Research Article

Antidiabetic and Antihyperlipidaemic Activity of Ethanol extract of *Polycarpaea corymbosa* (L.) Lam Whole Plant in Alloxan Induced Diabetic Rats

Karuppasamy Balamurugan, Antony Nishanthini and

Veerabahu Ramasamy Mohan*

Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin -628 008, Tamil Nadu, India.

ABSTRACT

The ethanol extract of *Polycarpaea corymbosa* whole plant (Family: Caryophyllaceae) was investigated for its antihyperglycemic and antihyperlipidaemic effect in Wistar Albino rats. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg, i.p). The ethanol extract of *Polycarpaea corymbosa* at a dose of 150 and 300mg/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 *Polycarpaea corymbosa* whole plant on blood glucose, serum insulin, urea, creatinine, glycosylated haemoglobin, serum lipid profile [total cholesterol (TC), 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), Serum Glutamate Oxaloacetate Transaminases (SGOT), and Alkaline Phosphatase (ALP)], were measured in the diabetic rats. The ethanol extract of *Polycarpaea corymbosa* 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 *Polycarpaea corymbosa* possesses significant antihyperglycemic and antihyperlipidaemic effects in alloxan induced diabetic rats.

Keywords: *Polycarpaea corymbosa*, Antidiabetic, Antihyperlipidaemic, SGOT, SGPT and HbA_1C.

INTRODUCTION

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease. Diabetes mellitus is caused by the abnormalities of carbohydrates metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin[1]. It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million worldwide. In addition to elevated blood glucose levels, diabetes is generally accompanied with lipid metabolism abnormality communally known as diabetic dyslipidaemia, increase the risk for coronary heart disease[2]. In addition, prolonged hyperglycemia causes increased protein glycation, which has been known to be a source of free radicals.

Recently, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulfonylureas, d-phenylalanine and α-glucosidase inhibitors in addition to insulin. However, due to unwanted side effects in the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes[3]. Hence plants have been suggested as a rich, as yet unexplored source of potentially useful antidiabetic drugs. Many traditional plants treatment for diabetes are used throughout the world. Moreover, the World Health Organization (WHO) estimates that 80% of people in developing countries depend on traditional medicine for their health needs and 85% of traditional medicine involves the use of plant extracts. In other words, about 4 billion people in the world rely on plants as source of drugs. This has led researchers to continue their search for the “miracle drug” for treatment of diabetes from plants[4].

Polycarpaea corymbosa(L.) Lam. belongs to "Caryophyllaceae" is commonly known as "Palllipoondu" in Palliyar tribals of Sirumalai hills,Western Ghats Tamil Nadu. Paste prepared from the whole plant is taken once in a day for period of 2-3 weeks to treat jaundice by the palliyars[5]. Whole plant extract of *Polycarpaea corymbosa* were respond for the biological activities such as hepatoprotective and antiinflammatory [6,7]. Literature reviews indicated that the antidiabetic activity of whole plant of *Polycarpaea corymbosa* has not been scientifically evaluated so far. Hence in the present study, the ethanol extract of *Polycarpaea corymbosa* whole plant has been investigated for antihyperglycemic and antihyperlipidaemic activity in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Material

The whole plant of *Polycarpaea corymbosa* (L.) Lam were collected from Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. With the help of local flora, a voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

Preparation of plant extract for phytochemical screening and antidiabetic studies

The *Polycarpaea corymbosa* whole plant was shade dried at room temperature and the dried whole plant was powdered in a Wiley mill. Hundred grams of powdered whole plant was packed in a Soxhlet apparatus and extracted with ethanol. The extracts were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures[8,9]. 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±2ºC) 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. Study was carried out as per IAFC approval No: 82/PHARMA/SCRI, 2010.

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[10]. 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 Diabetes in Experimental animal

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg)[11]. 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 *Polycarpaea corymbosa* whole plant (150mg/kg body weight)

Group IV: Diabetic rats given ethanol extract of *Polycarpaea corymbosa* whole plant (300mg/kg body weight)

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

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.

Estimation of insulin, glucose, urea, creatinine and glycosylated haemoglobin

Serum glucose was measured by the Otoluidine method[12]. Insulin level was assayed by Enzyme Linked Immuno Sorbant Assay (ELISA) kit[13]. Urea estimation was carried out by the method of Varley[14]; serum creatinine was estimated by the method of Owen et al[15]. Glycosylated haemoglobin (HBA1C)

estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan[16].

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

Serum protein[17] 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[18]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong[19].

Estimation of lipids and lipoprotein

Serum total cholesterol (TC)[20], total triglycerides (TG)[21], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL- C)[22], high density lipoprotein cholesterol (HDL-C)[23] and phospholipids[24] 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.

RESULTS

Preliminary phytochemical screening and acute toxicity

The ethanol extract of whole plant of *P.corymbosa* subjected for phytochemical study showed the presence of alkaloids, coumarin, glycosides, flavonoids, steroids, saponin, phenols, tannins and xanthoprotein. The acute toxicity study carries out in wistar albino rats showed that ethanol extract of *P.corymbosa* whole plant was safe up to 2000mg/kg body weight. No death was observed until the end of the study.

Fasting Blood Glucose (FBG) Levels

The measured blood glucose levels of nondiabetic and alloxan induced diabetic rats are shown in Table 1. The administration of ethanol extract of *P.corymbosa* whole plant to diabetic rats produced a significant (p<0.05) reduction in blood glucose levels in a dose dependent manner as compared with alloxan induced control group (Group II).

Blood glucose level and other parameters levels

Table 2 shows the levels of blood glucose, serum insulin, urea, creatinine and glycosylated hemoglobin of normal, diabetic control and drug treated rats. There was a significant (p<0.01) increase blood glucose level in alloxan induced diabetic rats (Group II) when compared with normal rats (Group I). Administration of whole plant extract of *P.corymbosa* (Group III& IV) and glibenclamide (Group V) tends to bring the parameters significantly (p<0.05, p<0.01) towards the normal. Serum insulin level of diabetic control group was significantly (p<0.01) decreased when compared to normal control group (Group I). The extract and glibenclamide group of diabetic rats significantly (p<0.01) increased the serum insulin. A significant (p<0.05) elevation in urea and creatinine was observed in alloxan induced diabetic rats (Group II), when compared to control rats. The *P.corymbosa* extracts were administrated orally to diabetic rats for 14 days reversed the urea and creatinine level to near normal. Administration of ethanol extract of *P.corymbosa* whole plant (300mg/kg) and glibenclamide significantly (p<0.05) reduced HbA1C level compared to diabetic control rats.

Biochemical parameters levels changes in diabetic rats

The decreased protein, albumin and globulin levels were noticed in diabetic control rats (Group II) compared to control rats. Administration of ethanol extract of *P.corymbosa* whole plant 150 and 300mg/kg and glibenclamide increased protein, albumin and globulin levels compared to diabetic control rats (Table 3). Also, the serum SGOP, SGPT and ALP levels were elevated significantly (p<0.05) in alloxan induced diabetic rats compared to control rats. Both the doses of ethanol extract of *P.corymbosa* whole plant and glibenclamide treatment reduced above parameters compared to diabetic control rats.

Lipid profiles levels changes in diabetic rats

In the present study, induction of diabetes, significantly (p<0.01) altered the normal lipid profile levels compared to control rats. Administration of both doses of ethanol extract of *P.corymbosa* whole plant and glibenclamide significantly (p<0.05) decreased TC, TG, LDL, VLDL, PL levels and also significantly increased the HDL level compared to diabetic control rats (Table 4).

DISCUSSION

Alloxan is widely used for the induction of diabetes mellitus in experimental animals. It is

postulated to induce diabetes by degeneration and necrosis of β- cells of the islets of Langerhans of pancreas, which leads to reduction in insulin release[25]. It has been reported that using medicinal plant extract to treat alloxan induced diabetic rats result in activation of β-cells and insulinogenic effects[26]. *P.corymbosa* whole plant may also have brought about hypoglycemic action through stimulation of surviving β-cells islets of langherhans to release more insulin. This was clearly evidenced by the increased levels of plasma insulin in diabetic rats treated with *P.corymbosa*. Since the percentage fall in plasma glucose levels was different in models with varying intensity of hyperglycaemia, it implies that the antihyperglycaemic effect of that plant is dependent on the dosage of diabetogenic agent, which in turn leads to β-cells destruction[27]. A number of other plants have also been observed to exert hypoglycemic activity through insulin release stimulatory effects[28-30].

In diabetes, elevated levels of serum urea and creatinine are observed which may be due to renal damage caused by abnormal glucose regulation or elevated glucose and glycosylated protein tissue levels[31]. In present study, significant increase in serum urea and creatinine levels were observed in diabetic rats compared to normal control rats which indicate impaired renal function in diabetic rats. The treatment with ethanol extract of *P.corymbosa* lowered the above parameters significantly compared to diabetic control rats and it showed protective effect of ethanol extract of *P.corymbosa* on the kidneys.

In diabetes, HbA1C is considered as a diagnostic marker and helps to know about degree of protein glycation, long-term blood sugar level and correlation of diabetes associated complications[32, 33]. 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[30]. The rate of glycation is proportional to the concentration of blood glucose. In present study, alloxan induced diabetic rats showed significant increase (p<0.01) glycosylated haemoglobin (HbA1C) level compared with normal rats. The ethanol extract of *P.corymbosa* whole plant treated rats showed a significant decrease (p<0.05) in the content of glycosylated haemoglobin that could be due to an improvement in glycemic status.

Elevation of serum biomarker enzymes such as SGOT, SGPT and ALP was observed in diabetic rats indicating impaired liver function, which is obviously due to hepatocellular necrosis. The decreased total protein content in alloxan induced diabetic rats also substantiated the hepatic damage by alloxan. Diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activities[34]. The 14 days treatment with ethanol extract of *P.corymbosa* whole plant restored all the above mentioned hepatic biochemical parameters towards the normal levels in a dose dependent manner.

The concentration of lipids, such as cholesterol, TG, LDL-Cholesterol was significantly increased, whereas HDL-Cholesterol was decreased in the diabetic rats than normal rats. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids. Further it has been reported that diabetic rats treated with insulin shows normalized lipid levels[35]. Diabetic rats treated with ethanol extract of *P.corymbosa* whole plant and glibenclamide also normalized lipid levels. Thus, the results indicate that ethanol extract of *P.corymbosa* whole plant also may possess insulin like action by virture of the ability to lower the lipid levels. These results are similar to earlier reports observed with the other plant[36].

In conclusion, the preliminary investigation on the antidiabetic efficacy of ethanol extract of *P.corymbosa* whole plant will be significant to proceed further in this path for the isolation of active principles responsible for antidiabetic activity. The present study emphasizes that the ethanol extract has more antidiabetic effect than aqueous extract and it contains potent and safe antihyperglycemic principles unlike synthetic drugs. Further studies will be carried out to elucidate the exact mechanism of action of ethanol extract of *P.corymbosa* whole plant on diabetes and its antiperoxidative effect.

ACKNOWLEDGEMENT

The authors are thankful to Dr.R.Sampathraj, Honorary Director, Dr. Samsun Clinical Research Laboratory, Tirupur, for providing necessary facilities to carry out this work.

Table 1: Effect of *Polycarpaea corymbosa* whole plant extract on the body weight and fasting blood glucose in normal, diabetic and diabetic treated rats

Treatment groups	Mean initial body weight (g)	Mean final body weight (g)	Mean weight Gain (G↑) / loss(L↓) (g)	Fasting Blood glucose (mg/dl)	
				Initial	Final(after 2wks)
Group I	189.54±5.11	197.65±6.36	8.12↑	69.88±1.92	73.95±1.64
Group II	192.63±8.24	173.94±5.24	18.69↓**	248.56±2.84	263.16±5.36
Group III	208.44±7.66	216.80±5.08	8.36↑a	228.36±5.96	146.14±4.56b
Group IV	201.34±8.54	211.05±6.27	9.71↑a	221.93±5.28	131.64±5.16b
Group V	196.58±9.34	216.42±7.39	19.84↑a	229.66±6.16	104.53±4.35b

Each Value is SEM of 5 animals * $p < 0.05$ comparison with Normal control vs diabetic and drug treated : a $p< 0.05$ Diabetic control vs drug treated : b $p<0.05$ comparison with initial vs final

Table 2: Effect of *Polycarpaea corymbosa* whole plant extracts on the serum insulin, glucose, urea, creatinine and HbA1c level of normal, diabatic induced and drug treated rats

Parameter	Insulin (mIu/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	GlycolytedHb (%)
Group I	21.83±1.13	73.81±3.54	12.05±1.04	0.73±0.12	4.61±0.52
Group II	9.19±0.948**	198.39±2.61**	29.63±1.85*	2.45±0.35*	9.89±0.25**
Group III	13.26±1.56a	136.32±1.21a	22.06±1.14	1.94±0.36	7.08±0.36ns
Group IV	16.85±1.86a	124.43±1.73a	16.84±1.84	1.38±0.24	6.27±0.16ns
Group V	19.73±1.09aa	109.38±1.84aa	13.05±1.56a	1.05±0.11a	5.21±0.62a

Each Value is SEM of 5 animals :* Comparison made between normal control to diabetic control and drug treated groups; * $p < 0.05$,**$p<0.01$ a ,Comparison made between diabetic control to drug treated groups .a: $p<0.05$;aa $p<0.01$:ns :Not significant

Table 3: Effect of *Polycarpaea corymbosa* whole plant extracts on the serum protein, albumin, globulin, SGOT, SGPT and ALP level of normal, diabetic induced, and drug treated rats

Groups	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	7.93±0.16	4.63±0.14	3.30±0.11	18.53±1.62	20.16±1.33	184.93±3.65
Group II	6.02±0.19*	3.84±0.21*	2.18±0.24	97.18±3.54*	89.25±1.91*	226.16±4.84*
Group III	6.84±0.13ns	4.02±0.13	2.82±0.11	42.14±2.14	46.62±2.36	203.14±3.14[a]
Group IV	7.54±0.24ns	4.14±0.34	3.40±0.39	33.64±2.14	38.63±2.64	213.19±3.63ns
Group IV	7.96±0.38ns	4.03±0.45	3.93±0.24	21.94±1.12	25.88±1.65	173.94±2.16[a]

Each Value is SEM of 5 animals:* Comparison made between normal control to diabetic control and drug treated groups; * $p < 0.05$, **$p<0.01$ a ,Comparison made between diabetic control to drug treated groups . *a*: $p<0.05$;ns :Not significant

Table 4: Effect of *Polycarpaea corymbosa* whole plant extracts on the serum Lipid profile of normal, diabetic induced, and drug treated rats

Groups	TC (mg/dl)	TG(mg/dl)	HDL(mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)	PL (mg/dl)
Group I	103.34±2.18	93.61±1.64	42.98±2.16	41.64±1.32	18.72±1.03	159.97±3.51
Group II	214.26±4.76**	198.16±4.27**	26.36±1.65**	148.27±3.68**	39.63±1.26*	258.69±5.33**
Group III	174.36±1.76[a]	166.28±1.51[aa]	32.08±1.96[a]	102.10±0.96[a]	33.36±1.28[a]	214.11±1.78[a]
Group IV	163.16±1.94[a]	181.33±2.19ns	39.65±2.44[a]	87.24±1.39a	36.27±1.34*	213.21±2.83[a]
Group V	119.57±1.21[aa]	124.74±2.68[aa]	23.54±1.88	71.08±1.21[aa]	24.95±1.23[a]	174.41±1.92[aa]

Each Value is SEM of 5 animals :* Comparison made between normal control to diabetic control and drug treated groups; * $p < 0.05$,**$p<0.01$ a ,Comparison made between diabetic control to drug treated groups . a: $p<0.05$;aa $p<0.01$:ns :Not significant

REFERENCES

1. Maiti R, Jana D, Das UK and Ghosh D. Antidiabetic effect of aqueous extract of seed of Tamarindus indica in streptozotocin induced diabetic rats. J Ethnopharmacol. 2004;52:85-91.
2. Dangi KS and Mishra SN. Antihyperglycemic, antioxidant and hypolipidemic effect of Capparis aphylla stem extracts in Streptozotocin induced diabetic rats. Biol Med. 2010;2:35-44.
3. Ali MA, Sagar HA, Khatun MCS, Azad AK, Begum K and Wahed MII. Antihyperglycemic and analgesic activities of ethanol extract of Cassia fistula (L.) Stem bark. Int J Pharmac Sci Res. 2012; 3: 416-423.
4. Eseyin O, Ebong P, Eyong E, Awofisayo O and Agboke A. Effect of Telfairia occidentalis on oral glucose tolerance in rats. Afr J Pharm Pharmacol 2010;4:368-372.
5. Maruthupandian A, Mohan VR and Kottaimuthu R. Ethnomedicinal plants used for the treatment of diabetics and jaundice by palliyar tribals in Sirumalai hills, Western Ghats, Tamil Nadu, India. Indian J Nat Prod Resour. 2011; 2: 493 -497.
6. Nishanthini A, Balamurugan K and Mohan VR. Hepatoprotective and Antioxidant effect of Polycarpaea corymbosa against CCl4 induced hepatotoxicity in rats. Int J Adv Life Sci. 2012; 5: 104-111
7. Balamurugan K, Sakthidevi G and Mohan VR. Anti-inflammatory activity of whole plant of Polycarpaea corymbosa (L.) Lam (Caryophyllaceae). Pharmascience Monitor. 2012; 3: 3336- 3341.
8. Brinda P, Sasikala P and Purushothaman KK. Pharmacognostic studies on Merugan kizhangu. Bull Med Ethnobot Res 1981;3:84-96.
9. Lala PK. Lab manuals of Pharmacognosy CSI Publishers and Distributers, Kolkata 1993.
10. OECD. Organisation for Economic cooperation 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.
11. Nagappa AN, Thakurdesai PA, Venkat Rao N and Sing J. Antidiabetic activity of Terminalia catappa Linn. fruits. J Ethnopharmacol. 2003;88:45-50.
12. Sasaki T, Masty S and 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.
13. Anderson L, Dinesen B, Jorgonsen PN, Poulsen F and Roder ME. Enzyme immune assay for intact human insulin in serum or plasma. Clin Chem. 1993;39:578-582.
14. Varley H. Practical clinical biochemistry, Arnold Heinemann Publication Pvt. Ltd., 1976; 452.
15. Owen JA, Iggo JB, Scangrett FJ and Steward IP. Determination of creatinine in plasma serum, a critical examination. J Biochem. 1954;58:426-437.
16. Karunanayake EH and 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.
17. Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ. Protein measurement with the Folin's phenol reagent. J Bio Chem. 1951;193:265-275.
18. Reitman S and Frankel SA. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer J Clin Path. 1957; 28: 56-63.
19. King EJ and Armstrong AR. Determination of serum and bile phosphatase activity. Cannad Med Assoc J. 1934;31:56-63.
20. Parekh AC and Jung DH. Cholesterol determination with ferric acetate, uranium acetate and sulphuric acid, ferrous sulphate reagent. Anal Chem.1970;112:1423-1427.
21. Rice EW. Triglycerides in Serum In: Standard Methods. Clinical Chemistry. 9ed Roderick MP, Academic press, New York. 1970;215-222.
22. Friedwald WT, Levy RI and 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.
23. Warnick GR, Nguyan T and Albers AA. Comparison of improved precipitation methods for quantification

of high density lipoprotein cholesterol. Clin Chem. 1985;31:217.
24. Takayama M, Itoh S, Nagasaki T and Tanimizu I. A new enzymatic method for determination of serum phospholipids. Clin Chem Acta. 1977; 79:93-98.
25. Kundusem S, Gupta M, Mazumder UK, Halder PK, Saha P, Bhattacharya S, Kan B and Bala A. Antihyperglycemic effect and antioxidant property of Citrus maxima leaf in streptozotocin induced diabetic rats. Diabetologia croatica. 2011;40:113-120.
26. Anitha M, Rajalakshmi K, Muthukumarasamy S and Mohan VR. Antihyperglycemic, antihyperlipidaemic and antioxidant activity of Cynoglossum zeylanicum (Vahl Ex Hornem) Thurnb Ex lehrn in alloxan induced diabetic rats. Int J Pharm Pharm Sci. 2012;4:490-495.
27. Grover JK, Vats V and Rathi SS. Antihyperglycemic effect of Eugenia jambolana and Tinospora cordifolia in experimental diabetes and their effects on leaf metabolic enzymes involved in carbohydrate metabolism. J Ethnopharmacol. 2000;73:461-470.
28. Kala SMJ, Tresina PS and Mohan VR. Antioxidant, antihyperlipidaemic and antidiabetic activity of Eugenia floccosa Bedd leaves in alloxan induced diabetic rats. J Basic Clin Pharmacy. 2012;3:235-240.
29. Shajeela PS, Kalpanadevi V and 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.
30. Alagammal M, Nishanthini A and Mohan VR. Antihyperglycemic and Antihyperlipidaemic effect of Polygala rosmarinifolia Wright & Arn on alloxan induced diabetic rats. J App Pharmaceu Sci 2012; 2: 143-148.
31. Lal SS, Sukla Y, Singh A, Andriyas EA and Lall AM. Hyperuricemia, high serum urea and hypoproteinemia are are the risk factor for diabetes. Asian J Med Sci. 2009; 1: 33-34.
32. Deguchi Y and Miyazaki K. Anti-hyperglycemic and anti-hyperlipidemic effects of guava leaf extract. Nutr Metabol. 2010;7:1-10.
33. Lanjhiyana S, Garabadu D, Ahirwar D, Bigoniya P, Chandrana A and Chandrapatra K. Antidiabetic activity of methanolic extract of stem bark of Elaeodendron glaucum Pers. in alloxanized rat model. Adv Appl Sci Res. 2011;2:47-62.
34. Ghosh S and Suryawansi SA. Effect of Vinca rosea extracts in treatment of alloxan diabetes in male albino rats. Indian J Exp Biol. 2001; 39:748-759.
35. Ragini V, Prasad KVSRG and Bharathi K. Antidiabetic and antioxidant activity of Shorea tumbuggaia Rox. Int J Innovation Pharmac Res. 2011; 2: 113-121.
36. Maruthupandian A and Mohan VR. Antidiabetic, antihyperlipidaemic and antioxidant activity of Pterocarpus marsupian Roxb. in alloxan induced diabetic rats. Asian J Pharm Tech. 2011; 1:34-39.