicals, reagent and medicine used

Alloxan monohydrate (2,4,5,6-tetraoxypyrimidine,5,6-dioxyuracil) was purchased from Appli Chem GmbH Darmstadt, Germany. Single intraperitoneal injection of alloxan was used to induce type 1 diabetes in overnight fasted mice of 150 mg/kg dose. After 72 hours of this injection fasting blood glucose levels were monitored from tail vein of mice with the help of glucometer (Optium Xceed, Diabetes Monitoring system by Abbott), mice showed glucose level ≥200 mg/dL were selected for further respective treatment of the work done. Antidiabetic medicine 'glibenclamide' as product name *Doanil* (5mg/kg) of Sanofi-aventis Pakistan Ltd, were used as positive control. All other reagents and chemicals used were of analytical grade and purchased from

authentic dealer of Department of Biochemistry, University of Karachi.

Grouping of mice

The mice were divided into seven groups (six animals each) as follows;

Group I: normal control mice: (NC – animals receiving only distilled water (1mL/kg) with no induction of diabetes through alloxan).

Group II: diabetic control: (alloxan-induced diabetic mice - treated with distilled water (1mL/kg).

Group III: negative control: alloxan-induced diabetic mice treated with 0.05% DMSO (1 mL/kg).

Group IV: positive control: alloxan-induced diabetic mice treated with glibenclamide (5 mg/kg).

Group V: 1st test group: alloxan-induced diabetic mice - treated with HyMREt (50 mg/kg).

Group VI: 2nd test group: alloxan-induced diabetic mice - treated with HyMREt (100 mg/kg).

Group VII: 3rd test group: alloxan-induced diabetic mice - treated with HyMREt (150 mg/kg).

Each treatment was given to its respective group orally once in a day for 14 days consecutively. At the end of animal trial, mice were sacrificed to collect whole blood, serum and liver tissues that were used to analyze hematological and biochemical parameters.

Body Weight Determination and Biochemical Parameters

Body weights of animals of each group were measured at initial day as well as on final (14th) days of trial by using balance (Kitchen scale 1800) and recorded in comparative manner. Fasting blood glucose levels were monitored in each group by glucometer at initial day and final (14th) day of trial. Other parameters including serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein-cholesterol (HDL-c) concentrations were determined by commercially available enzymatic assay kits (Randox, United Kingdom). Whereas low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were calculated by Friedewald formulae (Azmi and Qureshi, 2012b). The serum insulin levels were determined by cobas e411 analyzer, Hitachi (Roche Diagnostics GmbH, Mannheim, Germany) and expressed as pmol/L.

Determination of Antiatherogenic, Cardioprotective and Coronary Risk Indices

Cardioprotective index (CPI) was estimated in term of HDL-c to LDL-c and TG to HDL-c ratios (Azmi and Qureshi, 2012b). Whereas coronary risk indices (CRI) were calculated by the following formula (Azmi and Qureshi, 2012b):

$$CRI = \frac{\text{Total Cholesterol}}{\text{HDL - c Cholesterol}}$$

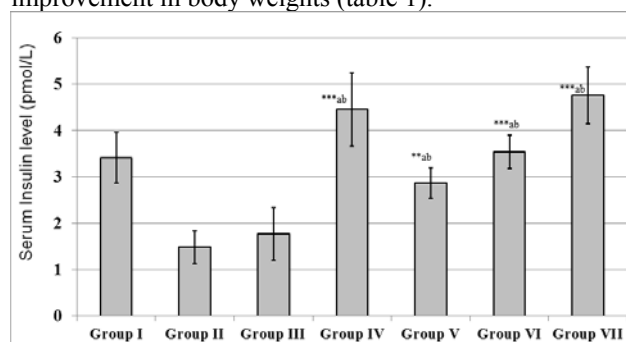
STATISTICAL ANALYSIS

Results of the present study are expressed as mean \pm SD (standard deviation). The data were analyzed with Statistical Package for Social Sciences (SPSS version 18) by using one-way ANOVA followed by LSD (least significant difference) test at $P < 0.05$. The differences were considered significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$ when compared with respective controls.

RESULTS

Effect of HyMREt on Body weight of Mice, FBG and Serum Insulin of Mice

HyMREt test (50, 100 and 150 mg/kg) doses progressively and significantly ($p < 0.05$ & $p < 0.01$) showed improvement in the body weights of mice. Especially magnitude of test dose 150 mg/kg showed considerable gain in total body weights of mice were observed as compared to controls (both diabetic and negative). These control (diabetic and negative) groups showed noticeable decrement in total body weights when compared to control (distilled water) group animals. In group IV (positive control) consecutive treatment with glibenclamide of type 1 diabetic mice showed highest improvement in body weights (table 1).



Values are expressed as mean \pm SD ($n = 6$). $**p < 0.01$ and $***p < 0.0001$, when compared with respective group II (a) and III (b).

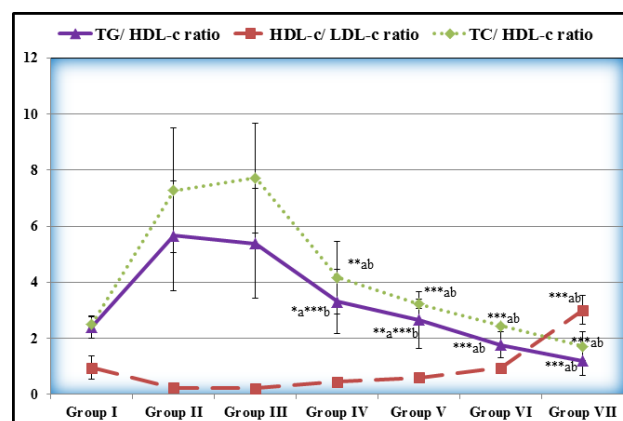
Fig. 1: Effect of HyMREt on Serum Insulin Levels of Alloxan-Induced Diabetic Mice

All the three selected doses of HyMREt i.e., 50, 100 & 150 mg/kg showed prominent ($p < 0.0001$) reduction in FBG levels of test groups when evaluated against the respective controls (diabetic and negative). Moreover, major ($p < 0.0001$) reduction was also observed in FBG level of group IV (positive control) animals (table 2). Similarly, good ($p < 0.0001$) improvement in the levels of serum insulin was also noticed in all three selected groups of HyMREt doses (50, 100 & 150 mg/kg) when evaluated with respective diabetic and negative control groups (fig. 1).

Effect of HyMREt on Serum Lipid markers of type 1 diabetic mice

HyMREt test doses (50, 100 & 150 mg/kg) produced a sequential and significant ($p < 0.0001$) decrement in a

dose-dependent manner in serum TG, TC, VLDL-c, LDL-c, levels and increment in HDL-c level in test doses respectively ranging from 132.87-90.36, 169.50-128.57, 26.57-18.08, 89.85-43.12 mg/dl and 53.08-83.33 mg/dl in comparison with control (diabetic and negative) groups. Mice group treated with glibenclamide (positive control group) exhibited almost the same significant representation of all of these lipid markers (table 3).



Values are expressed as mean \pm SD ($n = 6$). $*p < 0.05$, $**p < 0.01$ and $***p < 0.0001$, when compared with respective group II (a) and III (b).

Fig. 2: Effect of HyMREt on Cardioprotective Indices of Alloxan-Induced Diabetic Mice

Effect of HyMREt on Cardioprotective Indices of type 1 diabetic mice

Dose dependently significant ($p < 0.05$, $p < 0.01$ & $p < 0.0001$) lowered levels of TG/ HDL-c ratio was computed from the range of 3.31 to 1.19 in mice group treated with glibenclamide (positive control group) and test groups treated with HyMREt doses (50, 100 & 150 mg/kg) respectively. Similarly, good improvement ($p < 0.05$, $p < 0.01$ & $p < 0.0001$) was also noticed in TC/HDL-c ratio and HDL-c/LDL-c and in HyMREt test groups from 3.22-1.71 and 0.59-3.01 respectively compared with controls (diabetic and negative) which showed least values (0.23 to 0.21) of HDL-c/LDL-c and elevated ranges (7.28 to 7.72) of TC/HDL-c ratio. (fig. 2).

DISCUSSION

Most common type 1 DM inducer in animal models of diabetes is alloxan which is a pyrimidine based oxygenated derivative (Sun *et al.*, 2008; Azmi and Qureshi, 2012b). Its various formulation is used for major destruction of insulin synthesizing (β -cells) unit of pancreas resulted in preliminary hyperglycemia, and other dyslipidemia as well as oxidative complications through reactive oxygen species (Rohilla and Ali, 2012). The loss in the body weight is most visible symptom of type 1 DM, because at metabolic level body is utilizing triacylglycerides instead of glucose residues in order for the maintaining the energetic demands of body (Bishop *et*

Table 1: Effect of HyMREt on Body Weights of Alloxan-Induced Diabetic Mice

Groups	Treatment	Body weights in (g)	
		Initial day	Final day
Group I	Distilled water (1 ml/kg)	28.54 ± 2.75	31.14 ± 2.42
Group II	Alloxan (150 mg/kg)	29.81 ± 2.54	26.76 ± 2.33
Group III	Alloxan (150 mg/kg) + 0.05% DMSO (1 ml/kg)	29.48 ± 3.12	26.20 ± 1.34
Group IV	Alloxan (150 mg/kg) + Glibenclamide (5 mg/kg)	29.64 ± 1.77	31.59 ± 1.24
Group V	Alloxan (150 mg/kg) + HyMREt (50 mg/kg)	30.06 ± 2.40	27.99 ± 2.63
Group VI	Alloxan (150 mg/kg) + HyMREt (100 mg/kg)	29.47 ± 1.63	29.04 ± 0.77
Group VII	Alloxan (150 mg/kg) + HyMREt (150 mg/kg)	29.68 ± 2.14	30.74 ± 0.66

Values are expressed as mean ± SD ($n = 6$). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.0001$, when compared with respective group II (a) and III (b).

Table 2: Effect of HyMREt on Blood Glucose Levels of Alloxan-Induced Diabetic Mice

Groups	Treatment	Blood glucose level (mg/dl)	
		Initial day	Final day
Group I	Distilled water (1 ml/kg)	108.89 ± 17.87	108.29 ± 13.76
Group II	Alloxan (150 mg/kg)	194.75 ± 9.95	224.25 ± 11.79
Group III	Alloxan (150 mg/kg) + 0.05% DMSO (1ml/kg)	199.50 ± 4.51	216.25 ± 8.99
Group IV	Alloxan (150 mg/kg) + Glibenclamide (5 mg/kg)	200.50 ± 9.57	114.25 ± 11.93***ab
Group V	Alloxan (150 mg/kg) + HyMREt (50 mg/kg)	225.50 ± 43.38	133.50 ± 5.74***ab
Group VI	Alloxan (150 mg/kg) + HyMREt (100 mg/kg)	211.75 ± 49.12	116.75 ± 27.11***ab
Group VII	Alloxan (150 mg/kg) + HyMREt (150 mg/kg)	253.25 ± 94.73	105.25 ± 8.66***ab

Values are expressed as mean ± SD ($n = 6$). * $p < 0.0001$, when compared with respective group II (a) and III (b).

Table 3: Effect of HyMREt on Lipid Profile of Alloxan-Induced Diabetic Mice

Groups	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Group I	154.15 ± 21.27	147.30 ± 17.35	62.70 ± 14.22	72.47 ± 23.11	29.46 ± 3.45
Group II	255.73 ± 28.32	195.12 ± 16.71	39.02 ± 17.70	181.23 ± 27.64	39.03 ± 3.34
Group III	253.34 ± 27.52	206.70 ± 18.41	34.74 ± 10.84	173.68 ± 36.02	41.34 ± 3.68
Group IV	175.71 ± 32.11***ab	139.55 ± 23.79**a***b	44.04 ± 8.73	104.83 ± 20.85***a***b	27.91 ± 4.76**a***b
Group V	169.50 ± 22.52***ab	132.87 ± 27.23**a***b	53.08 ± 9.77	89.85 ± 18.19***ab	26.57 ± 5.45**a***b
Group VI	146.31 ± 4.26***ab	105.87 ± 29.51***ab	60.18 ± 0.65*ab	65.10 ± 2.54***ab	21.18 ± 5.89***ab
Group VII	128.57 ± 23.80***ab	90.36 ± 14.01***ab	83.33 ± 23.52***ab	43.12 ± 26.11***ab	18.07 ± 2.79***ab

Values are expressed as mean ± SD ($n = 6$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.0001$, when compared with respective group II (a) and II (b)

al., 2010). The same was noticed in present type 1 diabetic mice which experienced rapid reduction in their body weights. Body weights of mice treated with experimental test doses of HyMREt revealed prominent improvement ($p < 0.05$ & $p < 0.01$) which may be a potential role of this plant extract to inhibit tissue protein catabolism and maintaining the body weight (Azmi and Qureshi, 2012b).

In type DM one of the prominent symptom is hyperglycemia which is mainly linked with the disturbance in glucose metabolism (uptake of glucose) specifically caused with the deficiency of insulin (Daneman, 2006). After 72 hours administration of alloxan has direct impact on glucose metabolism which elevated the FBG level (23-28%) in control (diabetic)

groups through interfering the mechanism of insulin release from pancreas (β -cells) either by totally or incompletely tear down the physiology of these cells. Beside this, the experimental extract of HyMREt (50-150mg/kg) induced significant ($p < 0.0001$) reduction in glucose levels (i.e., from -39 to -53%). These finding support and validates the previous outcomes achieved in glucose tolerance as well as the longitudinal study on MREt of *R. serpentina* which reported the improvement in glucose tolerance (mice model) either by inhibiting absorption of glucose (in intestine) or speeding its utilization (peripheral tissues and hepatic tissues) (Azmi and Qureshi, 2012a). Glibenclamide (5mg/kg) the secretagogues (sulphonylurea) medicinal analogue used in DM, responsible for enhancing the release of insulin from pancreas (β -cells) (Kaku *et al.*, 1995) also found effective

and exhibited significant glucose reduction in positive controls. This outcome indicates another possible action of antidiabetic mechanism of root extracts as they may possess pancreatic potential. In relation to the above scenario, possibly it may be linked with the fact that some of the β -cells remain physiologically alive through which the test extract mediates its action by uplift the release of insulin, similarly like glibenclamide showed in positive control group. For validating this action, levels of serum insulin were also investigated experimentally tested groups treated with each extract (50, 100, 150mg/kg) in dose-dependent manner that showed a significant ($p < 0.01$ & $p < 0.0001$) increment in their levels in comparison with control (diabetic) groups which revealed very low levels of this peptide hormone. Findings from this investigation elaborately evident that the test extract of *R. serpentina* may have both pancreatic and extra-pancreatic actions to normalize blood glucose level as similar as the glibenclamide possess.

Obtained hypolipidemic effect is attributed more likely due to concurrent hypoglycemia (Azmi and Qureshi, 2012b), inhibition of rate-limiting enzyme HMG-CoA reductase for cholesterol biosynthesis (Azmi and Qureshi, 2016) and /or may be due to the inhibition of Acetyl CoA carboxylase for lipogenesis (Lateef and Qureshi, 2014; Lateef and Qureshi, 2016) or speeding the mechanism of action of lipase (hormone sensitive) (Lateef and Qureshi, 2016). Values from coronary risk and other indices suggests the decrease coronary risk of atherosclerosis as dose-dependent increased HDL-c contents through test doses may be link with the suppression of free circulating cholesterol moieties via promoting the activity of reverse cholesterol transport mechanism through lecithin-cholesterol acyltransferase (Azmi and Qureshi, 2012b; Lateef and Qureshi, 2016).

CONCLUSION

The present work concludes that HyMREt extract of *R. serpentina* is effective in improving the blood glucose, lipid profile, serum insulin levels and suppressing the coronary risk in animal model of type 1 DM.

REFERENCES

Alagumanivasagam G and Veeramani P (2015). A review on medicinal plants with hypolipidemic activity. *Int. J. Pharm. Ana. Res.*, **4**: 129-134.

Azmi MB and Qureshi SA (2012a). Methanolic root extract of *Rauwolfia serpentina* Benth improves the glucose tolerance in Wister mice. *J. Food Drug Anal.*, **20**: 54-58.

Azmi MB and Qureshi SA (2012b). Methanolic root extract of *Rauwolfia serpentina* Benth improves the glycemic, antiatherogenic, and cardioprotective indices in alloxan-induced diabetic mice. *Adv. Pharmacol. Sci.*, article ID 376429: 11 pages.

Azmi MB and Qureshi SA (2013). *Rauwolfia serpentina* ameliorates hyperglycemic, haematinic and antioxidant status in alloxan- induced diabetic mice. *J. App. Pharm. Sci.*, **3**: 136-141.

Azmi MB and Qureshi SA (2014). Glucose lowering potential of hydromethanolic extract of *Rauwolfia*. *World J. Pharmaceut. Sci.*, **2**: 219-223.

Azmi MB and Qureshi SA (2016). *Rauwolfia serpentina* improves altered glucose and lipid homeostasis in fructose-induced type 2 diabetic mice. *Pak. J. Pharm. Sci.*, **29**: 1619-1624.

Azmi MB, Qureshi SA and Imtiaz F et al., (2017). Categorization of relative risk of disease with relation to stress, body mass index and anthropometric markers: A cross-sectional study in general population of Karachi, Pakistan. *Int. J. Med. & Medical Sci.*, **9**(5): 51-60.

Azmi MB, Qureshi SA, Rais S and Sultana S (2015). Methanolic root extract of *Rauwolfia serpentina* lowers atherogenic dyslipidemia, arteriosclerosis and glycosylation indices in type 1 diabetic mice. *J. Appl. Pharmaceut. Sci.*, **5**: 61-67.

Bailey CJ and Day C (2004). Metformin: its botanical background. *Pract Diabetes Int.*, **21**: 115-117.

Bishop ML, Fody EP and Schoeff L (2010) Clinical Chemistry: Principles, Procedures, Correlations, Lippincott Williams & Wilkins, 6th edition.

Daneman D (2006). Type 1 diabetes. *Lancet*, **367**: 847-58.

Davis SN (2006). Insulin, oral hypoglycemic agents, and the pharmacology of the Endocrine Pancreas. In "Goodman and Gilman's The Pharmacological Basis of Therapeutics." 11th eds. Brunton LL, Lazo JS and Parker KL. McGraw- Hill Co. Incorp. U.S.A. pp. 1613-1645.

Kaku K, Inoue Y, Kaneko T (1995). Extrapancratic effects of sulfonylurea drugs. *Diabetes Res Clin Prac.*, **28**:S105-S108.

Kolluru GK, Bir SC and Kevil CG (2012). Endothelial dysfunction and diabetes: Effects on angiogenesis, vascular remodeling and wound healing. *Int. J. Vasc. Med.*, 918267.

Lateef T and Qureshi SA (2014). Ameliorative effect of *Withania coagulans* on experimentally-induced hyperlipidemia in rabbits. *J. Nat. Remedy*, **14**: 83-88.

Lateef T and Qureshi SA (2016). *Centratherrum anthelminticum* and *Withania coagulans* improves lipid profile and oxidative stress in triton X-100 induced hyperlipidemic rabbits. *Int. J. Pharmacog. & Phytochem. Res.*, **8**(6): 933-940.

Malviya N, Jain S and Malviya S (2010). Antidiabetic potential of medicinal plants. *Acta. Pol. Pharm.*, **67**: 113-118.

Marshall SM and Flyvbjerg A (2006). Prevention and early detection of vascular complications of diabetes. *BMJ.*, **333**(7566): 475-480.

Mukhopadhyay MK, Banerjee P and Nath D (2012). Phytochemicals biomolecules for prevention and

- treatment of human diseases-a review. *Int. J. Scienti. Eng. Res.*, **3**: 1-32.
- Qureshi SA, Nawaz A and Udani SK *et al.* (2009). Hypoglycemic and hypolipidemic activities of *Rauwolfia serpentina* in alloxan-induced diabetic rats. *Int. J. Pharmacol.*, **5**: 323-326.
- Rang HP, Dale MM, Ritter JM and Moore PK (2003). The Endocrine Pancreas and the control of blood glucose,” in *Pharmacology*, J. G. Hardman and L. E. Limbird, Eds., p. 385, Churchill Livingstone, New York, NY, USA, 5th edition.
- Rohilla A and Ali S (2012). Alloxan-induced diabetes: Mechanisms and effects. *Int. J. Res. Pharmaceu. Biomed. Sci.*, **3**: 819-823.
- Seifu D, Assefa F and Abay SM (2012). Medicinal plants as antioxidant agents: Understanding their mechanism of action and therapeutic efficacy (Capasso A, ed). pp.97-145.
- Shaikh ZA, Shaikh MZ and Ali G (2011). Diabetic patients, awareness about life style modifications. *Prof. Medical. J.*, **18**(2): 265-268.
- Shaw JE, Sicree RA and Zimmet PZ (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diab. Res & Clin. Prac.*, **87**(1): 4-14.
- Sun JE, Ao ZH and Lu ZM *et al.*, (2008). Antihyperglycemic and antilipidperoxidative effects of dry matter of culture broth of *Inonotus obliquus* in submerged culture on normal and alloxan-diabetes mice. *J. Ethnopharmacol.*, **118**(1): 7-13.
- Young BA, Lin E and Korff MV *et al.* (2008). Diabetes complications severity index and risk of mortality, hospitalization, and healthcare utilization. *Am. J. Manag Care*, **14**: 15-24.
- Zhou B, Lu Y, Hajifathalian K, Bentham J, Di Cesare M, Danaei G, Bixby H, Cowan MJ, Ali MK and Taddei C *et al.* (2016). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet*, **387**(10027): 1513-1530.