Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 1, 2013

Research Article

POTENTIAL ANTIDIABETIC, HYPOLIPIDAEMIC AND ANTIOXIDANT EFFECTS OF *XANTHOSOMA SAGITTIFOLIUM* EXTRACT IN ALLOXAN INDUCED DIABETIC RATS

SHAJEELA. P.S.[1], KALPANADEVI. V[2] AND MOHAN. V.R.[2*]

[1]PG & Research Department of Botany, St. John's College, Palayamkottai 627002, Tamil Nadu, India, [2]Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin-628008, Tamil Nadu, India. Email: vrmohan_2005@yahoo.com

Received: 08 May 2012, Revised and Accepted: 12 Jun 2012

ABSTRACT

The present study was designed to investigate the possible antidiabetic, hypolipidemic and antioxidant effects of ethanol extract of *Xanthosoma sagittifolium* corm. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150 mg/kg, body weight i.p). The ethanol extract of *Xanthosoma sagittifolium* corm at a dose of 200mg/kg and 500mg/kg 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 *Xanthosoma sagittifolium* corm extract on blood glucose, plasma insulin, urea creatinine, glycosylated haemoglobin, serum lipid profile [total cholesterol (TC), triglyceride (TG), low density lipoprotein- cholesterol (LDL-C), very low density lipoprotein- cholesterol (VLDL-C), high density lipoprotein- cholesterol (HDL-C)and phospholipids (PL)], serum protein, albumin, globulin, serum enzymes [serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transaminases (SGOT) and alkaline phosphate (ALP)], lipoprotein peroxidation (LPO), blood reduced glutathione (GSH), oxidative glutathione (GSSG), GSH/GSSG ratio, erythrocytes glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione S-transferase (GST) were measured in the diabetic rats. The ethanol extract of *Xanthosoma sagittifolium* corm elicited significant (p<0.05) reductions of blood glucose, lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C and antioxidant. The extract also caused significant increase in plasma insulin (p<0.05) in the diabetic rats. In conclusion, ethanol extract of *Xanthosoma sagittifolium* corm offers promising antidiabetic and hypolipidemic effects that may be mainly attributed to its potent antioxidant potential. Further studies will be needed in future in order to determine which one or more of its active constituents have the main antidiabetic and hypolidemic effects.

Keywords*:* Diabetes, Alloxan, Antioxidant, *Xanthosoma sagittifolium*.

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, nephropathy, neuropathy and angiopathy and several disorders[2]. India has today, become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025[3]. Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes and high oxidative stress induced damage to pancreatic beta cells. These metabolic disturbances result in acute and long-term diabetic complications which are responsible for premature death and disability[4].

In traditional practice, medicinal plants are used in many countries to control Diabetes mellitus. The National Center for Complementary and Alternative Medicine, established in 1998 by the United States Government where development of herbal medicines is one of the important subjects of study[5]. Many herbal products have been described for the cure of diabetes mellitus. The antidiabetic properties of some plants like *Momordica charantia*, *Azadiracta indica*, *Ocimum sanctum* and *Allium saivum* are well known in India. These herbal mixtures have been cited as dietary supplements and are quoted as especially useful for lowering the blood glucose level in diabetic patients[6,7]. This study was designed to investigate the protective effect of new herbal extracts namely, *Xanthosoma sagittifolium* corm in antidiabetic, hypolipidemic and antioxidant activities in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant material

Xanthosoma sagittifolium corms were freshly collected from the Injikuzhi, Agasthiarmalai Biosphere Reserve, Western Ghats, 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 *Xanthosoma sagittifolium* corms were shade dried at room temperature and the dried corms were powdered in a Wiley mill. Hundred grams of powdered *Xanthosoma sagittifolium* corm 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 standard procedures[8,9,10].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±2º C) and light and dark (12: 12 h).Rats were feed 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[11].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 intraperitioneal dose of alloxan monohydrate (150 mg/kg)[12].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 investigation, a total of 25 rats (20 diabetic surviving rats and 5 normal rats) were taken and divided in to five groups of 5 rats each.

Group I: Normal, untreated rats.

Group II: Diabetic control rats

Group III: Diabetic rats given ethanol extract of *Xanthosoma sagittifolium* corm (200 mg/kg of body weight).

Group IV: Diabetic rats given ethanol extract of *Xanthosoma sagittifolium* corm (500 mg/kg of body weight).

Group V: Diabetic rats given standard drug glibenclamide (600µg/kg of 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[13]. Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit[14]. Urea estimation was carried out by the method of Varley[15]; serum creatinine was estimated by the method of Owen *et al*[16]. Glycosylated haemoglobin (HbA_1C) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan[17]. Serum total cholesterol (TC)[18], total triglycerides (TG)[19], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL- C)[20], high density lipoprotein cholesterol (HDL-C)[21]and phospholipids[22]were analyzed. Serum protein[23] 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[24]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong [25]. Lipid peroxidation (LPO)[26], reduced glutathione (GSH)[27], Oxidized glutathione[28], glutathione reductase (GR)[29], glutathione peroxidase (GPx)[30], and glutathione S-transferase (GST)[31]were analyzed in the normal, diabetic induced and drug treated rats.

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 AND DISCUSSION

The phytochemical screening of ethanol extract of *Xanthosoma sagittifolium* corm revealed the presence of alkaloids, flavonoids, glycosides,terpenoids, tannins, phenols, saponins and steroids. Acute toxicity study revealed the non-toxic nature of the ethanol extract *Xanthosoma sagittifolium* corm. Table 1 shows the levels of blood glucose, plasma insulin, urea, creatinine and glycosylated haemoglobin levels, while the plasma insulin level decreases significantly in alloxan induced diabetic rats (Group II) when compared with normal rats (Group I). Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptoztocin. Alloxan has a destructive effect on the beta cells of the pancreas[32,33]. Alloxan causes a massive reduction in the insulin release by the destruction of beta cells of the islets of largerhans, thereby inducing hyperglycemic[34]. Insulin deficiency leads to various metabolic alterations in the animals viz, increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases[35,36,37]·[38]. The results of the present study indicated that *Xanthosoma sagittifolium* corm extract was found to reduce the glucose level and increase the plasma insulin significantly. The hypoglycemic effect of *Xanthosoma sagittifolium* corm was found to be inducing insulin release from pancreatic cells of diabetic[39]. It is evident from this study that, there was an increase in insulin levels in diabetic rats treated with plant extract.

Table 1: Effect of ethanol extracts of *Xanthosoma sagittifolium* corm on the serum glucose, insulin, urea, creatinine and glycosylated Hb level of normal, diabetic induced and drug treated adult albino rats.

Parameter	Insulin (MIu/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Glycosylated Hb
Group I	20.40 ± 1.2	72.40 ± 3.8	13.34 ± 1.2	0.73 ± 0.1	4.80± 0.8
Group II	08.30 ± 0.8*	186.60 ± 9.7*	34.33 ± 2.8*	1.01 ± 0.4*	11.40±0.7*
Group III	15.3 0± 0.9[a]	118.40 ± 7.8[a]	16.43 ± 1.4[a]	0.79 ± 0.3	7.80±0.4
Group IV	16.50 ± 0.9[a]	81.40 ± 3.1[aa]	13.31 ± 1.9	0.71 ± 0.4	6.80±0.3
Group V	16.54±0.5[a]	73.56±1.1[aa]	14.57±2.6	0.73±0.24	4.10±0.5[a]

Each value is SEM of 5 animals, Comparisons were made between normal control to diabetic control, * $p < 0.05$ and comparisons were made between diabetic control to drug treated groups: [a] $p<0.05$; [aa] $p<0.01$ level.

A significant elevation in serum constituents, urea and creatinine were observed in alloxan induced diabetic rats (Group II) when compared to control rats. The results from the present study also indicated that *Xanthosoma sagittifolium* corm extract can reduce the levels of serum urea and creatinine and confirms the protection of vital tissues (Kidney and liver) including the pancreas, thereby reducing the causation of diabetes in the experimental animals.

Alloxan induced diabetic rats showed significant increased ($p<0.05$) glycosylated haemoglobin (HbA_1C) level compared with normal rats. The ethanol extract of *Xanthosoma sagittifolium* treated rats showed the decrease in the content of glycosylated haemoglobin. Glycosylated haemoglobin determinations are self monitoring of blood glucose therefore play important complementary roles for the management of diabetes mellitus[40].

Table 2: Effect of ethanol extracts of *Xanthosoma sagittifolium* corm on the protein, albumin, globulin, SGPT, SGOT and ALP level of normal, diabetic induced and drug treated adult albino rats.

Parameter	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	6.71 ± 0.2	3.70 ± 0.1	3.0 ± 0.2	16.30 ± 5.8	24.70 ± 5.2	132.40 ± 4.9
Group II	3.74 ± 0.4	2.90 ± 0.2	1.10 ± 0.4	96.10 ±3.4*	88.30 ±7.3*	182.32 ± 6.4*
Group III	6.51 ± 0.3	3.50 ± 0.5	2.90 ± 0.3	32.40 ± 9.2	25.40 ± 2.8	116.41 ± 5.4
Group V	7.20 ± 0.2	3.70 ± 0.3	3.50 ± 0.7	19.40 ± 6.9	18.20 ± 7.2	114.63 ± 3.1
Group VII	7.10±0.3	4.0±0.2	3.10±0.1	17.50±2.1[a]	16.30±0.5[a]	103.20±5.8

Each value is SEM of 5 animals, Comparisons were made between normal control to diabetic control and drug treated groups * $p < 0.05$ and comparisons were made between diabetic control to drug treated groups: [a] $p<0.05$.

The levels of serum protein, albumin and globulin of control, alloxan induced diabetic rats and drug treated rats were presented in Table 2. Reduction in serum protein, albumin and globulin were observed in alloxan induced diabetic rats (Group II) when compared to control (Group I). There values showed tendency to retrieve towards near normal values in ethanol extract of *Xanthosoma sagittifolium* corm administrated groups (Group III and IV) and glibenclamide (Group V) rats. These results were in accordance with the effect of *Wattakaka volubilis* and *Pterocarpium marsupium* in diabetic rats[41,42].

Table 2 summarized the effect of alloxan on the activity of the hepatic marker enzymes in serum. In the present study, SGPT, SGOT and ALP levels were increased significantly (p<0.05) in alloxan induced diabetic rats in respect to control group. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan[43]. In the present study, these elevated parameters in serum were come towards control level after treatment with *Xanthosoma sagittifolium* corm extract. The restorations of SGPT, SGOT and ALP to their respective normal levels after treatment with both glibenclamide and ethanol extract of *Xanthosoma sagittifolium* corm, further strengthen the antidiabegenic effect of these extract. Moreover SGPT and SGOT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver. Since the alloxan can also affect the liver by free radical mechanism.

Hypercholesterolemia and hypertriglyceridemia are independent risk factors that alone or together can accelerate the development of coronary artery disease and progression of atherosclerotic lesions[44]. Diabetes mellitus is one of the most common metabolic diseases and the derangements in lipid metabolism[45].

The levels of serum lipid profiles, total cholesterol (TC), triglycerides (TG), LDL-C, VLDL-C, HDL-C and PL in control and diabetic rats were investigated (Table 3). Alloxan induced diabetic rats showed significantly increased serum lipid profile except HDL-C, when compared with normal rats. The glibenclamide and ethanol extract of *Xanthosoma sagittifolium* corm treated rats showed a significant decrease in the content of lipid profile, when compared with diabetic induced rats. Similarly HDL-C level decreased in alloxan induced diabetic rats when compared with normal rats. On administration of ethanol extract of *Xanthosoma sagittifolium* corm and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normal. HDL helps to scavenge cholesterol from extra hepatic tissues[46]. Decreased HDL can contribute to the increased cholesterol levels. A greater increase of LDL may cause a greater decrease of HDL as there is a reciprocal relation between the concentration of LDL and HDL.

Table 3: Effect of ethanol extracts of *Xanthosoma sagittifolium* corm on the TC, TG, LDL-C, VLDL-C, HDL-C and PL level in the plasma of normal, diabetic induced and drug treated adult albino rats.

Parameter	TC (mg/dl)	TG(mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	HDL -C (mg/dl)	PL (mg/dl)
Group I	98.67±2.98	72.33±3.98	34.56 ± 1.98	14.46 ± 1.32	49.65 ± 2.11	155.83±5.66
Group II	172.81 ± 8.56*	236.66 ±9.45*	105.93 ±4.78*	47.33 ± 2.02*	19.55 ± 1.78*	208.23±8.89*
Group III	118.56± 2.12[a]	163.21 ± 1.5[a]	35.73±2.66[a]	32.64±1.89	50.23 ±2.22[a]	168.74±2.12[a]
Group IV	97.56± 3.43[a]	117.34±4.67[aa]	20.80±1.88[aa]	23.46±1.98[a]	53.34±2.76[a]	156.31±5.90[a]
Group V	104.45±6.98	98.56±3.45	39.43±1.67	19.65±1.22	46.34±1.98	160.56±5.78

Each value is SEM of 5 animals, Comparisons were made between normal control to diabetic control, * p < 0.05 and comparisons were made between diabetic control to drug treated groups: [a] p<0.05; [aa] p<0.01 level.

Phospholipids (PL) were increased in alloxan induced diabetic rats. Phospholipids are present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasm environment and non-polar lipoprotein or lipoprotein core[47]. Increased phospholipids levels in tissues were reported by[48,49] in Streptozotocin diabetic rats. Administration of ethanol extract of *Xanthosoma sagittifolium* corm and glibenclamide decreased the levels of phospholipids.

The increases in the levels of LPO due to the effects of diabetes are shown in Table 4. The results obtained showed that lipids of the diabetic rats are vulnerable to peroxidation due to the increased oxidative stress during diabetes. LPO plays an important role in aging, atherosclerosis and in a number of diabetic complications[50,51]. As diabetes and its complications are associated with free radical medicated cellular damage[52], herbal hypoglycemic agents are administered to diabetic rats to assess their antioxidant potential. In the present study, *Xanthosoma sagittifolium* corm extract not only have hypoglycemic activity but these compounds also significantly control the LPO levels in diabetic rats.

The level of GSH, GSSG, GSH/GSSG ratio in the blood and GR, GPx, GST in the erythrocytes of normal, diabetic induced and drug treated rats were studied. The highly significant reduction of the activity of scavenging mitochondrial enzymes are observed in alloxan induced rats. These adverse changes were reversed to near normal values in ethanol extract of *Xanthosoma sagittifolium* (Table-4).

Table 4: Effect of ethanol extracts of *Xanthosoma sagittifolium* corm on the GSH, GSSG, GSH/GSSG, LPO, GR, GPx and GST activity of normal, diabetic induced and drug treated rats.

Parameter	Blood				Erythrocytes		
	GSH mol/mL	GSSG mol/mL	GSH/GSSG Ratio	LPO (nmol/mL)	GR nmol/min/mg protien	GPx nmol/min/mg protien	GST nmol/min/mg protien
Group I	1.56±0.08	0.08±0.004	19.5	0.89±0.067	4.845±0.87	74.67±11.23	62.45±6.56
Group II	1.02±0.04*	0.21±0.008*	4.8	2.78±0.037*	1.12±0.31*	46.67±10.32**	11.23±5.87**
Group III	1.67±0.09[a]	0.11±0.006	15.1	1.78±0.023	1.98±0.23	58.69±08.38	34.51±4.67
Group IV	1.78±0.12	0.07±0.009[a]	25.4	1.11±0.034[a]	4.45±0.49[a]	49.56±08.71[a]	46.64±6.64[a]
Group V	1.58±0.05	0.09±0.005	17.5	1.21±0.071	3.97±0.34	69.67±08.64	52.76±6.23

Each value is SEM of 5 animals, Comparisons were made between normal control to diabetic control, * p < 0.05 ** p<0.01 and comparisons were made between diabetic control to drug treated groups: [a] p<0.05level.

Mitochondria are the energy reservoir of the cells and the damage inflicted in mitochondria would ultimately result in the reduction of energy production and thereby leading to cell death[53]. Subcellular membrane associated with thiol bearing enzymes, represents sensitive riskes for detoxification causing perpetuation of cellular function[54]. Reactive oxygen species can themselves reduce the activities of antioxidant defence mechanism. In the present study ethanol extract of *Xanthosoma sagittifolium* have enhanced mitochondrial enzymatic antioxidant activity and suppressed lipid peroxidation.

GSH is a major non- protein thiol in living organisms which plays a central role in coordinating the body's antioxidant defence processes. Perturbation of GSH status of a biological system has been reported to lead to serious consequences[55]. Decline in GSH content in the serum of diabetic induced rats, and its subsequent return towards near normally in plant extracts treated rats reveal the antioxidant effect of *Xanthosoma sagittifolium*. Explanations of the possible mechanism underlying the antioxidant properties of this drug include the prevention of GSH depletion and destruction of free radicals[56]. These two factors are believed to attribute to the antioxidant properties of *Xanthosoma sagittifolium.*

GPx is a seleno-enzyme two third of which (in liver) is present in the cytosol and one third in the mitochondria. In hyperglycemia, glucose undergoes autooxidation and produces superoxide and it produces free radical that in turn leads to lipid peroxidation in lipoproteins. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. In the present study, decline in the activities of these enzymes in alloxan-induced diabetic animals and attainment of near normalcy in diabetic induced plant extracts treated groups indicate the oxidative stress elicited by alloxan had been nullified due to the effect of the extracts. This observation perfectly agrees with those of hypoglycemic and antioxidant activity of *Salacia oblonga*[57].

GSSG of blood was increased significantly in diabetic induced animals in comparison with control group. The ethanol extract of *Xanthosoma sagittifolium* treated rats decreased the levels of GSSG when compared with diabetic rats. The GSH / GSSG ratio in blood was also decreased significantly in diabetic induced animals, but in the plant extract treated group the decrease was less, in comparison with the control animals. Whenever GSH / GSSG ratio decreases, there is an adverse effect on several key enzymes of glycolysis[58]. Increased amount of GSSG are transported out of cells to maintain the normal ratio[59] but when accumulated inside the cell, GSSG creates oxidative stress, and various cellular components become vulnerable to damage by reactive oxygen species mainly membrane lipids, protein and DNA. GSH / GSSG ratio is maintained by enzymatic activities of GR and GPx. GR converts GSSG to GSH in the presence of NADPH, while GPx acts as an antioxidant. Glutathione – S-transferase (GST) activity was decreased significantly in the diabetic induced rats. The decrease in GR activity in diabetic induced animals may be responsible for the higher levels of GSSG. The present study indicates a reduction in the activity of GR, GPx, and GST in alloxan induced rats. These results revealed the protective role of plant extract in decreasing lipid peroxidation and by normalizing antioxidant system.

In conclusion, ethanol extract of *Xanthosoma sagittifolium* corm offers a promising therapeutic value in prevention of diabetes. These effects could be mainly attributed to its antioxidant properties as shown by significant quenching impact on the extract of lipid peroxidation along with, enhancement of antioxidant defense systems in pancreatic tissue. The antioxidative properly of *Xanthosoma sagittifolium* extract certainly is due to its chemical constituents. Phytochemical investigations of *Xanthosoma sagittifolium* have demonstrated the presence of flavonoids and phenolic compounds as main active ingredients having potent antioxidant activities. Further studied will be needed in future to determine the main active ingredient having the beneficial antidiabetic, hypolipidemic and antioxidant effects.

ACKNOWLEDGEMENT

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

REFERENCES

1. Edem DO. Hypoglycemic effects of ethanolic extracts of Alligator Pear seed (*Persea Americana* Mill) in rats. Euro J Sci Res 2009; 33: 669-678.
2. Kristova V, Liskoya S, Sotnikava S, Vojtko R and Kurtansky A. Sulodexide improve Endothelial Dysfunction in Streptozotocin-induced diabetes in rats. Physiol Res 2008; 5: 491-498.
3. Sridhar GR. Diabetes in India: Snapshot of a panorama. Curr Sci 2000; 83: 791.
4. Aravind K, Pradeepa R and Deepa R. Diabetes and coronary artery disease. Ind J Med Res 2002; 116: 163-176.
5. Yoon SL, Horne CH and Adams C. Herbal product used by African American older women. Clin Nurs Res 2004; 13: 271-288.
6. Khosla P, Sangeeta B, Singh J, Seth S and Srivastava RK. A study of hypoglycemic effects of *Azadirachta indica* (Neem) in normal and alloxan diabetic rabbits. Ind J Physiol Pharmacol 2000; 44: 69-74.
7. Chandra A, Singh RK, Singh VK, Tewari L and Mahdi AA. Antioxidative potential of herbal hypoglycemic agents in diabetes- An overview SFRR- Ind Bull 2004; 3: 24-26.
8. Brinda P, Sasikala P and Purushothaman KK. Pharmacognostic studies on *Merugan kizhangu*. Bull Med Ethnobot Res 1981; 3:84-96.
9. Anonymous. Phytochemical investigation of certain medicinal plants used in Ayurveda. Central Council for Research in Ayurveda and Siddha, New Delhi. 1990.
10. Lala PK. Lab manuals of Pharmacognosy CSI Publishers and Distributers, Kolkata. 1993.
11. 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.
12. Nagappa AN, Thakurdesai PA, Venkat Rao N and Sing J. Antidiabetic activity of *Terminalia catappa* Linn. Fruits. J Ethnopharmacol 2003; 88: 45-50.
13. Sasaki T, Matsuy S and Sanae A. Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose determination. Ransho Kagajci 1972; 1: 346-350.
14. 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.
15. Varley H. Practical clinical biochemistry, Arnold Heinemann Publication Pvt. Ltd. 1976; p, 452.
16. Owen JA, Iggo JB, Scangrett FJ and Steward IP. Determination of creatinine in plasma serum, a critical examination. J Biochem 1954; 58: 426-437.
17. 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.
18. Parekh AC and Jung. Cholesterol determination with ferric acetate, uranium acetate and sulphuric acid, ferrous sulphate reagent. Anal Chem 1970; 112: 1423-1427.
19. Rice EW. Triglycerides in Serum In: Standard Methods. Clinical Chemistry. 9ed Roderick MP, Academic press, New York. 1970; 215-222.
20. 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.
21. Warnick GR, Nguyan T and Albers AA. Comparison of improved precipitation methods for quantification of high density lipoprotein cholesterol. Clin Chem 1985; 31: 217.
22. 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.
23. Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ. Protein measurement with the folin's phenol reagent. J Bio Chem 1951; 193: 265-275.
24. 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.
25. King EJ and Armstrong AR. Determination of serum and bile phosphatase activity. Cannad Med Assoc J 1934; 31: 56-63.
26. Rehman S. Lead – induced regional lipid peroxidation in brain. Toxicol Lett 1984; 21: 333-337.
27. Prins HK and Loos JA. In Glutathione; Biochemical methods in red cell genetics, edited by J.J Yunis. Academic Press, New York. 1969; 127-129.

28. Owen Joshua B, Butterfield D A. Measurement of Oxidized/ Reduced Glutathione Ratio. Methods Mol Biol 2010; 648: 269-77.
29. Goldbery DM, Spooner RJ. Glutathione Reductase, In: Methods in Enzymatic Analysis, VCH Weinhem, Germany. 1983; 258-265.
30. Pagila DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin med 1967; 70: 158-169.
31. Habig WH, Pabst MJ and Jacob WB. Glutathione s-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-7139.
32. Jelodar G, Mohren M and Shahram S. Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan- induced diabetic rats. Afr J Trad Com Alt med 2003; 3: 299-305.
33. Prince SM and Menon VP. Hypoglcemic and other related actions of *Tinospora cardifolia* roots in alloxan induced diabetic rats. J Ethnopharmacol 2000; 70: 9-15.
34. Grover JK, Vats V and Rathi SS. Antihyperglycemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism, J Ethnopharmacol 2000; 73:461-470.
35. Begum W and Shanmugasundaram KR. Tissue phosphates in experimental diabetes. Arogya: J Health science 1978; 4: 129-139.
36. Shanmugasundaram KR, Panneerselvam SP and Shanmugasundaram ERB. Enzyme changes and glucose utilization in diabetic rabbit. The effect of *Gymnema sylvestrae*, R,Br. J Ethnopharmacology 1983; 7: 205-216.
37. Sharma N and Garg V. Antidiabetic and antioxidant potential of ethanolic extract of *Butea monosperma* leaves in alloxan induced diabetic mice. Ind J Biochem Biophys 2009; 46: 99-105.
38. Masih M, Banerjee T, Banerjee B and Pal A. Antidiabetic activity of *Acalypha indica* Linn. on normal and alloxan induced diabetic rats. Int J Pharm Pharm Sci 2011; 3: 51-54.
39. Sarasa D, Sridhar S and Prabakaran E. Effect of an antidiabetic extract of *Trigonella foenum graceum* on alloxan induced diabetic mice. Int J Pharm Pharm Sci 2012; 4: 63-65.
40. Thai AC, Yeo PPB, Chan L, Wang KW, Tan BY and Jocobs E. Glycoslated haemoglobin and diabetic control. Singapore Medical Journal 1983; 24: 210-212.
41. Maruthupandian A, Mohan VR and Sampathraj R. Antidiabetic, antihyperlipidaemic and antioxidant activity of *Wattakaka volubilis* (L.F) Stapf. Leaf. Int J Pharm Sci Res 2010; 11: 83-90.
42. Maruthupandian A and Mohan VR. Antidiabetic, antihyperlipidaemic and antioxidant activity of *Pterocarpus marsupium* Roxb. In alloxan induced diabetic rats. Int J PharmTech Res 2011; 3: 1681-1687.
43. Stanely P, Prince M and Menon V. Hypoglycemic and other related actions of *Tinospora cordifolia* roots in alloxan induced diabetic rats. J Ethnopharmacol 1999; 70: 9-15.
44. Mc Kenney JM. Pharmacotherapy of dyslipidemia. Cardiovasc Durg Ther 2001; 15: 413-422.
45. Fumelli P, Romagnoli F, Carlino G, Fumelli C and Bomei M. Diabetes mellitus and chronic heart failure. Arch Gerontol Geriatr 1996; 23: 277-281.
46. Brewer HB. Focus on high density lipoproteins in reducing cardiovascular risk. Am Heart J 2004; 148: 514-518.
47. Cohn RM and Roth KS. Lipid and lipoprotein metabolism In: *Biochemistry and Diseases*. Williams and Wilkins publishers, Baltimore 1996. p. 280.
48. Venkateswaran S, Pari L and Saravanan G. Effect of *Phaseolus vulgaris* on circulatory antioxidants and lipids in streptozotocin-induced diabetic rats. J Med Food 2002; 5: 97-104.
49. Pari L and Satheesh AM. Antidiabetic effect of *Boerhavia diffusa:* effect on serum and tissue lipids in experimental diabetes. J Med Food 2004; **7:** 472-476.
50. Kesavulu MM, Rao Bk, Giri R, Vijya JS and Subramanyam ACH. Lipid peroxidation and antioxidant enzymes status in type 2 diabetics with coronary heart disease. Diabetes Res Clin Prac 2001; 53: 33-39.
51. Matkovics B, Varga SI, Szabo L and Witas H. The effect of diabetes on the activities of the peroxide metabolism enzymes. Horm Metab Res 1982; 14: 77-79.
52. Yu BP. Cellular defense against damage from reactive oxygen species. Physiological Rev 1994; 74: 139-162.
53. Sohal R and Dubey A. Mitochondria oxidative damage, hydrogen peroxide release and aging. Free Rad Biol Med 1994; 16: 621-626.
54. Kyu BK and Byung ML. Oxidative stress to DNA, protein and antioxidant enzymes in rats treated with Benzo(a)Pyrene Cancer Lett 1997; 113: 205-212.
55. Uday B, Das D and Banerjee K. Reactive oxygen species: oxidative damage and pathogenesis. Curr Sci 1999; **77:** 658-665.
56. Valenzuela A, Lagos C, Schmidt K and Videla K. Silymarin Protection against hepatic lipid peroxidation induced by acute ethanol intoxication in the rat. Biochem Pharmacol 1985; 3: 2209-2212.
57. Krishnakumar K, Augusti KT and Vjayammal PL. Hypoglycemic and anti-oxidant activity of *Salacia oblonga* wall. Extract in streptozotocin-induced diabetic rats. Indian J Physiol Pharmacol 1999; 43: 510-514.
58. Gilbert HF. Biological disulfides: the third messenger: Modulation of phosphofructokinase activity by thiol / disulfide exchange. J Biol chem 1982; 257: 12086-12091.
59. Eklow L, Thor H and Orrenius S. Formation and efflux of glutathione disulfide studied in isolated rat hepatocytes. FEBS Let 1981; 127: 125-129.