International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol. 3, No.2, pp 747-756, April-June 2011

Antidiabetic, antihyperlipidaemic and antioxidant activity of *Senna auriculata* (L.) Roxb. leaves in alloxan induced diabetic rats

Shanmugasundaram R., Kalpana Devi V., Tresina Soris P.,

Maruthupandian A., Mohan V.R.*

Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu, India – 628008

Corres. author E-mail: vrmohan_2005@yahoo.com

Abstract: The ethanol extract of *Senna auriculata* (L.) Roxb. (Family: Fabaceae) leaf was investigated for its antidiabetic effect in wistar Albino rats. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg, i.p).The ethanol extract of *Senna auriculata* at a dose of 150mg/kg of body weight was administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of *Senna auriculata* leaf on blood glucose, plasma insulin, creatinine and urea, 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)], lipid peroxidation (LPO) antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione peroxidase (GPx) were measured in the diabetic rats. The ethanol extract of *Senna auriculata* leaf elicited significant reductions of blood glucose ($P<0.05$), lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C and antioxidant enzymes. The extracts also caused significant increase in plasma insulin ($P<0.05$) in the diabetic rats. From the above results, it is concluded that ethanol extract of *Senna auriculata* possesses significant antidiabetic, antihyperlipidaemic and antioxidant effects in alloxan induced diabetic rats.

Key words: Antidiabetic, antihyperlipidaemic and antioxidant activity, *Senna auriculata,* Alloxan induced Diabetic rats.

Introduction:

Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting from malfunction in insulin secretion and /or insulin action both causing by impaired metabolism of glucose, lipids and protein [1]. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs [2]. The number of people suffering from the disease world wide is increasing at an alarming rate with a projected 366 million peoples likely to be diabetic by the year 2030 as against 191 million estimated in 2000 [3]. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations.

In the present investigation, *Senna auriculata* (L.) Roxb leaves were tested for their antidiabetic, antihyperlipidaemic and antioxidant efficacy. *Senna auriculata* (L.)Roxb (Family: Fabaceae) is widely used in Indian traditional medicines and flowers are used for diabetes [4]; leaves and flowers to treat skin diseases [5]; leaf juice is used to reduce body heat [6].The flower and leaf extracts have antidiabetic activity in experimentally induced diabetes rats [7,8]. In view of above medicinal properties, the present study was conducted to investigate the antidiabetic,

antihyperlipidaemic and antioxidant activities of ethanol extract of *Senna auriculata* leaf in alloxan induced diabetic rats.

Materials and Methods:

Plant material:

Senna auriculata (L.) Roxb. leaves were freshly collected from the 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 *Senna auriculata* leaves were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *Senna auriculata* 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 [9,10,11].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 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 [12]. 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 1000 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)[13]. 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 24 rats (18 diabetic surviving rats and 6 normal rats) were taken and divided into four groups of 6 rats each.

Group I: Normal, untreated rats.

Group II: Diabetic control rats

Group III: Diabetic rats given ethanol extract of *Senna auriculata* leaf (150 mg/kg of body weight).

Group IV: Diabetic rats given standard drug glibenclamide (100mg/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 [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 [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]. Catalase (CAT) [26], superoxide dismutase (SOD) [27], lipid peroxidation (LPO) [28], reduced glutathione (GSH) [29] and glutathione peroxidase (GPx) [30] 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.

Table 1: Effect of ethanol extract of *Senna auriculata* leaf on the insulin, blood glucose, urea, creatinine level of normal, diabetic induced and drug treated rats

Parameter	Insulin (MIu/ml)	Blood glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Group I	20.4±1.2	71.4 ±3.8	17.34±1.2	0.53±0.1
Group II	8.3±0.8*	235.6 ±9.7*	37.33±2.8*	0.91±0.4*
Group III	16.3 ± 0.9*	111.6±7.3*	15.43±1.9*	0.79±0.3
Group IV	19.8±0.9*	98.4±5.3*	16.78±1.8	0.89±06

Each Value is ± SEM of 6 animals * P < 0.05 – Normal control vs diabetic control; Diabetic control vs treatment groups.

Table 2: Effect of ethanol extract of *Senna auriculata* leaf on the glucose tolerance in normal, diabetic induced and drug treated rats at different time intervals

Experimental animals	Blood Glucose levels (mg/dl)			
	Pre - treatment		Post treatment	
	FBG	1 hour	2 hour	3 hour
Group I	88.40±5.2	91.3±2.9	164.3±5.6	124.4±3.9
Group II	194.5±3.9*	215.5±3.1*	236.0±3.1*	206.4±2.4*
Group III	118.3±2.6	139.4±2.9	130.4±3.1	121.4±2.8*
Group IV	98.3 ±2.6*	123.6±3.6*	104.4±2.6*	96.5±1.9*

Each Value is ±SEM of 6 animals * P < 0.05 – Normal control vs diabetic control; Diabetic control vs treatment groups.

Note: Blood samples were collected just prior to glucose administration taken as 0hr value and after 1hr, 2hr and 3hr of glucose loading and their glucose levels were measured.

Table 3: Effect of ethanol extract of *Senna auriculata* leaf on blood glucose level of normal, diabetic induced and drug treated rats at different week intervals

Groups	Blood glucose level in mg/dl		
	0 day	1 week (after 7 days)	2 week (after 14 days)
Group I	92.53±2.50*	89.31±3.10	82.41±2.60
Group II	210.25±3.75*	221.31±3.70*	219.43±2.92*
Group III	231.25±4.3	156.31±3.70*	103.41±2.91*
Group IV	216.31±3.71	171.34±4.21	93.11±3.21*

Each Value is ±SEM of 6 animals * P < 0.05: Normal control vs diabetic control; Diabetic control vs treatment groups.

Table 4: Effect of ethanol extract of *Senna auriculata* leaf on the protein, albumin, globulin, SGPT, SGOT and ALP level of normal, diabetic induced 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	7.70 ± 0.2	3.7± 0.2	3.0 ± 0.2	66.3 ± 4.8	72.4 ± 5.9	142.4 ± 4.9
Group II	5.40 ± 0.4	2.9 ± 0.2	2.2 ± 0.4	256.1 ± 3.4*	198.3 ± 6.3*	196.32 ±7.4*
Group III	7.10 ± 0.2	3.5 ± 0.5	3.6 ± 0.3	82.4 ± 10.2*	65.4 ± 2.8*	176.41 ± 7.4
Group IV	7.12± 0.9	4.0±0.3	3.1±0.7	74.5±8.8*	56.5±1.9*	168.5±6.9

Each Value is ± SEM of 6 animals * P < 0.05: Normal control vs diabetic control; Diabetic control vs treatment groups

Table 5: Effect of ethanol extract of *Senna auriculata* leaf on the TC, TG, LDL-C, VLDL-C, HDL-C and PL in the plasma of normal, diabetic induced and drug treated 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	83.1 ± 4.2	62.4± 2.9	27.6 ± 1.3	12.41 ± 1.9	43.31 ± 1.9	141.56 ± 5.9
Group II	151.31± 3.9*	184.11±4.1*	93.61±5.1*	37.21±1.6*	21.56 ± 2.3*	202.39 ± 6.9
Group III	91 ± 0.2	73.13 ± 3.6	30.31 ± 2.4	15.59 ± 1.2	46.37 ± 1.9*	148.59 ± 5.4
Group IV	88± 0.9*	60.14 ± 4.3*	27.4±1.9	12.51±0.9	49.31 ±1.8	146.63±6.3

Each value is ±SEM 6 animals. * P < 0.05: Normal control vs diabetic control; Diabetic control vs treatment groups.

Table 6: Effect of ethanol extract of *Senna auriculata* leaf on the LPO and activities of SOD, CAT, GPx and GSH enzymes in the plasma of normal, diabetic induced and drug treated rats

Parameter	LPO (mmole/dl)	SOD (unit x/mg protein)	CAT (unit y/mg protein)	GPx (unit z/mg protein)	GSH (mg/dl)
Group I	0.61 ± 0.5	5.31 ± 0.9	0.56 ± 3.8	6.12 ± 0.6	82.09±5.04
Group II	2.20 ± 1.3*	2.01 ± 0.3*	3.91 ± 4.2*	2.98 ± 0.9*	39.50±7.27**
Group III	0.91 ± 0.6	3.91 ± 0.4*	0.41 ± 4.1*	5.54 ± 1.1*	71.66±3.85
Group IV	0.74 ± 0.4	4.91 ± 0.3	0.45 ± 3.9	5.81 ±1.6	69.62±3.85

*p< 0.05: Normal control vs diabetic control; Diabetic control vs treatment groups. ** p <0.01

Values are given as means ± S.D from six rats in each groupx- **x**; One unit of SOD is defined as the enzyme concentration which gives 50% inhibition of NBT reduction in one minute.**y-** One unit of CAT is defined as the mole of hydrogen peroxide consumed per minute.**z-** One unit of GPx is defined as the µg of glutathione consumed per minute

Results:

The phytochemical screening of ethanol extract of *Senna auriculata* leaf revealed the presence of alkaloid, anthraquinone, catechin, flavonoid, phenol, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotien. Acute toxicity study revealed the non-toxic nature of the ethanol extract of *Senna auriculata* leaf.

Table 1 shows the levels of blood glucose, plasma insulin, urea and creatinine of normal and experimental rats. There was a significant elevation in blood glucose, urea and creatinine levels, while the plasma insulin level decreased significantly in alloxan induced diabetic rats (Group II) when compared with normal rats (Group I). Administration of leaf extract of *Senna auriculata* (Group III) and glibenclamide (Group IV) tends to bring the parameters significantly towards the normal. The effect of *Senna auriculata* leaf extract at the dose of 150 mg/ kg body weight was highly significant in restoring to normalcy and therefore, the dose was used for further biochemical studies.

Table 2 depicts the effect of *Senna auriculata* leaf extract and glibenclamide, on glucose tolerance upto 3h diabetic rats. This helps in deciding the dose of 150 mg/ kg body weight as the most effective dose for the study of severe diabetic models. The fall of blood glucose level (BGL) observed after 2 hrs of glucose administration was 130.4 mg/dl and 104.4 mg/dl with the doses of 150, and 100 mg/kg body weight respectively. Since, the initial fall found at 1h was 139.4 mg/dl and 123.6 mg/dl from dose of 150, mg/ kg body weight and 100 mg /kg body weight respectively, suggesting thereby that 150 mg kg^{-1} body weight is the most effective dose for assessing the antidiabetic potential of ethanol extract in diabetic induced rats.

The impact of repeated oral administration of *Senna auriculata* leaf extract and glibenclamide on normal and diabetic rats was shown in **Table3.** The fasting plasma glucose (FBG) levels remain practically the same before and after the treatment with vehicle (saline only) in case of normal control rats. Whereas, in diabetic control rats the fasting plasma glucose levels rises gradually in 2 weeks after treatment with vehicle (saline only). Moreover, the 2-week treatment with the most effective dose (150, mg/ kg body weight) of the *Senna auriculata* leaf extract decreases FBG significantly from 231.25 mg/dl to103.41 mg/dl. This sharp fall of fasting plasma glucose levels was a clear evidence of significant antidiabetic effect of *Senna auriculata* leaf extract. Further, the same trend was

observed in the known antidiabetic drug glibenclamide treated group

The levels of total protein, albumin, globulin, and liver marker enzymes such as SGPT, SGOT and ALP in the serum of diabetic rats are presented in the **Table 4.** The diabetic rats (Group II) had decreased levels of serum total protein, albumin, globulin and elevated level of liver marker enzymes such as SGPT, SGOT and ALP when compared with normal control rats (Group I). After treatment with *Senna auriculata* leaf extract, glibenclamide, total protein, albumin, globulin, and liver marker enzymes were brought back to near normal levels (Group III & IV).

Table 5 shows the levels of TC, TG, LDL-C, VLDL-C, HDL-C and PL in the serum of diabetic rats. The diabetic rats had elevated levels of serum TC, TG, LDL-C, PL and VLDL-C and decreased level of HDL-C as compared with normal control rats. Diabetic rats treated with *Senna auriculata* leaf extract and glibenclamide reversed serum lipid profiles to near normal levels.

The activities of LPO, SOD, CAT, GPx and GSH in the alloxan induced diabetic rats were illustrated in **Table 6.** In the present study, the alloxan induced diabetic rats had shown increased activities of LPO. The levels of SOD, CAT, GPx and GSH in the serum were significantly reduced in alloxan induced rats. Treatment with *Senna auriculata* leaf extract and glibenclamide showed reversal of all these parameters to near normal levels.

Discussion:

Diabetes mellitus is one of the most common chronic diseases associated with carbohydrate metabolism. It is also an indication of co-morbidities such as obesity, hypertension, and hyperlipidemia which are metabolic complications of both clinical and experimental diabetes. Alloxan, a beta cytotoxin induces chemical diabetes (Alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release, which paves way for the decreased utilization of glucose by the tissues [31, 32,33,34,35]. The prevention of diabetes is an urgent worldwide health concern. The period preceding the onset of type 2 diabetes is typically characterized by obesity and insulin resistance induced by over reacting and physical inactivity.

The ethanol extract of *Senna auriculata* (Group III) was treated on alloxan induced diabetic rats (Group II). The results were compared with control (Group I) and the positive control glibenclamide (Group IV) after fourteen days of treatment based on biochemical parameters. After the alloxan induction, glucose, insulin, lipid profiles, protein and antioxidant were restored to control levels with the administration of the known drug glibenclamide and plant extracts *Senna auriculata*. The result from the present study shows the significant changes in biochemical parameter during the experimentally inducted diabetes. Blood glucose, serum insulin, urea, creatinine levels were determined in control and ethanol extract and glibenclamide treated rats (Table 13). The administration of ethanol extract of *Senna auriculata* leaf decreases the blood glucose level whereas; serum insulin level was increased in treated rats compared to control rats. The hypoglycemic ethanol extract of *Senna auriculata* leaf was found to be inducing insulin release from pancreatic cells of diabetic rats [36.37] have fed the ethyl acetate-soluble fraction of an absolute ethanol extract of *Pterocarpus marsupium* wood, which significantly lowered blood sugar level with corresponding increase in insulin level in alloxan induced diabetic rats. It is evident from this study that, there was an increase in insulin levels in diabetic rats treated with plant extract. Earlier many plants have been studied for their hypoglycemic and insulin release stimulatory effects [38, 39].

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 extract of *Senna auriculata* leaves were administered orally (Group III) to rats for fourteen days, reversed the urea and creatinine level to near normal. The administration of glibenclamide also decreased the levels of urea and creatinine to some extent.

Alloxan is taken as indications of an abnormal glomerular function where a single injection of cisplatin at a dose of 5 mg/kg body weight in rabbits caused a marked reduction in the glomerular filtration rates, which was accompanied by an increase in the serum creatinine level, indicating the induction of acute renal failure. It is confirmed that there is a significant increase in serum creatinine in albino rats 14 days after alloxan administration. The present result shows that, the treatment with ethanol extract of *Senna auriculata* leaf was effective in preventing alloxan induced increase in serum creatinine level when compared with the control.

A significant reduction in serum protein, albumin and globulin were observed in alloxan induced diabetic rats (Group II), when compared to control (Group I) and glibenclamide treated rats (Group IV). On administration of ethanol extract of *Senna auriculata* leaf to the diabetic rats, protein, albumin and globulin levels were found to be restored in normal. These results were in accordance with the effect of *Wattakaka volubilis* leaf in diabetic rats [40]. The increased level of serum protein, albumin and globulin in alloxan induced diabetic rats are presumed

to be due to increased protein catabolism and gluconeogenesis during diabetes [41].

The animals treated with alloxan developed hepatic damage which was evident from the increase in the enzyme activities. Pretreatment with ethanol extract of *Senna auriculata* leaf and glibenclamide resulted in a decrease of transaminase activities in alloxan treated rats. The serum AST and ALT levels increases as a result of metabolic changes in the liver, such as administration of toxin, cirrhosis of the liver, hepatitis and liver cancer including diabetes [42]. Similarly in the present study, it was observed that the levels of SGPT and SGOT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan [43].AST and ALT were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats[44] .

In this study, the ethanol extracts of *Senna auriculata* leaf regulated the activity of SGPT, SGOT and ALP in liver of rats intoxicated with alloxan. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study [45].

Alloxan induced diabetic rats showed significantly increased serum lipid profiles except HDL-C, when compared with normal rats. The glibenclamide and ethanol extract of *Senna auriculata* leaf treated rats showed a significant 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. On administration of ethanolic extract of *Senna auriculata* leaf and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normal. The level of serum lipid profiles are usually raised in diabetic rats in the present study and such elevation represents a risk factor for coronary heart diseases [46]. Lowering the serum lipid level through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease[47].

During diabetes, enhanced activity of the enzyme, increased lipolysis and releases more fatty acids into the circulation [48].The increased fatty acid concentration also increases the β-oxidation of fatty acids, producing more acetyl Co-A and cholesterol during diabetes. In normal condition, insulin increases receptor-mediator removal of LDL-cholesterol and decreased activity of insulin, during diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglycedemia have been reported to occur in diabetic rats[46]. The increased concentration of free fatty acid may be due to lipid break-down and this may cause increased generation of NADPH-dependent microsomal lipid peroxidation. Phospholipids 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 plasma environment and non-polar lipoprotein of lipoprotein core [49].Increased phospholipids levels in tissues were reported by Venkateswaran[50]; Pari and Satheesh, [51] in streptozotocin diabetic rats. Administration of ethanol extract of *Senna auriculata* leaf and glibenclamide decreased the levels of phospholipids.

The results showed increased lipid peroxidation (LPO) of alloxan induced diabetic rats. Earlier studies have reported that there was an increased lipid peroxidation in liver, kidney and brain of diabetic rats[52, 53]. This may be because the tissues contain relatively high concentration of early peroxidizable fatty acids. In the present study, an increase in the levels of LPO was found and these levels were significantly reduced after the supplementation of the ethanol extract of *Senna auriculata* leaf and glibenclamide (Table 18). These indicate that, plant extract inhibit oxidative damage due to the antiperoxidative effect of ingredients present in ethanol extract of *Senna auriculata*. This could be correlated with previous study reported that *Cassia auriculata* flower [54]*Syzigium cuminii*[55, 56]; *Tinospora cardifolia* [57]and *Scoparia dulcis* [58] has antiperoxidative and antihyperlipidemic effect of diabetic animals. Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the lipid metabolism. Insulin is a potent inhibitor of lipolysis, since it inhibits the activity of hormone sensitive lipase in adipose tissue and suppresses the release of free fatty acids [59].

The level of serum superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GPx) and reduced glutathione (GSH) in control and experimental rats were studied. A highly significant reduction in the activity of scavenging mitochondria enzymes is observed in alloxan induced rats. These adverse changes were reversed to near normal values in ethanol extract of *Senna auriculata* leaf treated group III as well as glibenclamide treated rats group IV.

Mitochondria are the energy reservoir of the cell and the damage inflicted in mitochondria would ultimately result in the reduction of energy production and thereby leading to cell death [60]. Subcellular membrane, associated with thiol bearing enzymes, represents sensitive sites for detoxification causing perpetuation of cellular function [61]. Reactive oxygen species can themselves reduce the activities of antioxidant defence mechanism. In the present study, ethanol extract of *Senna auriculata* have enhanced mitochondrial enzymatic antioxidant activity and suppressed lipid peroxidation.

Free radical reacts with lipids causing peroxidation, resulting in the release of products such as malondialdehyde, hydroperoxide and hydroxyl radicals. These extracts have the capacity to scavenge free radicals directly or interfering with generation of free radicals [62, 63]. Thus, the inhibitory effects of these extracts on oxidative damage may be attributed to the suppression induced peroxidation [64]. It is well known that CAT, SOD and GPx play an important role as protective enzymes against free radical formation in tissues [65]. Several investigators have reported that the reduced activities of CAT and SOD genes are induced by free radicals and also by certain humoral factors [66, 67]. The present study indicates the reduction in the activity of SOD, CAT, GPx and GSH in alloxan induced rats. These results reveal the protective role of plant extract in decreasing lipid peroxidation and by normalizing antioxidant system.

It is concluded that, medicinal plants have been reported to possess antihyperglycemic activity; *Senna auriculata* leaf is gaining much importance in diabetic control as it has been used as a traditional medicine for diabetes; since the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, terpenoids, glycosides, steroids, saponin and phenols. Several authors reported that flavonoids, steroids/terpenoids, phenolic acids are known to be bioactive antidiabetic principles [68, 69]. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues [70, 71]. Saponin reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intraluminal physicochemical reaction. Hence, it has been reported to have hypocholesterolemic effect and thus may aid a lessening metabolic burden that would have been placed in the liver [72]. In the present study, the phytochemical analysis of ethanol extract of *Senna auriculata* leaf clearly points out the presence of above said active principles. The preliminary investigation on the antidiabetic efficacy of ethanol extract of *Senna auriculata* leaf will be significant to proceed further in this path for the isolation of active principles responsible for antidiabetic activity.

Acknowledgement:

Thanks to Dr. Sampathraj, Honorary Advisor, Samsun Clinical Research Laboratory, Tirupur for their assistance in animal studies.

References:

1. Scheen, J.A. Drug treatment of non-insulin dependent diabetes mellitus in the 1990 s Achievement and function development. Drug, 54, 1997: 355-368.
2. Lyra R., Oliverira M., Lins D and Cavalcarti N. Prevention of type 2 diabetes mellitus. Arq. Bras.Endocrinol. Metabo. 50, 2006: 239-249.
3. Wild S.G., Roglic A., Green R., and King H. Global prevalence of diabetes. Estimated for the year 2000 and projection for 2030. Diabetes Care, 27, 2004: 1047-1054.
4. Shanmugam S., Manickandan K. and Rajendran K. Ethnomedicinal survey of medicinal plants used for the treatment of diabetics and jaundice among the villagers of Sivagangai district, Tamil Nadu. Ethnobotanical Leaflets. 13**,** 2009: 189 – 194.
5. Ragupathy S. and Newmaster S.G. Valorizing the 'Irulas' traditional knowledge of medicinal plant the Kodiakarai reserve forest. International Journal of Ethnobiology and Ethnomedicine. 5, 2009: 1 – 17.
6. Sandhya B., Thomas S., Isabel W. and Shenbagarathai R. Ethnomedicinal plants used by the Valaiyan community of Piranmalai hills (Reserved Forest), Tamil Nadu, India – A pilot study. African Journal of Traditional and Complementary Medicine. 3, 2006: 101 – 114.
7. Latha M. and Pari L. Antihyperglycaemic effect of *Cassia auriculata* in experimental diabetics and its effects on key metabolic enzymes involved in carbohydrate metabolism. Clinical Experimental Pharmocological Physiology 30, 2003a: 38 – 43.
8. Uma Devi P., Selvi S., Suja S., Selvam V. and Chinnaswamy P. Antidiabetics and hypolipidemic effect of *Cassia auriculata* in Alloxan induces diabetic rats. International Journal of Pharmacology 6**,** 2006: 661 – 607.
9. Brinda P., Sasikala P. and Purushothaman KK: Pharmacognostic studies on *Merugan kizhangu*. Bulletin in Medical Ethnobotanical Research. 3, 1981:84-96.
10. Anonymous: Phytochemical investigation of certain medicinal plants used in Ayurveda. Central Council for Research in Ayurveda and Siddha, New Delhi. 1990.
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 A.N., Thakurdesai P.A., Venkat Rao N. and Sing J .Antidiabetic activity of *Terminalia catappa* Linn. Fruits. Journal of Ethnopharmacology. 88, 2003: 45-50.
14. Sasaki T, Masty S. and Sonae A. Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. Rinshbo Kagaku. 1, 1972: 346-353.
15. Anderson L., Dinesen B., Jorgonsen P.N., Poulsen F. and Roder M.E. Enzyme immune assay for intact human insulin in serum or plasma. Clinical Chemistry. 39, 1993: 578-582.
16. Varley H. Practical clinical biochemistry, Arnold Heinemann Publication Pvt. Ltd., 1976: p, 452.
17. Owen, J.A., Iggo, J.B., Scangrett, F.J. and Steward I.P. Determination of creatinine in plasma serum, a critical examination. Journal of Biochemistry 58**,** 1954: 426-437.
18. Parekh A.C. and Jung. Cholesterol determination with ferric acetate, uranium acetate and sulphuric acid, ferrous sulphate reagent. Analytical Chemistry. 112, 1970: 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 W.T., Levy R.I. and Fredrickson D.S. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultra centrifuge. Clinical Chemistry. 18, 1972: 499-502.
21. Warnick G.R., Nguyan T. and Albers A.A. Comparison of improved precipitation methods for quantification of high density lipoprotein cholesterol. Clinical Chemistry. 31, 1985: 217.
22. Takayama M., Itoh S., Nagasaki T. and Tanimizu I. A new enzymatic method for determination of serum phospholipids. Clinical Chemistry Acta. 79**,** 1977: 93 – 98.
23. Lowry O.H., Rosenbrough N.J., Farr A.L. and Randall R.J. Protein measurement with the folin's phenol reagent. Journal of Biological Chemistry. 193, 1951: 265-275.
24. Reitman S. and Frankel S.A. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 28, 1957: 56-63.
25. King E.J. Armstrong A.R. Determination of serum and bile phosphatase activity. Cannadian Medical Association Journal. 31, 1934: 56-63.
26. Bergmayer H.U. UV method of catalase assay. In Methods of Enzymatic Analysis, Weiheim Deer field Beach, Florida, Bansal. 3, 1983: 273.
27. Madesh M. and Balasubramanian K.A. Microtitre plate assay for superoxide dismutase using MTT reduction by superoxide. Indian Journal of Biochemistry and Biophysics. 35, 1998: 184-188.
28. Rehman S. Lead – induced regional lipid peroxidation in brain. Toxicology Letter. 21, 1984: 333-337.
29. Prins H.K. and Loos J.A. In Glutathione; Bio-chemical methods in red cell genetics, edited by J.J Yunis. Academic Press, New York. 1969:127-129.
30. Pagila DE, Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical medicine 70, 1967: 158-169.
31. Baynes J.W. 1991. Role of oxidative stress in the development of complications in diabetes. Diabetes. 40: 405-412.
32. Saravanan R. and Pari L. Antihyperlipidemic and antiperoxidative effect of Diasulin, a polyherbal formulation in alloxan induced hyperglycemic rats. BMC Complementary and Alternative Medicines 5, 2005**:** 1-8.
33. Bierman E.L., Amaral J.A.P. and Balknap B.H. Hyperlipidemia and diabetes mellitus. Diabetes. 25, 1975: 509 – 515.
34. Omamoto H., Ucgigata Y. and Hiroskitckan. STZ and alloxan induces DNA strand breaks and poly (ADP ribose) synthatase in pancreatic islets. Nature. 294, 1981: 284-286.
35. Gurusamy K., Kokilavani R., Sowmya C. and Arumugasamy K. Non enzymatic antioxidant properties of ethanolic extract of polyherbal formulation (PHF) against CCL_4 induced rats. Research Journal of Biotechnology 3, 2008: 51-54.
36. Sharma N. and Garg V. Antidiabetic and antioxidant potential of ethanolic extract of *Butea monosperma* leaves in alloxan induced diabetic mice. Indian Journal of Biochemistry and Biophysics. 46, 2009: 99-105**.**
37. Ahmed F., Khalid P., Khan M.M., Meena C., Rastogi A.K. and Kidwai J.R. Hypoglycemic activity of *Pterocarpus marsupium* wood. Journal of Ethnopharmacology, 35**,** 1991: 71-75.
38. Ayoola G.A., Ngene I.E., Awobajo F.O., Olatunji-Bello I.I. and Odugbemi T.O. Hypogylcaemic effect of the aqueous extract of the leaves of

Allanblackia floribunda Oliv. (Guttiferae). Journal of Phytology, 1, 2009: 260-266.

39. Awobajo F.O. and Olatunji- Bello I.I. Hypoglycemic activities of aqueous and methanol leaf extract of Hybanthus enneaspermus and Paquetina nigrescens on normal and alloxan induced diabetic female Sprague dawley rats. Journal of Phytology, 2, 2010: 01-09.
40. Maruthupandian A., Mohan V.R., and Sampathraj R. Antidiabetic, antihyperlipidaemic and antioxidant activity of *Wattakaka volubilis* (L.f) Stapf leaves in alloxan induced diabetic rats. International Journal of Pharmaceutical Sciences and Research, 1, 2000: 83-90.
41. Palanivel R., Thangavel M., Selvendran K. and Sakthisekaran D. Insulinomimetric effect of ammonium paratungstate on protein metabolism in streptozotocin induced diabetic rats. Biomed 21, 2001**:** 23-30.
42. Chalasani N., Aljadhey H., Kesterson J., Murray M.D. and Hall S.D. Patients with elevated liver enzymes are not act high risk for station hepatotoxicity, Gastroenterology. 126, 2004: 1287 – 1292.
43. Stanely P., Prince M. and Menon V. Hypoglycemic and other related actions of *Tinospora cordifolia* roots in alloxan induced diabetic rats. Journal of Ethnopharmacology 70, 1999: 9 – 15.
44. Hwang H.J., Kim S.W., Lim J.M., Joo J.H., Kim H.O., Kim H.M. and Yun J.W. Hypoglycemic effect of crude epoxypolysaccharides produced by a medicinal mushroom *Phellinus baumii* in streptozotocin induced diabetic rats. Life Science 76**,** 2005: 3069 – 3080.
45. Preethi K.C. and Kuttan R. Hepato and reno protective action of *Calendula officinalis* L. flower extract. Indian Journal of Experimental Biology 47, 2009: 163 – 168.
46. Mironova M.A., Klein R.L., Virella G.L. and Lopes-Virella M.F. Anti-modified LDL antibodies, LD -containing immune complexes and susceptibility of LDL to *in vitro* oxidation in patients with type 2 diabetes. Diabetes 49**,** 2000: 1033-1049.
47. Scott M. and Grundy MD. 1999. Diabetis Cardiovascular Disease. Circulation 100: 1134-1146.
48. Agardh C.D., Bjorgell P. and Nilson E.P. The effect of tolbutamide on lipoproteins and lipoproteinlipase and hormone sensitive lipase. Diabetes Research Clinical Practice 46, 1999: 99-108.
49. Cohn R.M. and Roth K.S. Lipid and lipoprotein metabolism In: *Biochemistry and Diseases,* Williams and Willkins publishers, Baltimore, 1996: p 280.
50. Venkateswaran S., Pari, L. and Saravanan G. Effect of *Phaseolus vulgaris* on circulatory antioxidants and lipids in streptozotocin-induced diabetic rats. Journal of Medicinal Food. 5, 2002: 97 – 104.
51. Pari L. and Satheesh A.M. Antidiabetic effect of *Boerhavia diffusa*: effect on serum and tissue lipids in experimental diabetes. Journal of Medicinal Food. **7,** 2004: 472 – 476.
52. Latha M. and Pari L. Preventive effects of *Cassia auriculata L*. flowers on brain lipid peroxidation in rats treated with streptozotocin. Molecular Cell and Biochemistry 243, 2003b: 23-28.
53. Ananthan R., Latha M., Ramkumar K.M, Pari L., Basker L. and Narmatha Bai V. Antidiabetic effect of *Gymnema montanum* leaves: effect on lipid peroxidation induced oxidative stress in experimental diabetes. Nutrition. 6, 2004: 379 – 386
54. Pari L. and Latha M. Effect of *Cassia auriculata flowers* on blood sugar levels, serum and tissue lipids in Streptozotocin Diabetic Rats. Singapore Medical Journal 43**,** 2002: 617-621.
55. Prince P.S.M. and Menon V.P. Hypoglycemic activity of *Syzigium cumini* seeds; effect on lipid peroxidation in alloxan diabetic rats. Journal of Ethnopharmacology 61**,** 1998: 1 – 7.
56. Prince P.S.M., Kamalakkannan N. and Menon V.P. Antidiabetic and antihyperlipidaemic effect of alcoholic *Syzigium cumini* seeds in alloxan induced diabetic albino rats. Journal of Ethnopharmacology 91, 2004: 209 – 213.
57. Prince P.S.M., Menon V.P. and Gunasekaran G. Hypolipidemic action on *Tinospora cordifolia* root extract in alloxan diabetic rats. Journal of Ethnophamacology 14, 1999: 4 – 16.
58. Latha M. and Pari L. Modulatory effect of *Scopaia dulcis* in oxidative stress induced lipidperoxidation in streptozotocin diabetic rats. Journal of Medicinal Food. 6**,** 2003c**:** 379 – 386.
59. Loci A.S., Shaabha M., Khazraji A.L., Hussain A. and Twaija A. Hypoglycemic effect of a valuable extract of *Artemisia herba-alba* II. Effect of a valuable extract on some blood parameters in diabetic animals. Journal of Ethnopharmacology 43, 1994: 167 - 171.
60. Sohal R. and Dubey A. Mitochondria oxidative damage, hydrogen peroxide release and aging.

Free Radical Biology and Medicine 16**,** 1994: 621-626.

61. Kyu B.K. and Byung M.L. Oxidative stress to DNA, protein and antioxidant enzymes in rats treated with Benzo(a) Pyrene. Cancer Letters 113**,** 1997: 205-212.
62. Reddy A.C. and Lokesh B.R. Studies on spices principles as antioxidants in the inhibiting of lipid peroxidation of rat liver microsomes. Molecular Cell and Biochemistry 111**,** 1992: 117-124.
63. Dhuley J.N., Raman P.H., Mujumda M. and Naik S.R. Inhibition of lipid peroxidation by piperine during experimental inflammation in rats. Indian Journal of Experimental Biology 31**,** 1993: 443-445.
64. Selvendiran K., Senthilnathan P., Magesh V. and Sakthisekaran D. Modulatory effect of piperine on mitochondrial antioxidant system in Benzo(a) pyrene-induced experimental lung carcinogenesis. Phytomedicine. 11**,** 2004: 85-89.
65. Oberly W.R. and Buettner R.G. Role of superoxide dismutase in cancer. Cancer Research. 35**,** 1974: 1141-1149.
66. Anderson D., Yu T.W., Phillips J.B. and Schmezer D. The effect of various antioxidants and other modifying agents on oxygen radical generated DNA damage in human lymphocytes. Mutation Research 307, 1994: 261 – 271.
67. Slaga T.J. Inhibition of the induction of cancer by antioxidants. Advances in Experimental Medical Biology 369, 1995: 167-174.
68. Oliver-Bever B. Medicinal plants in tropical West Africa, Cambridge University press, London. 1986, pp: 245 – 267.
69. Rhemann A.V. and Zaman K. Medicinal plants with hypoglycemic activity, Journal of Ethnopharmacology. 26, 1989: 1 – 55.
70. Chakravarth, B.K., Gupta S., Gambir S.S. and Gode K.D. Pancreatic beta cell regeneration. A novel antidiabetic mechanism of *Pterocarpus marsupium* Roxb. Indain Journal of Pharmacology 12**,** 1980: 123 – 127.
71. Geetha B.S., Mathew B.C. and Augusti K.T. Hypoglycemic effects of leucodelphinidin derivative isolated from *Ficus bengalensis* Linn. Indian Journal of Physiological Pharmacology. 38**,** 1994: 220 – 222.
72. Price K.R., Johnson L.I. and Ferewick H. The chemical and biological significant of saponins in food and feeding staffs .CRC Critical Reviews of Food Science and Nutrition 26**,** 1987: 127 – 135.

*****