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Research Article

ANTIDIABETIC AND ANTIHYPERLIPIDAEMIC EFFECT OF *POLYGALA JAVANA* DC ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The ethanol extract of *Polygala javana* whole plant (Family: Polygalaceae) was investigated for it antidiabetic and antihyperlipidaemic effect in Wistar Albino rats. Diabetes was induced in albino rats by administration of alloxan monohydrate (150mg/kg, i.p). The ethanol extracts of *Polygala javana* at a dose of 100 and 200mg/kg of body weight were administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of *Polygala javana* whole plant extract on blood glucose, serum insulin, urea, creatinine, glycosylated haemoglobin, serum lipid profile [total cholesterol (TR), triglycerides (TG), low density lipoprotein – cholesterol (LDL-C), very low density lipoprotein – cholesterol (VLDL-C), high density lipoprotein – cholesterol (HDL-C) and phospholipid (PL)] serum protein, albumin, globulin, serum enzymes [serum glutamate pyruvate transaminases (SGPT), and serum glutamate oxaloacetate transaminases (SGOT), and alkaline phosphatase (ALP)], were measured in the diabetic rats. The ethanol extract of *Polygala javana* whole plant elicited significant reductions of blood glucose ($P<0.05$), lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C. The extracts also caused significant increase in serum insulin ($P<0.05$) in the diabetic rats. From the above results, it is concluded that ethanol extract of *Polygala javana* possesses significant antidiabetic and antihyperlipidaemic effects in alloxan induced diabetic rats.

Keywords: Antihyperlipidaemic, Antidiabetic, *P.javana*, Alloxan and Glibenclamide.

INTRODUCTION

Diabetes is a major degenerative disease in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders[1]. Diabetes mellitus is also associated with long term complications, including retinopathy, neuropathy and angiopathy and several disorders[2]. Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities, raised metabolic rate, defect in reactive oxygen species scavenging enzymes and high oxidative stress include damage to pancreatic beta cells[3]. Diabete mellitus is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken in to account[4]. The World Health Organization (WHO) reported that 300 million peoples world suffer from diabetes mellitus by the year 2025 [5].

The search for an effective and safer hypoglycemic agents with a protective effect from diabetic complication has continuous to be a research topic of interest [6]. The world health organization has recommended and encouraged the use of alternative therapy especially in countries where access to the conventional treatment of diabetes is inadequate [7].

Plant medicines (Phytotherapies) have a long history as treatments for diabetes. With a disturbing rise in the prevalence of this metabolic disease and associated health care costs, interest in alternative or complementary therapies has grown [8]. Over the two decades, numerous phytotherapies and their combination demonstrate multiple beneficial antidiabetic mechanism, including modulation of carbohydrate metabolism, restoration of beta-cell integrity and function, insulin - releasing activity, improvement in glucose uptake/utilization antioxidant properties and a reduction in the risk of cardiovascular disease[7-8].

One of these anti-diabetic phytotherapies is *Polygala javana* DC. *Polygala javana* belongs to Polygalaceae family. It is commonly known as "Palpiranthi". Paste prepared from fresh leaves is applied by kanikkar tribal woman on the breast twice a day for 2-3 days to check lactation and to get relief from the pain developed while shopping[9]. The current investigation is an attempt to study the antidiabetic and antihyperlidaemic activities of ethanol extract of *Polygala javana* DC whole plant in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Material

The leaves of *Polygala javana* DC were freshly collected from the well grown healthy plants inhabiting the natural region of Scott Christian college, Nagercoil, Kanyakumari district, Tamil Nadu. The plant were identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for phytochemical screening and antidiabetic studies

The *Polygala javana* leaves were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *Polygala javana* leaves was packed in a Soxhlet apparatus and extracted with ethanol The extract were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures[10-12]. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

Animals

Normal healthy male Wistar albino rats (180- 240g) were housed under standard environmental conditions at temperature (25±2° C) and light and dark (12: 12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [13]. The animals were kept fasting for overnight

and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100, and 2000 mg/kg body weight.

Induction of Experimental Diabetes

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg) [14]. Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental Design

In the present investigation, a total of 30 rats (24 diabetic surviving rats and 6 normal rats) were taken and divided into five groups of 6 rats each.

Group I: Normal untreated rats

Group II: Diabetic control rats

Group III: Diabetic rats given ethanol extract of *P. javana* leaf (100mg/kg body weight)

Group IV: Diabetic rats given ethanol extract of *P. javana* leaf (200mg/kg body weight)

Group V: Diabetic rats given standard drug glibenclamide (600µg/kg body weight).

Biochemical analysis

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes. Serum glucose was measured by the O-toluidine method[14]. Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit[15]. Urea estimation was carried out by the method of Varley[16]; serum creatinine was estimated by the method of Owen et al[17]. Glycosylated haemoglobin (HBA_1C) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan[18].

Estimation of protein, albumin, globulin, SGPT, SGOT, ALP

Serum protein [19] and serum albumins was determined by quantitative colorimetrically method by using bromocresol green. The total protein minus the albumin gives the globulin, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel [20]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong [21].

Estimation of lipids and lipoprotein

Serum total cholesterol (TC) [22], total triglycerides (TG) [23], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL- C) [24], high density lipoprotein cholesterol (HDL-C) [25] and phospholipids [26] were analyzed.

STATISTICAL ANALYSIS

The data were analyzed using student's t-test statistical methods. For the statistical tests a p values of less than 0.01 and 0.05 was taken as significant.

Table1. Effect of *Polygala javana,* extracts on serum Insulin, Glucose, Urea, Creatinine and HbA_1C level in the normal, diabetic induced and drug treated rats

Groups	Glucose (mg/dl)	Insulin (MIu/ml)	Urea (mg/dl)	Creatinine (mg/dl)	Hb A_1c (%)
I	83.16±2.11	14.27±0.74	18.16±0.94	0.69±0.03	3.91±0.11
II	231.84±18.43**	5.21±0.24**	36.69±1.14**	0.93±0.04*	8.59±0.14**
III	144.36±1.21[a]	9.11±0.16[a]	24.31±0.54	0.89±0.01	5.96±0.15[a]
IV	113.69±3.16[a]	11.36±0.39[a]	14.16±1.16	0.84±0.01	4.63±0.21[a]
V	94.66±1.84[aa]	13.63±0.18[aa]	16.24±0.39[a]	0.77±0.06	4.07±0.14[a]

Each Value is SEM ± 5 individual observations * P < 0.05 ; ** P<0.01 Compared normal control vs -Diabetic rats : a -P < 0.05 ; aa - P<0.01 Compared -Diabetic rats vs drug treated

Table 2. Effect of *Polygala javana,* extracts on the protein, albumin, globulin serum GOT, GPT, and ALP in the normal, diabetic and drug treated rats

Parameter	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	8.14±0.17	4.50±0.18	3.64±0.13	18.31±0.61	21.66±0.34	120.16±4.11
Group II	5.27±0.11*	2.32±0.13*	2.95±0.16*	29.11±0.36**	34.16±0.71*	191.20±3.92**
Group III	6.20±0.09	2.94±0.11	3.26±0.14	24.15±0.56	31.14±0.36	156.11±1.36
Group IV	7.27±0.17	4.08±.012	3.19±0.23	19.16±0.71	28.33±0.54	136.14±2.88
Group V	8.01±0.31	4.23±0.14	3.78±0.25	18.16±0.23	24.30±0.14	124.66±2.74

Each Value is SEM ± 5 individual observations * P < 0.05 ; ** P<0.01 Compared normal control vs -Diabetic rats

Table 3. Effect of *Polygala javana,* extracts on serum lipid profile in the normal, diabetic and drug treated rats

Groups	TC (mg/dl)	TG (mg/dl)	HDL – C (mg/dl)	LDL – C (mg/dl)	VLDL –C (mg/dl)	PL (mg/dl)
I	103.11±2.56	92.66±1.87	21.11±1.32	63.47±2.13	18.53±1.15	159.76±2.67
II	214.19±1.84**	178.26±2.19**	06.29±2.36**	92.25±2.56*	35.65±1.45	258.62±3.46
III	156.94±1.36[a]	10.14±1.44[a]	18.39±1.32[a]	81.06±1.56[a]	24.15±1.14	194.61±3.12
IV	113.62±2.04a	81.14±1.04a	24.33±1.16a	73.07±1.73a	16.22±1.37	169.12±2.55
V	116.84±1.63a	91.55±1.14a	25.34±1.11a	73.19±1.59a	18.31±1.08	171.98±2.57

Each Value is SEM ± 5 individual observations * P < 0.05 ; ** P<0.01 Compared normal control vs -Diabetic rats
a -P < 0.05 ; Compared -Diabetic rats vs drug treated

RESULTS AND DISCUSSION

The phytochemical screening of ethanol extract of *Polygala javana* whole plant revealed the presence of alkaloid, catechism, tannin, saponin, steroid, flavonoid, phenol, sugar, glycoside and xanthoprotein. Aucte toxicity study revealed the non-toxic nature of the ethanol extract of *Polygala javana*. Table1 shows the levels of blood glucose, serum insulin, urea creatine and glycosylated haemoglobin of

normal, diabetic rats and drug treated rats. The alloxan induced diabetic rats elicited significant rise in blood glucose from 83.16 to 231.84 mg/dl ($p<0.05$) and a significant decrease in serum insulin level from 14.27 to 5.21 ($p<0.01$). On the other hand, diabetic rats treated with ethanol extract of *Polygala javana* whole plant exhibited decrease in blood glucose and increase in serum insulin significantly ($p<0.05$) at a dose of 100 mg/kg and 200mg/kg body weight. It was observed that ethanol extract of *Polygala javana* reversed these effects in diabetic rats. Alloxan causes diabetes through its ability to destroy the insulin producing β cells of the pancrease[27]. Diabetogenic effect of alloxan is due to excess production of reactive oxygen species (ROS) leading to cytotoxicity in pancreatic β cells which reduces the synthesis and release of insulin[28].In this study, administration of ethanol extract of *Polygala javana* whole plant14 days produced a significant increase in insulin levels along with a decrease in blood glucose levels in alloxan induced diabetic rats. Earlier many plants have been studied for their hypoglycemic and insulin release stimulatory effects [29-32].

A significant elevation in serum constituents, urea and creatinine were observed in alloxan induced diabetic rats (Group II) when compared to control rats. The ethanol extracts of *Polygala javana* whole plant were administrated orally (100mg/kg body weight Group III and 200 mg/kg body weight Group IV) to rats for 14 days reversed the urea and creatinine level to near normal. The administration of gllibenclamide (GroupV) also decreased the levels of urea and creatinine to some extent. Renal impairment is one of the serious and common diabetic complications; the diabetic rats had increased levels of serum urea and creatinine, which are considered as significant markers of renal function important[33]. Treatment with ethanol extract of *Polygala javana* indicated a significant decrease in levels of serum urea and creatinine which could be decreased metabolic disturbance as the extract improved glycemic control.

Alloxan induced diabetic rats showed significant increase ($p<0.05$) glycosylated haemoglobin (HBA, C) level compared with normal rats. The ethanol extract of *P. javana* whole plant treated showed a significant decrease ($p<0.05$) in the content of glycosylated haemoglobin .Glycosylated haemoglobin has been found to be increased over a long period of time in diabetes. During diabetes, the excess of glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin[34]. The rate of glycation is proportional to the concentration of blood glucose[34]. Alloxan induced diabetic rats showed significant increase ($p<0.05$) glycosylated haemoglobin (HBA, C) level compared with normal rats. The ethanol extract of *P. javana* whole plant treated showed a significant decrease ($p<0.05$) in the content of glycosylated haemoglobin. Glycosylated haemoglobin determinations are self-monitoring of blood glucose therefore play an important complementary roles for the management of diabetes mellitus[35].

The levels of serum protein, and globulin of control, alloxan induce diabetic rats and drug treated rats were presented in Table 2. A significant reduction in serum protein, albumin and globulin were observed in alloxan induce diabetic rats (Group II) when compared to control (Group I) and glibenclamide treated rats (Group IV). On administration of ethanol extract of *P. javana* whole plant to be diabetic rats, the levels of protein, albumin and globulin were found to be restored in normal. There results were in accordance with the effects of Wattakaka volubilis, Senna auriculata, Pterocarpus marsupium and Eugina floccose[36-38,32].

Among the parameters of protein metabolism, the present study showed a sharp decline in total protein serum albumin and globulin in alloxan induced diabetic rats. This is in agreement with hypoalbuminemia observed in diabetes [39-40]. On the other hand, in the extract treated diabetic rats protein metabolism never derivated from normal range. Hypoalbuminemia is a common problem in diabetic animals and is generally attributed in the presents of nephropathy. An overall reduction in serum total protein in diabetic animals and albumin were observed in the present study. The corroboratus earlier reports[41]. The reversal of these changes by ethanol extract of *Polygala javana* whole plant therapy proved that insulin deficiency had been grossly corrected.

Table 2 summarized the effect of alloxan on the activity of the hepatic marker enzymes in serum. In the present study, the levels of SGPT and SCOT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating in to the circulation by the adverse effects of alloxan[42]. Asperateanimo transaminases and Alanine transaminases were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats [43]. In this study the ethanol extract of *Polygala javana* regulated the activity of SGPT and SCOT in liver of rats intoxicated with alloxan. The restoration of SGPT and SGOT to their respective normal levels after treatment with both glibenclamide and ethanol extract of *Polygala javana*, further strengthen the antidiabegenic effect of this extract. Moreover, SGPT and SGOT levels also act as indicators of liver function and restoration of normal levels of this parameter indicate normal functioning of liver, since the alloxan can also affect the liver by the free radical mechanism.

In addition to the assessment of SGPT and SGOT levels during diabetes the measurement of enzymatic activities of phosphatases such as acid phosphatase (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants. In the present study, serum ALP increased in alloxan induced diabetic rats (Table 2). Elevated level of this enzyme in diabetes may be due to extensive damage to liver in the experimental animal by alloxan. Treatment with ethanol extract of *Polygala javana* in alloxan induced diabetic rats produces a decline in ALP level.

The level of serum lipid profile, total cholesterol(TC), triglycerides(TG), LDL-C, VLDL-C, HDL-C and PL in control, diabetic induced and drug treated rats were presented in Table 3. Serum triglycerides, total cholesterol and VLDL-cholesterol level were found to be decreased in alloxan treated diabetic rats. Treatment with ethanol extract of *Polygala javana* produced a significant reduction in elevated TG, TC, LDL, VLDL within 14 days in diabetic rats. There was significant increase in HDL levels within 14 days. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. A variety of dearrangement in metabolic and regulatory mechanism, due to insulin deficiency, are responsible for the observed accumulation of lipids[44]. Diabetic rats treated with ethanol extract of *Polygala javana* and gliibenclamide also normalized lipids levels. Thus, the results indicate that ethanol extract of *Polygala javana* also may possess insulin like action by virtue of the ability to lower the lipid levels. The results are similar to earlier reports observed with the other plants [45,32].

To conclude it is suggested that the ethanol extract of *Polygala javana* whole plant shows antidiabetic and antihyperlipidaemic properties in animal models. A

preliminary phytochemical flavonoids, saponins, phenols and terpenoids and a GC-MS analysis squalene and 1H-Perimidine, 2,3- dihydro-2-(2,4,5,trimethoxyphenyl) having antioxidant activity which are known to enhance free radical scavenging leading to antidiabetic effect. Detailed studies on isolation, characterization and structure determination of the exact chemical compounds could lead to pharmacological potent drug molecules.

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REFERENCES

1. Edem DO. Hypoglycemic effects of ethanolic extracts of Allijator pear seed (*Persea americana* Mill) in rats. European J Sci. Res 2009; 33:669-678.
2. Kristova V, Liskoya S, Sotnikova S, Vojtko R, Kurtansky A. 2008. Sulodexide improves endothelial dys function streptozotocin-induced diabetic rats. Int J Pharmaceu Sci Rev Res 2008 1:18-22.
3. Sharma VK, Kumar S, Patel HS, Hugan S. Hypoglycemic activity of *Ficus glomerata* in alloxan induced diabetic rats. Int J Pharmaceu Sci Rev Res 2010; 1: 18-22.
4. Trivedi NA, Majumder B, Bhalt JD, Hemavathi KG. Effect of Shilajt on blood glucose and lipid profile in alloxan-induced diabetic rats. Indian J Pharmacol.2004; 36: 373-376.
5. Pradeepa R, Mohan V. The changing of the diabetes epidemic implications for India. Indian J Med Res 2002; 116:121-132.
6. Krishna B, Nammi S, Kota MK, Krishna Rao RV. Evaluation of hypoglycemic and antihypoglycemic effects of *Datura metal* linn seeds in normal and alloxan induced diabetic rats. J Ethnopharmacol 2004; 9:95-98.
7. Dallak M, Bin-Jaliah I. 2010.Antioxidnt activity of *Cittrullu scolocynthis* pulp extract in the RBC'S of alloxan, induced diabetic rats. Pale J Physiol 2010; 6: 1-5.
8. Mansi K ,Lahham J. Effect of *Artemisia siebeni* Besser (*A.herbalba*) on heart rate and some hematological values in normal and alloxan – induced diabetic rats. J Basic Appl Sci 2008; 4:57-62.
9. Lalitha rani S, Kalpanadevi V, Tresinasoris P, Maruthupandian A, Mohan VR. Ethnomedicinal plants used by Kanikkar of Agasthimalai biosphere Reserve Western Ghats. J Ecobiotech 2011; 3: 16-25 .
10. Brinda P, Sasikala P, Purushothaman KK. Pharmacognostic studies on Merugankizhangu. Bull Med EthnobotRes 1981; 3: 84-96.
11. Lala PK. Lab manuals of Pharmacognosy CSI Publishers and Distributers, Kolkata 1993.
12. OECD. (Organisation for Economic co-operation and Development). OECD guidelines for the testing of chemicals/Section 4: Health Effects Test No. 423; Acute oral Toxicity- Acute Toxic Class method. OECD. Paris 2002.
13. Nagappa AN, Thakurdesai PA, VenkatRao N, Sing J. Antidiabetic activity of *Terminalia catappa* Linn.fruits**.** J Ethnopharmacol 2003; 88: 45-50.
14. Sasaki T, Masty S, Sonae A. Effect of acetic acid concentration on the colour reaction in the Otoluidine boric acid method for blood glucose estimation. Rinshbo Kagaku 1972; 1: 346-353.
15. Anderson L, Dinesen B, Jorgonsen PN, Poulsen F, Roder ME. Enzyme immune assay for intact human insulin in serum or plasma. Clin Chem 1993; 39: 578-582.
16. Varley H. Practical clinical biochemistry**,** Arnold Heinemann Publication Pvt. Ltd., 1976; p, 452.
17. Owen JA, Iggo JB, Scangrett FJ, Steward IP. Determination of creatinine in plasma serum, a critical examination. J. Biochem 1954; 58: 426-437.
18. Karunanayake EH, Chandrasekharan NV. An evaluation of a colorimetric procedure for the estimation of glycosylated haemoglobin and establishment of reference values for Sri Lanka. J Nat Sci Coun Sri Lanka 1985; 13: 235-258.
19. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin's phenol reagent. J Bio Chem 1951; 193: 265-275.
20. Reitman S, Frankel SA.Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases.Amer. J Clin Path 1957; 28: 56-63.
21. King EJ, Armstrong AR. Determination of serum and bile phosphatase activity. Cannad Med Assoc J 1934; 31: 56-63.
22. Parekh AC. Jung. Cholesterol determination with ferric acetate, uranium acetate and sulphuric acid, ferrous sulphate reagent. Anal Chem 1970; 112: 1423-1427.
23. Rice EW. Triglycerides in Serum In: Standard Methods. Clinical Chemistry. 9ed Roderick MP, Academic press, New York. 1970; 215-222.
24. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultra centrifuge. Clin Chem 1972; 18: 499-502.
25. Warnick GR, Nguyan T, Albers AA. Comparison of improved precipitation methods for quantification of high density lipoprotein cholesterol. Clin Chem 1985; 31: 217.
26. Takayama M, Itoh S, Nagasaki T, Tanimizu I. A new enzymatic method for determination of serum phospholipids. Clin Chem Acta 1977; 79: 93 – 98.
27. Lenzen S, Panten U. Alloxan: History and mechanisms of action. Deabetologica 1998; 31: 337-342.
28. Anne J, Becle IR, Bleck C, Wienbergen A. Derensitization of Insulin secretion by Deprlarizing Insulin secretagogues. Diabetes 2004; 53:140-150.
29. Rajini V, Prasad KVSRG, Bharathi K. 2011.Antidiabetic and antioxidant activity of *Shoreatum beaggaia* Rox. Int J Inno Pharmaceu Res 2011; 2:113-121.
30. Cao J, Li C, Zhang P, Cao Xu, Huang T, Bai Y, Chen K. Antidiabetic effect of burdock (*Aretium lappa* L.) root ethanolic effect on streptozotocin-induced diabetic rats. Afr J Biotech 2012; 11:9079-9085.
31. Kala SMJ, Tresina PS, Mohan VR. Antioxidant, antihyperlipidaemic and antidiabetic activity of *Eugenia floccose* Bedd leaves in Alloxan induced diabetic rats**.** J Baric Clin Pharmacy 2012; 3: 235-240.
32. Shajeela PS, Kalpanadevi V, Mohan VR. Potential antidiabetic, hypolipidaemic and antioxidant effects of *Nymphaea pubercens* extract in Alloxan induced diabetic rats. J Appl Pharmacu. Sci 2012; 2: 83-88.
33. Almdal TP, Vilstrup H. Strict insulin treatment normalizes the organic nitrogen contents and the capacity of urea-N synthesis in experimental diabetes rats. Diabetologica. 1988; 31:114-118.
34. Alyassin D, Ibrahim KA. Minor haemoglobin fraction and the level of fasting blood glucose. J Fac Med Gnive Baghdad 1981; 23: 373-380.
35. Thai AC, Yeo PPB, Chan L et al., Glycosylated haemoglobin and diabetic control. Singapore Medicinal Journal 1983; 24: 210-212.
36. Maruthupandian A, Mohan VR. Antidiabetic, antihyperlipidaemic and antioxidant activity of *Pterocarpus marsupian* Roxb. In alloxan induced diabetic rats. Int J Pharm Tech Res 2011; 3: 1681-1687.
37. Shanmugasundaram R, Kalpana Devi V, TresinaSoris P, Maruthupandian A, Mohan VR. Antidiabetic, antihyperlipidaemic and antioxidant activity of *Senna auriculata* (L.) Roxb leaves in alloxan induced diabetic rats. Int J PharmTech Res 2011**;** 3: 747-756.
38. Maruthupandian A, Mohan VR, Sampathraj R. Antidiabetic, antihyperlipidaemic and antioxidant activity of *Wattaka kavolubilis*(L.f) Stapf leaves in alloxan induced diabetic rats. Int J Pharmaceut Sci Res 2010**;** 1: 83-90.
39. Purte DJ, Halter JB. 1981 Text book of Entoerinology. WB saundersCO. Philadelphia1981; p.715.
40. Sivajothi V, Dey A, Jayakar B, Rajkapoor B. Antihyperglycemic, antihyperlipidemic and antioxidant effect of *Phyllanthus rheedii* on streptozotocim induced diabetic rats. Iranian J Pharmaceau Res 2008; 7: 53-59.
41. Soon YY, Tan BKH. Evaluation of the hypoglycemic and antioxidant activity of *Morinda officinalis* in streptozotocin-induced diabetic rats. Singapore Med J 2002; 43:77-85.
42. Stanely P, Prince M, Menon V. Hypoglycemic and other related actions of *Tinospora cordifolia* roots in alloxan induced diabetic rats**.** J Ethnopharmacol 1999; 70: 9-15.
43. Hwang HJ, Kim SW, Lim JM, Joo JH, Kim HO, Kim HM, Yun JW. Hypoglycemic effect of crude epoxy polysaccharides produced by a medicinal mushroom *Phellinus baumii* in streptozotocin induced diabetic rats. Life Science 2005; 76: 3069 – 3080.
44. Rajalingam R, Srinivasam N, Govindanajulu P. Effect of alloxan induced diabetes on lipid profiles in renal cortex and medulla of mature albino rats. Indian J Exp Biol 1993; 31:577-579.
45. Kaleem M, Kirmani D, Asif M, Ahmld Q, Bano B.Biochemiical effects of *Nigella sativa* L seeds in diabetic rats. Indian J Exp Biol 2006; 44:745-748.

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