CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1041986

Available online at: http://www.iajps.com Research Article

ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECTS OF METHANOL EXTRACT OF SESUVIUM PORTULACASTRUM. (AIZOACEAE)WHOLE PLANT IN ALLOXAN INDUCED DIABETIC RATS

Sheela. D and Uthayakumari Kalavathy*

*Head of the Department of Botany (Rtd) St. Mary's College. (Autonomous), Thoothukudi. Tamilnadu, India.

Abstract:

The methanol extract of Sesuvium portulacastrum whole plant (Family: Aizoaceae) was investigated for it antidiabetic and antihyperlipidemic effect in Wistar Albino rats. Diabetes was induced in albino rats by administration of alloxan monohydrate (150 mg/kg i.p). The Methanol extracts of Sesuvium portulacastrum at a dose of 150 and 300 mg/kg of body weight were administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of methanol extract of Sesuvium portulacastrum whole plant extract 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), and Serum glutamate oxaloacetate transaminases (SGOT), and alkaline phosphatase (ALP)] were measured in the diabetic rats. The Methanol extract of Sesuvium portulacastrum whole plant was non toxic at 2000 mg/kg in rats. The increased body weight, decreased blood glucose, glycosylated haemoglobin and other biochemical parameters level was observed in diabetic rats treated with both doses of methanol extract of Sesuvium portulacastrum whole plant compared to diabetic control rats. In diabetic rats, methanol extract of Sesuvium portulacastrum whole plant administration altered lipid profiles were reversed to near normal than diabetic control rats. From the above results, it is conducted that methanol extract of Sesuvium portulacastrum whole plant possess significant antidiabetic and antihyperlipidemic effects in alloxan induced diabetic rats.

Keywords: *Sesuvium portulacastrum, antidiabetic, Hypolipidemic, Alloxan, Glibenclamide.*

Corresponding author:

Dr.D.Sheela,

Head of the Department of Botany (Rtd),
St. Mary's College. (Autonomous),
Thoothukudi.
Tamilnadu, India.
Email – sheelajoshua77@gmail.com
Cell no: 9095361722.

QR code

*Please cite this article in press as Sheela .D and Uthayakumari K , **Antidiabetic And Antihyperlipidemic Effects of Methanol Extract of Sesuvium Portulacastrum. (Aizoaceae) Whole Plant in Alloxan Induced Diabetic Rats**, Indo Am. J. P. Sci, 2017; 4(11).*

INTRODUCTION:

Diabetes mellitus is one of the common metabolic disorders with micro- and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world. [1,2] and if not treated, it is responsible for many complications affecting various organs in the body [3] The Chronic hyperglyceamia of diabetes is associated with long term damage. Dysfunction and failure of various organs[4]. In diabetic rats, the utilization of impaired carbohydrate leads to accelerate lipolysis resulted in hyperlipidemia [5]. Despite the presence of known antidiabetic medicine in the pharmaceutical market diabetes and the related complications continued to be a major medical problem. Recently some Medicinal plants have been reported to be useful in diabetes world wide and have been used empirically as antidiabetic and antihyperlipidemic remedies. [6] Most of the plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc. that are frequently implicated as having antidiabetic effect [7]. However the study of plant for hypoglycemic, antioxidant and hypolipidemic activities may give new pharmacological approaches in the treatment of diabetes mellitus [8].

Sesuvium portulacastrum belong to the Aizoaceae family. It is also called as "Sea purslane". It is a perennial herb found on the sea coasts. It grows on the ocean side of the dunes down to the high tide mark. It is commonly called "Orputu and Vankaravacci". This plant is used in traditional medicine as a remedy for fever, kidney disorders and scurvy [9] by the indigenous people in Africa, Latin America and in Asian countries. The plant is used on the Senegal coast as a haemostatic and decoction of it is considered to be the best known antidote for stings of venomous fish. Leaves have acidulose flavor of sorrel as well as antiscorbutic [10].The essential oil from the fresh leaves of *S. portulacastrum* exhibited antibacterial, antifungal and antioxidant activity [11]. *S. portulacastrum* expresses fatty acid methyl esters (FAME extract) which can be used in medicine as a potential antimicrobial and antifungal agent [12]. There is no report on the antidiabetic and antihyperlipidaemic potential of this plant extract so far. The main objective of this study was to assess the antidiabetic and antihyperlipidaemic effect of methanol extracts of *Sesuvium portulacastrum* in alloxan induced diabetic rats.

MATERIALS AND METHODS:

Plant Material

The well grown whole plant of *Sesuvium pottulacastrum (L.)* L was collected from coastal regions of Thoothukudi district, Tamilnadu with the help of local flora, vocher specimens were identified and preserved in research department of botony, St.Mary's College, tutocorin Tamil Nadu for further references.Preparation of plant extract for phytochemical screening and antidiabetic studies

The whole plant of Sesuvium pottulacastrum (L.) was shade dried at room temperature and dried whole plants were powdered in a whiley mill. Hundered grams of powdered whole *Sesuvium pottulacastrum (L.)* plant was packed in a sohxle apparatus and extracted with methanol.

The extract was subjected to qualitative test for the identifiacation of various phytochemical constituents as per thr standard procedures[13,14].The methanol extracts were concentraded in a rotary evaporator.The concentrated methanol extracts were extract were used for antidiabetic studies.

Animals:

Normal healthy male Wistar rats (20-25gms) were housed under standard environmental conditions at temperature (25±20º C) and light and dark (12: 12 h). Rats were fed with standard pellet diet (Sai Durga Animal Feeds, Bangalore, India) and water *ad libitum.*

Acute Toxicity Study:

Acute oral toxicity study was performed as per OECD423guidelines. (Acute toxic class method), albinorats(n=6) of either sex selected by random sam pling were used for acute toxicity study [15]. The ani mals 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 same dose 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 intraperitonial route of all alloxan monohydrate (150 mg/kg)[16].

Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200,260 mg/100ml were taken for the study. All animals were allowed free acess 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 diab etic 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 methanol extract of *S.portulacastrum* whole plant (150mg/kg) body weight.
Group IV: Diabetic rats given methanol extract of *S.portulacastrum* whole plant (300mg/kg body weight).
Group V: Diabetic rats given standard drug glibencla mide (600µg/kg body weight).
The animals were sacrificed at the end of experiment al 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 O-toluidine method [17]. Insulin level was assayed by enzyme linked immunosorbant assay (ELISA) kit [18]. Urea estimation was carried out bythe method of Owen et al [20].Glycolated haemoglobin(HBA_1C) estimation was carried out by amodified colorimetric method of karunanayake and chandrasekharan [21].

Estimation of protein, albumin, globulin, SGPT, S GOT, ALP

Serum protein [22] and serum albumin were determined by quantitative colorimetrically method by using bromocresol green. The total protein minus the albumin gives the globulin, serum glutamate pyruvate transaminase transaminase (SGPT)and serum glutamate oxaloac etate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel [23]. Serum alkaline phosphatase (ALP) was measured by the method of King and Frankel [23].Serum alkaline phosphatase (ALP) was measured by the m ethod of King and Armstrong [24].

Estimation of lipids and lipoprotein

Serum total cholesterol (TC)[25], total triglycerides (TG) [26], low density lipoprotein cholesterol (LDL-C),very low density lipoprotein cholesterol (VLD-C)[27]high density lipoprotein cholesterol(HDL-C) [28] and phospholipids [29] were analyzed.

Statistical analysis

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

RESULTS:

Phytochemical constituents

The phytochemical screening of methanol extract of ***S.portulacastrum*** extracts whole plant revealed the presence of alkaloid, coumarin, flavonoid, phenols, saponins, steroids, tannins, terpenoids, sugar and glycoside.

Acute toxicity study

The methanol extrct was safe upto a dose of 2000mg/kg body weight. Behavior of the animals was clearly observed for the first 8 hours then at an inteeval of every 4 hours during the next 48 hours, the extract did not cause mortality on rats during 48 hours observation or any behavioral change.

Body weight and fasting blood glucose (FBG) level changes in diabetic rats

In the present study, alloxan induced diabetic rats showed significant ($p<0.05$) reduction in body weight (Table1). The administration of *S.portulacast rum* and glibenclamide to diabetic rats restored the changes in the levels of body weight. Table-1 shows the dose dependent antihyperglycemic activity of *S.portulacastrum* extracts. The FBG levels of diabetic rats were significantly ($p<0.001$) higher than those of normal control rats. When different doses of *S.portulacastrum*were tested for their gluco se lowering effects, the methanol extract at a dosage of 300 mg/kg body weight produced the maximum fall in the FBG levels of diabetic rats after 2 weeks of treatment.

Table 1: Effect of methanol extracts of Sesuvium portulacastrum whole plant on the Body weight and Fasting Blood Glucose in Normal, Diabetic induced and diabetic treated rats.

Parameter	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 2 wks)
Group I	213.56±9.42	218.06±8.36	5.09↑	68.34±1.31	78.65±6.54
Group II	226.83±8.16	204.54±9.89	22.29↓**	221.66±11.82***	214.59±9.64***
Group III	211.54±9.37	219.62±8.27	8.08↑	204.58±9.28***	163.62±2.64*ab
Group IV	204.55±8.36	209.31±7.84	4.76↑	198.68±7.36***	138.12±5.89aabb
Group V	208.16±9.27	214.28±9.66	6.12↑	198.16±7.58***	108.31±5.66aabb

Each Value is SEM of 5 animal: * *p<0.05 comparison with Normal Control vs Diabetic and Drug treated: *p<0.05;**p<0.01; ***p<0.001;ns- Not Significant a-p<0.05 Diabetic Control vs Drug treated;b-p<0.05 comparison with intial vs final*

Blood glucose and the other parameters levels of diabetic rats

Table.2 shows the levels of blood glucose, serum insulin, urea, creatinine and glycosylated haemoglobin of normal,diabetic control and drug treated rats. There was a significant (p<0.001) increase in blood glucose level in alloxan induced diabetic rats (Group.II) when compared with normal rats (Group.I).The administration of whole plant extract of *S.portulacastrum*(Group.III and IV) and glibenclamide(GroupV)tends to bring the parameters (p<0.05) towards the normal. Serum insulin level of diabetic control group was significantly (p<0.001) decreased when compared to normal control group (Group.I).The plant extract and glibenclamide group of diabetic rats significantly (p<0.05; p<0.01) increased insulin. A significantly elevation in urea and creatinine was observed in alloxan induced diabetic rats when compared to control rats.

The *S.portulacastrum*extracts were admistered orally to diabetic rats for 14 days reversed the urea and creatinine levels to near normal

The S.portulacastrum whole plant and glibenclamide (p<0.05; p<0.001) reduced HbA_1C.

Table 2: Effect of methanol extracts of *Sesuvium portulacastrum* on the Serum Insulin, Glucose, Urea, Creatinine and Glycosylated Haemoglobin level of Normal, Diabetic induced and diabetic treated rats.

Parameter	Insulin(mμ/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl	Glycosylated Hb
Group I	16.23±1.94	81.23±1.64	13.28±0.67	0.63±0.04	4.34±0.03
Group II	7.84±1.31**	224.88±8.93***	24.67±1.22*	2.98±0.07**	11.84±1.23**
Group III	9.54±1.84*	163.84±6.36^{**a}	20.16±1.08*	1.81±0.03*	10.29±0.94*
Group IV	11.98±1.22^{ns}	144.67±2.16^{*aa}	17.28±0.84^{ns}	1.14±0.02^{nsa}	8.13±0.54^{*ns}
Group V	15.27±1.93^{nsa}	93.54±3.54^{aaa}	15.18±0.78^{a}	0.93±0.07^{aa}	5.81±.013^{aa}

Each Value is SEM of 5 animal: * *p<0.05* ****p<0.001. Comparison made between Normal Control and Diabetic control and Drug treated group.* ^{a}*p<0.05;*^{aa} *-p<0.01- Comparison made between Diabetic Control and Drug treated group.*

Biochemical parameters levels in diabetic rats

The decreased total protein, albumin and globulin levels were noticed in diabetic control rats (Group II) (Table.3). The administration of *S.portulacastrum* Whole plant extract 150 and 300mg/mg and glibenclamide significant (p<0.05) increased total protein, albumin and globulin compared to diabetic control rats

Also, the SGPT,SGOT and ALP levels were elivated in alloxan induced diabetic rats compared to control rats.

Oral administration of *S.portulacastrum whole plant* extract 300mg/kg and glibenclamide treatment reduced above parameters compare to diabetic control rats.

Table 3: Effect of methanol extracts of *Sesuvium portulacastrum* on the Serum protein, Albumin, Globulin, SGOT, SGPT and ALP level of Normal, Diabetic induced and diabetic treated rats.

Parameter	Protein(g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (μ/l)	SGOT(μ/l)	ALP(μ/l)
Group I	9.16±1.13	4.83±0.14	4.28±0.31	13.22±0.67	19.36±0.13	213.16±6.93
Group II	6.04±0.81**	4.16±0.24	1.88±0.65**	31.62±1.39**	34.22±0.98*	263.93±11.96*
Group III	7.36±0.65	4.11±0.16	3.25±0.11	27.14±1.13	23.84±1.22	235.84±8.46
Group IV	7.13±0.63	4.84±0.38	2.29±0.13	26.84±0.93	15.62±1.93	229.56±9.19
Group V	8.68±0.16^{a}	5.08±0.39^{a}	3.60±0.16^{a}	15.93±0.81	17.16±0.17^{a}	194.51±6.73^{a}

Each Value is SEM of 5 animal. * *p<0.05. Comparison made between Normal Control and Diabetic control and Drug treated group.* ^{a} *p <0.05; Comparison made between Diabetic Control and Drug treated group.*

Table 4: Effect of methanol extracts of *Sesuvium portulacastrum* on the Serum Lipid profile of Normal, Diabetic induced and diabetic treated rats.

Parameter	TC(mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)	HDL(mg/dl)	PL(mg/dl)
Group I	128.55±4.83	112.84±2.93	34.63±1.21	22.56±1.34	71.36±2.16	182.40±3.54
Group II	184.65±5.95***	166.28±2.15**	21.84±1.05*	33.25±1.87*	129.56±2.87**	232.33±4.76**
Group III	162.13±3.73**	158.13±2.84**	26.33±1.16	31.62±1.22*	104.18±2.13*	212.29±2.12*
Group IV	130.89±4.66**	124.83±3.11^{aa}	30.88±1.84^{a}	24.96±1.08^{ns}	86.05±1.34^{a}	194.28±3.02^{a}
Group V	133.94±3.29^{aa}	124.63±2.56	31.14±1.24^{aa}	24.92±1.23^{a}	77.88±1.48^{a}	187.20±1.47^{aa}

Each Value is SEM of 5 animal. * *p<0.05;* ***p<0.01 Comparison made between Normal Control and Diabetic control and Drug treated group.* ^{a} *p <0.05;* ^{aa}*p<0.01- Comparison made between Diabetic Control and Drug treated group.*

Lipid profiles

Table – 4 shows the levels of TC,TG,LDL-C,VLDL-C,HDL-C AND PL in the serum of diabetic rats showed significantly ($p<0.05$) increased serum lipid profiles except HDL-C, when compared with normal rats. The methanol extract of *S.portulacastrum* whole plant treated rats showed a significant ($p<0.05$) decrease in the content of lipid profiles when compared with diabetic induced rats.

Similarly HDL-C level decreased in alloxan induced diabetic rats when compared with normal rats. The administration of methanol extract of *S.portulacastrum* whole plant and glibenclamide to the diabetic rats, HDL-C level found to be restored to normal.

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[30]. It has been reported that using medicinal plant extract to treat alloxan induced diabetic rats result in activation of β-cells and insulinogenic effects [31]. *S.portulacastrum* 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 *S.portulacastrum* . Since the percentage fall in plasma glucose levels was different in models with varying intensity of hyperglycemia, it implies that the antihyperglycemic effect of that plant is dependent on the dosage of diabetogenic agent, which in turn leads to β- cells destruction [32]. A number of other plants have also been observed to exert hypoglycemic activity through insulin release stimulatory effects [33,34,35] . 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 [36]. 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 methanol extract of *S.portulacastrum* lowered the above parameters significantly compared to diabetic control rats and it showed protective effect of methanol extract of *S.portulacastrum* 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 [37,38]. 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[39]. 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 methanol extract of *S.portulacastrum* 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 [39]. The 14 days treatment with methanol extract of *S.portulacastrum* 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 [40]. Diabetic rats treated with methanol extract of *S.portulacastrum* whole plant and glibenclamide also normalized lipid levels. Thus, the results indicate that methanol extract of *S.portulacastrum* 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 [41].

CONCLUSION:

The preliminary investigation on the antidiabetic efficacy of methanol extract of *S.portulacastrum* 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 methanol 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

methanol extract of *S.portulacastrum* whole plant on diabetes and its antiperoxidative effect.

ACKNOWLEDGEMENT

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

REFERENCES:

1. Vats V, Yadav S.P, Grover J.K. Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats. Journal of Ethnopharmacology.2004; 90, 1: 155-160.

2. Kumar G.PS, Arulselvan P, Kumar D.S, Subramanian S.P. 2006. Anti-diabetic activity of fruits of *Terminalia chebula* on streptozotocin induced diabetic rats. Journal of Health Science. 2006; 52, 3:283-291.

3. El-Hilaly J, Tahraoui A, Israili ZH, Lyoussi B. Acute hypoglycemic hypocholesterolemic and hypotriglyceridemic effects of continuous intravenous infusion of a lyophilised aqueous extract of *Ajuga iva* L. Schreber whole plant in streptozotocin-induced diabetic rats. Pakistan Journal of Pharmaceutical Sciences. 2007; 20(4): 261-268.

4. Lyra R, Oliveira M, Lins D, Cavalcanti N. Prevention of type 2 diabetes mellitus. *Arquivos Brasileiros* de Endocrinologia & Metabologia. 2006; 50(2): 239-249.

5. Morel DW, Chisolm GM. Antioxidant treatment of diabetic rats inhibits lipoprotein oxidation and cytotoxicity. The Journal of Lipid Research.1986. 30(12): 1827-1834.

6. Mitra SK, Gopumadhavan S, Muralidhar TS,Anturlikar SD, Sujatha MB. Effect of a herbomineral preparation
D-400 in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology. 1996; 54: 41-46.

7. Malviya N, Jain S, Malviya S. Antidiabetic potential of medicinal plants Acta pol pharm.2010; 67:113-118.

8.Dangi KS, Mishra SN. Antihyperglycemic antioxidant and hypolipidemic effect of *Capparis aphylla* stem extracts in Streptozotocin induced diabetic rats. Bio Med. 2010; 2:35-44.

9. Rojas A, Hernandez L, Rogeho PM, Mata R. Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. J Ethnopharmacol.1992;35: 127-149.

10.Hammer K, Aizoaceae. In: Hanelt P.Instituteof Plant Genetics and Crop Plant Research(eds) Mansfeld's encyclopedia on agricultural and horticultural crops, vol 1. Springer Verlag,Berlin, Heidelberg, New York:1986;223-227.

11. Michael L.M, Mazuru G, Nyasha G,Godfred H. Chemical composition and biological activities of essential oil from the leaves of Sesuvium portulacastrum. J.Ethnopharmacol.2006;103: 85-9.

12. Chandrasekaran M, Senthilkumar A, Venkatesalu V. Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. Eur RevMed Pharmacol Sci.2011;7: 775-80.

13. Vasantha K, Priyavardhini S,Tresina Soris P, Mohan VR. Phytochemical analysis and Antibacteri al activity of *Kedrostis foetidissima* (Jacq) Cogn. *Bioscience Discovery.2012;*3: 06-16.

14.Murugan M. Mohan VR. Evaluation of phytoch emical analysis and antibacterial activity of *Buahi nia purpurea* L. and *Hiptage benghalensis* L.Kurz. *J. Appl. Pharmeu. Sci* .2011; 01: 157-160.

15. OECD. Organisation for Economic cooperation a nd Development). OECD guidelines forthe testing of chemicals/Section 4: HealthEffects Test No. 423; A cute oral Toxicity Acute Toxic Class method. OECD .Paris. 2002

16. Nagappa AN, Thakurdesai PA, Venkat Rao N, SingJ. Antidiabetic activity of *Terminalia catappa* Linn. fruits. *J. Ethnopharmacol* .2003; 88: 45-50.

17.Sasaki T, Masty S, Sonae A. Effect of acetic acid concentration on the colour reaction in the Otoluidine boric acid method for bloodglucose estimation. *Rinshbo Kagaku.* 1972; 1: 346-353.

18.Anderson L, Dinesen B, Jorgonsen PN, Poulsen F, Roder ME. Enzyme immune assay for Intact human insulin in serum or plasma. *Clin Che m.* 1993; 39: 578-582.

19.Varley H.Practical clinical biochemistry, Arnold HeinemannPublication Pvt. Ltd., 1976; 452.

20. Owen JA, Iggo JB, Scangrett FJ, Steward IP. Determination of creatinine in plasma serum, a Critical examination. *J . Biochem.* 1954; 58: 426-437.

21.Karunanayake EH, Chandrasekharan NV. An evaluation of a colorimetric procedure for the estimation of glycosylated haemoglobin and establi shment of reference values for Sri Lanka. *J Nat Sci Coun SriLanka.* 1985; 13: 235-258.

22.Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin's Phenol reagent. *J Bio Chem.* 1951; 193: 265-275.

23.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.

24. King EJ, Armstrong AR.Determination of serum and bile phosphatase activity. *Cannad Med Assoc J. 1934*; 31: 56-63.

25. Parekh AC, Jung. Cholesterol determination with ferric acetate, uranium acetate and sulphuric acid, ferrous sulphate reagent. *Anal Chem,1970*; 112: 14 23-1427.
26. Rice EW. Triglycerides in Serum In: Standard M ethods. Clinical Chemistry. 9ed Roderick MP, Acade mic press, New York. 1970; 215-222.
27.Friedwald WT, Levy RI, Fredrickson DS. Esti mation of the concentration of low density Lipoprote in cholesterol in plasma without use of the preparative ultra centrifuge.*Clin Chem.1972* ;18: 499-502.
28.Warnick GR, Nguyan T, Albers AA.Comparis on of improved precipitation methods for quantific ation of high density lipoprotein cholesterol. *Clin Chem.*1985; 31: 217.
29.Takayama M, Itoh S, Nagasaki T, Tanimizu I. A new enzymatic method for determination of serum phospholipids. *Clin Chem Acta.* 1977; 79: 9 3 – 98.
30. Kundusem S, Gupta M, Mazumder UK, Halder PK, Saha P, Bhattacharya S, Bala A. Antihyperglycemic effect and antioxidant property of Citrus maxima leaf in streptozotocin induced diabetic rats. Diabetologia croatica.2011; 40:113-120.
31. Anitha M, Rajalakshmi K, Muthukumarasamy S, 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.2011; 4:490-495.
32. Grover JK, Vats V, 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.
33. Kala SMJ, Tresina PS, 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.
34. 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.
35. Alagammal M, Nishanthini A, Mohan VR. Antihyperglycemic and Antihyperlipidaemic effect of *Polygala rosmarinifolia* Wright & Arn on alloxan induced diabetic rats. J App Pharmaceu Sci.2012; 2: 143-148.
36. Lal SS, Sukla Y, Singh A, Andriyas EA, Lall AM. Hyperuricemia, high serum urea and hypoproteinemia are the risk factor for diabetes. Asian J Med Sci.2009;1: 33-34.
37. Deguchi Y, Miyazaki K. Antihyperglycemic and anti-hyperlipidemic effects of guava leaf extract. Nutr Metabol.2010;7:1-10.
38. Lanjhiyana S, Garabadu D, Ahirwar D, Bigoniya P, Chandrana A, 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.
39. Ghosh S, Suryawansi SA. Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats. Indian J Exp Biol. 2001;39:748-759.
40.Ragini V, Prasad KVSRG, Bharathi K. Antidiabetic and antioxidant activity of *Shorea tumbuggaia* Rox. Int J Innovation Pharmac Res.2011; 2: 113-121.
41. Maruthupandian A,Mohan VR. Antidiabetic, antihyperlipidaemic and antioxidant activity of *Pterocarpus marsupian* Roxb. in alloxan induced diabetic rats. Asian J Pharm Tech. 2011;1:34-39.