Original Article

Hepato-renal protective effects of hydroethanolic extract of *Senna alata* on enzymatic and nonenzymatic antioxidant systems in streptozotocin induced diabetic rats

Mohanasundaram Sugumar [a,*], *D.Victor Arokia Doss* [b],
P.N. Prasad Maddisetty [a]

[a] *Research & Development Centre, Bharathiar University, Coimbatore, India*
[b] *Department of Biochemistry, PSG College of arts and Science, Coimbatore, India*

ARTICLE INFO

ABSTRACT

Background: Oxidative stress induced tissue damage might be the major cause for diabetes mellitus and its associated complications. The management of such oxidative stress is the biggest challenge over the decade. The main objective was to analyze the protective effect of ethanolic extract of *Senna alata* L leaves on enzymatic and nonenzymatic antioxidant systems of hepatic and renal tissues in Streptozotocin-induced diabetes in rats.

Methods: The use of streptozotocin diabetes was induced in the experimental rats and the subsequent therapeutic effects of standard drug glibenclamide and *Senna alata* L were compared. The levels of plasma insulin, glucose, urea, uric acid, creatinine, vitamin C, vitamin E, reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-s-tranferase were assayed in control and experimental groups of rats.

Results: These alterations were detected throughout the study duration after the treatment with *Senna alata* L and glibenclamide. A significant raise followed by the treatment with *Senna alata* leaves in vitamin E, catalase, glutathione peroxidase and glutathione-s-tranferase was observed. It has been found that notable decline in the levels of vitamin C, reduced glutathione were observed in diabetic rats. The liver and kidney based antioxidant enzyme activities were significantly responsive to the treatment in diabetic rats. Apart from these antioxidant system, some vital changes were detected in the typical biochemical parameters such as level of protein, urea, uric acid, and creatinine from abnormal into normal in both the control and induced rats.

* *Corresponding author*. Department of Biotechnology, Karpaga Vinayaga, College of Engineering and Technology, Maduranthagam, Kanchipuram District, Tamil Nadu-603308, India.
    E-mail address: sbmohan2007@gmail.com (M. Sugumar).

Conclusion: From the above said observations, it was very clear that, *Senna alata* has helped to manage the oxidative tension in diabetic rats, which in turn may greatly support the hypoglycaemic potency of *Senna alata* L.

1. Introduction

Medicinal plants are the only promising source for developing challenging drugs for many diseases in this century. Though more than a few hundred medicinal plants are widely used as therapeutic sources for diabetes, only a very few of them have been well studied scientifically. Either as preventive or therapeutic medicine, a lot of phytochemicals have been extracted successfully from medicinal plants for a wide range of diseases such as cancer, heart disease, diabetes, and high blood pressure. It is expected that the diabetic population of this universe may reach 439 million by 2030. India, the "diabetes capital of the world" is having more diabetic patients and China and America are the subsequent followers.[1] In modern days, to manage diabetes, insulin injection is the most preferred one in case of Type 1 and oral hypoglycemic drugs are the choice for Type 2 diabetes with certain limitations. Generally, it was is well understood by elevated sugar level with imbalanced metabolic conditions followed by chronic organ failures. Few bioactive molecules were found successful and proven in protection against diabetes.[2] In recent times, the significance of antioxidants has increasingly received more attention as an important factor in the biochemical management of oxidative stress. Any substance which can inhibit or delay the undeserved peroxidation and prevent the oxidative damage to a particular cell or molecule is said to be an antioxidant. In general, tissue or cell damage is mainly caused by the elevated generation of free radicals. In general, free radicals are having the power to damage or destroy the major biochemical components of the tissues such as lipids, carbohydrates, proteins, and DNA. Antioxidant molecules have the tendency of removing these free radicals thereby preventing the cell from the damage. This removal mechanism was done by transferring one hydrogen atom to the reactive radical intermediate. Huge numbers of food stuffs have been detected to contain such antioxidants in the form of bioactive compounds.[3] Antidiabetic agents of naturals products, used both in recent and folklore medicine, are recommended by the World Health Organization (WHO). Alternative therapies have to be identified in order to manage and prevent diabetic complications. Therefore, medicinal plants screening towards identifying a new potent antidiabetic therapy is increasing day by day among scientific researchers. The importance of oxidative stress in diabetes has been studied extensively in recent times and it has been well understood that through many biochemical events excess of free radicals will be liberated during hyperglycemia.[4] Degeneration or extensive damage caused by streptozotocin in pancreatic beta cells is the main mechanism behind in the induction of Type 2 diabetes in experimental rats. It has been proven already that this degeneration is possible only when excess release of reactive oxygen species.[5]

The damage caused by the streptozotocin will usually result in hypoinsulinemia and poor glucose utilization by the cells. Liver, the major organ for glucose metabolism will suffer a lot due to the lack of insulin. This condition may further extend to damage the cellular membranes of liver cells due to the availability of huge free radicals.[6] Once the cells are damaged, it will affect the major cellular functional constituents such as enzymes, antioxidant systems and may result in the development of chronic illness. Hence, in most of the treatment ideas in diabetes, regeneration of the damaged cells and increased glucose utilization by the cells are considered to be the major breakthroughs. Many clinical findings have proven the usefulness of medicinal plants and their metabolites in the changeover of oxidative stress in diabetic individuals.[7] Due to the undesirable side effects of current antidiabetic agents, there is a demand and expectation for a safe antidiabetic drug discovery system especially focusing on natural plant sources, which can manage oxidative stress and diabetes mellitus without any other side effects.[8] While considering the above findings, it should be taken in consideration that antioxidants may show a useful pharmacological approach in controlling the diabetes.

The present study was aimed to examine the effect of *Senna alata* L on the enzymatic and nonenzymatic antioxidant defense systems in both liver and renal tissues of streptozotocin induced diabetic rats to understand the oxidative stress management potency.

2. Methods

2.1. Animals

Male albino Wistar rats (250–300 g) used in this study were housed in polypropylene cages of standard size and maintained under standard environmental conditions (14 hour dark /10 hour light cycles; temperature $25 \pm 2\,°C$; 35–60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. The animals were adapted to the environment for 2 weeks prior to study. Animals were fasted overnight prior to the experimental schedule with free access to water *ad libitum*. The experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines.

2.2. Drugs and chemicals

Streptozotocin was procured from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals used were of analytical grade.

2.3. Plant material

Fresh leaves of *Senna alata* were collected from in and around the regions of Thirukkalukundram taluk, Kanchipuram District, Tamil Nadu, India. The plant was authenticated by the regional Centre, Botanical Survey of India, Coimbatore.

2.4. Plant extract preparation

Fresh leaves of *Senna alata* L were collected from the village near Thirukkalukundram. The leaves of the plant were washed under the tap water and shade dried for 8–10 days. Then, the leaves of the plant was made into coarse powder using a mechanical grinder. Ten g of powdered plant leaves were taken and 100 mL of 50% ethanol was added and kept overnight in the shaker. The extract was filtered and the solvent was added again and kept in the shaker for next 7 hours. In this way the plant extract was collected and stored in the air tight container for further use.

2.5. Experimental induction of diabetes mellitus

Streptozotozin-induced diabetes mellitus was induced in overnight fasted wistar albino rats by a single intraperitoneal injection of streptozotozin (50 mg/kg of body weight.) dissolved in citrate buffer (0.01 M, pH 4.5). In order to stave off the hypoglycaemic shock during the 1st day after the streptozotozin administration, diabetic rats were given 5% glucose solution orally. It was noted that streptozotozin had induced hyperglycemia in experimental rats within 48 hours.[9] Diabetes was confirmed by the determining the fasting blood glucose concentration using glucose oxidase–peroxidase reactive strips and a glucometer after 48 hours of administration of streptozotozin. Animals observed with blood glucose levels above 250 mg/dL were considered diabetic and were selected for the study.

2.6. Experimental design

For antidiabetic evaluation, a total of 24 rats (18 diabetic surviving rats and 6 normal rats) were used. Animals were divided into four groups of six animals each and were treated as given below: (1) Group 1: normoglycemic control animals; (2) Group 2: streptozotozin (STZ)-induced diabetic animals; (3) Group 3: STZ induced animals followed by the treatment with glibenclamide (600 µg/kg bw); and (4) Group 4: STZ induced animals followed by the treatment with the ethanolic extract of *Senna alata* (400 mg/kg bw).

Groups 3 and 4 animals were orally administered with the standard drug glibenclamide (600 µg/kg bw) and the sample extract (400 mg/kg bw) once daily for 30 days. Day 3 of induction was designated as Day 1 for extract administration in diabetic rats. Blood samples were drawn by snipping marginal tail at weekly intervals during the study period. The levels of blood glucose and body weight were monitored periodically using glucometer (glucose oxidase method).

The animals were anesthetized with ketamine (80 mg/kg of body weight through intraperitoneally) and sacrificed by cervical decapitation. Serum and plasma were separated from the collected blood. The important organs such as liver and kidney were properly excised and preserved. One g of it was homogenized in 0.25 M Tris HCl buffer (pH 7.5) and centrifuged at 10,000 rpm for 20 minutes at 4°C. The clear supernatant obtained was used for measuring the antioxidant markers.

2.7. Analysis of common biochemical factors

The major and common biochemical factors includes plasma glucose,[10] insulin,[11] protein,[12] urea,[13] uric acid,[14] and creatinine[15,16] were analyzed by the specified proven methods and the significance in changes from abnormal to normal level was compared to understand therapeutic potency of *Senna alata* (L).

2.8. Measurement of nonenzymatic antioxidants

The changes in the levels of the most important nonenzymatic antioxidants includes vitamin C,[17] vitamin E,[18] and glutathione (GSH) [19] were detected by specified proven methods and the significance was compared.

2.9. Detection of enzymatic antioxidants

The significant changes in the levels of the enzymatic antioxidants includes super oxide dismutase,[20] catalase,[21] and glutathione peroxidase[22] and glutathione-s-transferase[23] were detected by the specified proven methods and statistically compared.

2.10. Statistical analysis

The results we obtained in our study were analyzed by one way analysis of variance (ANOVA) followed by least significant difference (LSD) test and it was considered statistically significant when $p < 0.05$. All the results were expressed as mean ± standard deviation (SD) for six animals in each group.

3. Results

3.1. Effect on total protein and albumin

We have observed a significant ($p < 0.05$) decline in the level of total protein and albumin in diabetic rats (Group 2) than normal control rats (Group 1). Administration of glibenclamide (Group 3) and *Senna alata* L extract (Group 4) notably ($p < 0.05$) raised into normal level (Table 1). It shows that the plant extract has some positive effect which has favored the normal level of protein synthesis followed by the treatment.

3.2. Effect on urea, uric acid, and creatinine levels

We have found a significant ($p < 0.05$) raise in the level of urea, uric acid and creatinine were observed in diabetic rats (Group 2) than normal control rats (Group 1). Administration of glibenclamide (Group 3) and *Senna alata* L extract (Group 4) notably ($p < 0.05$) decreased the urea, uric acid, and creatinine into normal level (Table 2). It shows that the plant extract has showed its inhibitory effect on unnecessary degradation of protein that may be released due to cell damage caused by free radicals.

Table 1 – Effect of *Senna alata* extract on serum total protein and albumin in experimental rats

Group	Method of treatment	Total protein (mg/dL)	Albumin (mg/dL)
1	Normal control	9.69 ± 0.48	5.66 ± 0.45
2	Diabetic control (STZ induced)	$4.36 \pm 0.42^*$	$4.47 \pm 0.27^*$
3	STZ + glibenclamide Treated	$8.11 \pm 0.45^*$	$5.12 \pm 0.33^*$
4	STZ + *Senna alata* treated	$6.07 \pm 0.48^*$	$4.81 \pm 0.31^*$

Values are given as mean ± SD for groups of six rats in each.
* Values are statistically significant when $p < 0.05$.
  Group 2 was compared with Group 1; Groups 3 and 4 were compared with Group 2.
  SD, standard deviation; STZ, streptozotozin.

Table 2 – Effect of *Senna alata* extract on urea and uric acid in experimental rats

Group	Method of treatment	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
1	Normal control	30.22 ± 1.48	1.36 ± 0.08	0.88 ± 0.09
2	Diabetic control (STZ induced)	$53.77 \pm 6.38^*$	$2.02 \pm 0.11^*$	$1.73 \pm 0.16^*$
3	STZ + glibenclamide Treated	$44.88 \pm 3.73^*$	$1.54 \pm 0.11^*$	$1.20 \pm 0.06^*$
4	STZ + *Senna alata* treated	$45.64 \pm 2.78^*$	$1.60 \pm 0.08^*$	$1.18 \pm 0.07^*$

Values are given as mean ± SD for groups of six rats in each.
* Values are statistically significant when $p < 0.05$.
  Group 2 was compared with Group 1; Groups 3 and 4 were compared with Group 2.
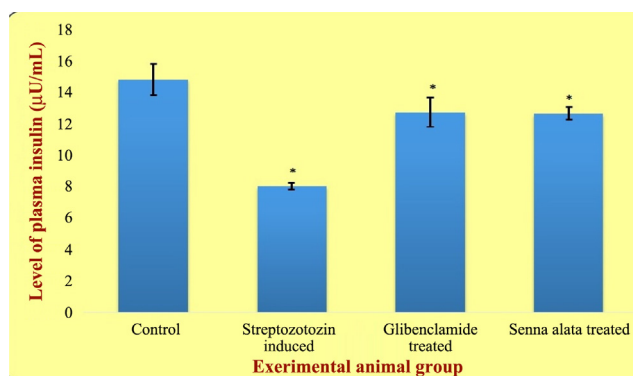 SD, standard deviation; STZ, streptozotozin.

3.3. Effect on plasma glucose and insulin

We have noticed a considerable ($p < 0.05$) elevation in blood glucose (Table 3) and the associated decline in insulin (Group 2) in diabetic rats than control rats (Group 1). Treatment with glibenclamide (Group 3) and *Senna alata* (Group 4) notably ($p < 0.05$) reduced the blood glucose and increased insulin (Fig. 1) to normal levels. Similarly, the decline in the body weight due to diabetes was rectified notably ($p < 0.05$) in all the induced rats (Table 4).

3.4. Levels of vitamin C, vitamin E, and GSH

A significant decrease ($p < 0.05$) in the level of natural antioxidants such as vitamin C, vitamin E, and GSH were observed in diabetic rats when compared with control rats. This decline might be the major fact for cell damage associated with diabetes as a result of undesirable peroxidation processes and subsequent free radicals generation. Group 3 and Group 4 rats tends to bring these levels into normal (Table 5). It was clearly observed in the experimental rats.

A considerable decline observed in the concentration of reduced glutathione in liver and renal tissues of induced

Fig. 1 – Effect of *Senna alata* extract on plasma insulin level in experimental rats.
Values are given as mean ± SD for groups of six rats in each.
* Values are statistically significant when $p < 0.05$.
Group 2 was compared with Group 1; Groups 3 and 4 were compared with Group 2.
SD, standard deviation.

Table 3 – Effect of *Senna alata* extract on plasma glucose level in experimental rats

Group	Method of treatment	D 0	D 3	D 10	D 20	D 30
1	Normal control	75.17 ± 1.00	74.17 ± 0.79	77.33 ± 0.64	79.83 ± 0.59	78.33 ± 1.26
2	Diabetic control (STZ induced)	$76.17 \pm 0.28^*$	$359.17 \pm 1.21^*$	$391.5 \pm 1.30^*$	$411.83 \pm 1.27^*$	$452 \pm 1.41^*$
3	STZ + glibenclamide treated	$69.5 \pm 0.57^*$	$408 \pm 0.83^*$	$389.83 \pm 2.46^*$	$254.33 \pm 1.34^*$	$172 \pm 1.02^*$
4	STZ + *Senna alata* treated	$83.67 \pm 9.77^*$	$378.53 \pm 2.85^*$	$399.12 \pm 2.01^*$	$269.08 \pm 2.35^*$	$180.65 \pm 3.32^*$

Values are given as mean ± SD for groups of six rats in each.
* Values are statistically significant when $p < 0.05$.
  Group 2 was compared with Group 1; Groups 3 and 4 were compared with Group 2.
  SD, standard deviation; STZ, streptozotozin.

rats was raised into normal level after the treatment (Table 5).

3.5. Effects on enzymatic antioxidants of liver SOD, CAT, GPx and GST

Significant ($p < 0.05$) reductions were observed in the activities of SOD (Superoxide Dismutase) (Fig. 2), CAT (Catalase) (Fig. 3), GPx (Glutathione Peroxidase) (Fig. 4), and GST (Glutathione S Transferase) (Fig. 5) in liver and renal tissues of all the experimental groups of rats. After administration of Senna alata extract and glibenclamide, the activities of these enzymes were raised to near normal in diabetic rats which clearly showed the antioxidant potency of our plant extract. This might be the major reason behind the oxidative stress management in diabetic rats in this study.

4. Discussion

Universally, diabetes is a complicated biochemical challenge affecting a huge number of human individuals. In current therapeutic choices, there are so many side effects and secondary failures have been found. Still, there is a gap in designing a promising way for the treatment of diabetes but, through many studies it has been proven already that the plant kingdom is a rich source of bioactive compounds with potency towards the therapy for diabetes.[24] We have examined the potency of Senna alata L by noticing its influences on the
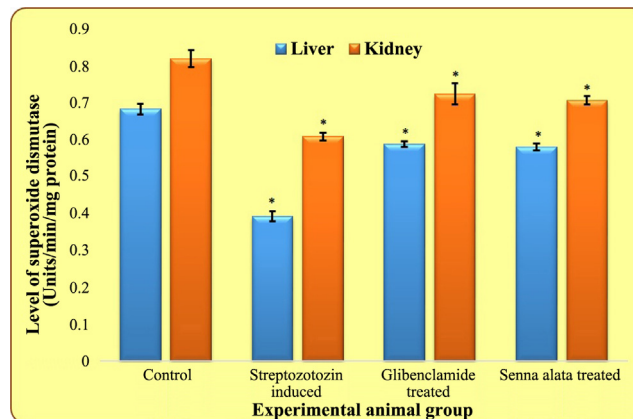

Fig. 2 – Effect of Senna alata extract on SOD (Superoxide dismutase) level in liver and renal tissues of experimental rats.
Values are given as mean ± SD for groups of six rats in each.
* Values are statistically significant when $p < 0.05$.
Group 2 was compared with Group 1; Groups 3 and 4 were compared with Group 2.
SD, standard deviation.

activities of key enzymes and other factors involved in the antioxidant defense systems of control and induced rats.[25]

In diabetes mellitus the declined insulin or its efficiency will result in impaired carbohydrate metabolism. In this study,

Table 4 – Effect of Senna alata extract on level of body weight gain in experimental rats

Group	Method of treatment	D 0 (g)	D 3 (g)	D 10 (g)	D 20 (g)	D 30 (g)
1	Normal control	182.35 ± 4.51	182.83 ± 3.43	185.0 ± 5.48	191.83 ± 4.12	203.71 ± 3.09
2	Diabetic control (STZ induced)	197.85 ± 2.35 *	191.17 ± 3.97 *	185.5 ± 1.94 *	174.83 ± 2.29 *	166.3 ± 2.31 *
3	STZ + glibenclamide treated	197.5 ± 1.38 *	195.17 ± 3.71 *	191.17 ± 4.79 *	190.05 ± 1.19 *	197.17 ± 1.67 *
4	STZ + Senna alata treated	193.97 ± 2.04 *	189.58 ± 0.97 *	188.22 ± 0.77 *	190.21 ± 1.02 *	192.35 ± 1.57 *

Values are given as mean ± SD for groups of six rats in each.
* Values are statistically significant when $p < 0.05$.
Group 2 was compared with Group 1; Groups 3 and 4 were compared with Group 2.
SD, standard deviation; STZ, streptozotozin.

Table 5 – Effects of Senna alata extract on nonenzymatic antioxidants of liver and kidney in experimental rats

Group	Method of treatment	Vitamin C (µg/mg of protein)		Vitamin E (µg/mg of protein)		GSH (µg/gm of Tissue)	
		Liver	Kidney	Liver	Kidney	Liver	Kidney
1	Normal control	88.11 ± 4.00	103.79 ± 6.32	2.53 ± 0.09	2.42 ± 0.14	26.47 ± 3.99	24.7 2 ± 2.20
2	Diabetic control (STZ induced)	72.75 ± 5.05 *	84.79 ± 5.72 *	1.83 ± 0.21 *	1.8 ± 0.16 *	14.73 ± 1.08 *	19.23 ± 0.58 *
3	STZ + glibenclamide treated	76.9 ± 3.23 *	98.57 ± 9.56 *	2.6 ± 0.17 *	2.46 ± 0.04 *	23.17 ± 1.72 *	20.56 ± 0.87 *
4	STZ + Senna alata treated	78.13 ± 6.90 *	93.6 ± 5.14 *	2.47 ± 0.06 *	2.31 ± 0.11 *	23.15 ± 1.04 *	20.62 ± 1.15 *

Values are given as mean ± SD for groups of six rats in each.
* Values are statistically significant when $p < 0.05$.
Group 2 was compared with Group 1; Groups 3 and 4 were compared with Group 2.
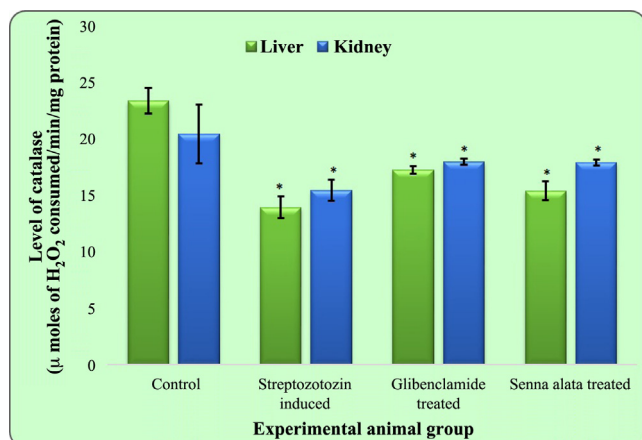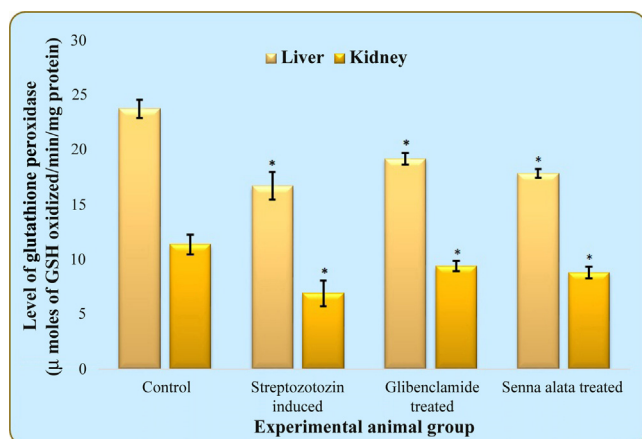SD, standard deviation; STZ, streptozotozin.

Fig. 3 – Effect of *Senna alata* extract on catalase in liver and renal tissues of experimental rats.
Values are given as mean ± SD for groups of six rats in each.
* Values are statistically significant when *p* < 0.05.
Group 2 was compared with Group 1; Groups 3 and 4 were compared with Group 2.
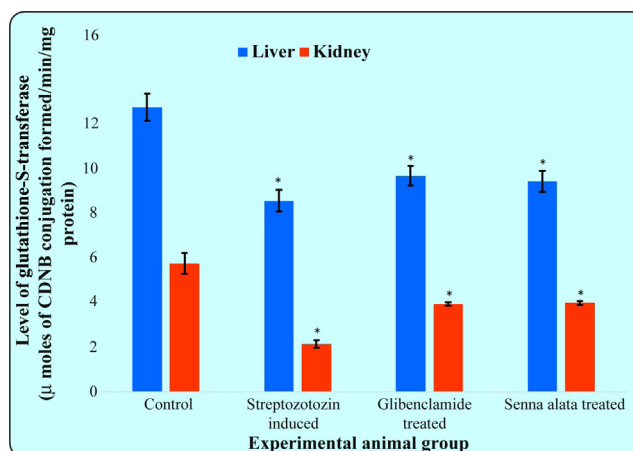**SD, standard deviation.**

Fig. 5 – Effect of *Senna alata* extract on GST level in liver and renal tissues of experimental rats.
Values are given as mean ± SD for groups of six rats in each.
* Values are statistically significant when *p* < 0.05.
Group 2 was compared with Group 1; Groups 3 and 4 were compared with Group 2.
GSH, glutathione; SD, standard deviation.

Fig. 4 – Effect of *Senna alata* extract on GPx (Glutathione peroxidase) level in liver and renal tissues of experimental rats.
Values are given as mean ± SD for groups of six rats in each.
* Values are statistically significant when *p* < 0.05.
Group 2 was compared with Group 1; Groups 3 and 4 were compared with Group 2.
SD, standard deviation.

a noticeable (*p* < 0.05) decline in blood glucose and rise in plasma insulin levels were observed in rats treated with our plant extract. *Senna alata* might trigger the beta cells of pancreas to discharge more insulin which is essentially needed to lower the elevated blood glucose. *Senna alata* tends to lower blood glucose because of the release of insulin by the redeveloped beta cells of pancreas. Otherwise, it should have inhibitory effects on ATP sensitive K^{+} channels such as glibenclamide so that it has released insulin from redeveloped beta-cells and regulates blood glucose level[26] in STZ-diabetic

rats. Hence, *Senna alata* was proven to be a notable option for blood glucose maintenance. Studies also suggests that subsequent insulin level is must for carbohydrate metabolism.[27]

It was clearly observable that there was significant (*p* < 0.05) decrease in the body weight in induced rats as a result of abnormal carbohydrate metabolism.[28] In an earlier study it was reported that a great reduction in protein synthesis was observed in almost all the tissues due to insufficient ATP and insulin.[29]

After treatment, in Groups 3 and 4, there was an improvement in body weight of rats due to improvement in hyperglycemic control. Due to the poor synthesis of protein and high degradation protein, the levels of urea, uric acid, and creatinine increased in diabetic rats. However, after successful administration with *Senna alata*, it had been greatly reduced to its normal level which clearly indicates that, protein degradation has been reduced significantly because of the treatment with *Senna alata*. In diabetes, tissue damage is an unavoidable issue as result of excessive generation of free radicals due to lack of insulin. This may further raise lipid peroxidation processes in the biological systems[30,31] and similar results were detected in our present study.

Normal GSH is needed to preserve the structural and functional integrity of cells. The distinctive reduction in GSH level of liver and kidney tissues in diabetic rats and its subsequent rise up to normal level in *Senna alata* extract administration shows that the protection was offered by *Senna alata* in battling oxidative stress which is comparable with previous studies.[32]

Reduction in SOD, GPx, GST, and CAT found in induced rats shows the possibility of free radical induced damage. As we know very well, whenever we fail to maintain the ratio of antioxidants and free radicals normally, there will be a definite deregulation of cellular damage and activities.[33] An antioxidant drug can alleviate this dysfunction issues. The rise in the

activities of the antioxidant enzymes in *Senna alata* administered rats showed the potency of the plant extract in managing oxidative stress in diabetic rats.

It has been well proven in many studies that through a diet with huge antioxidants, unnecessary peroxidation process can be avoided.[34] Treatment of *Senna alata* to induced rats preserved the antioxidant defense enzymes into normal condition which might be the result of antioxidant properties of the plant.

SOD, the key enzyme of antioxidant system of tissues prevents reactive oxygen species by catalyzing the dismutation of superoxide to H_2O_2[35] whereas catalase decays hydroxyl radicals and is widely dispersed in all the tissues especially in the RBC (Red Blood Cells) and liver.[36] Reduction in these enzyme activity outcomes in several harmful effects due to gathering of superoxide and hydroxyl radicals. In this attempt, a decline in the CAT activity has been noticed. In experimental rats, the unrestrained release of hydrogen peroxide owing to the auto-oxidation of glucose and lipid oxidation led to notable decline in the CAT activity.[37]

Glutathione peroxidase splits hydrogen peroxide into water and molecular oxygen through the oxidation of reduced glutathione.[38] It is a vital preventive response during huge oxidative stress. Due to ROS (Reactive Oxygen Species) mediated inactivation and glycation, there will be decline in glutathione activity in diabetes.[39] The decreased role of GPx could be observable in low content of glutathione in induced rats. *Senna alata* has elevated them by inhibiting lipid peroxidation significantly. Therefore, any compound with antioxidant potency can be tried against oxidative stress and which may be also be effective in STZ induced diabetes.

Another important enzyme of this antioxidant system GST which is decreased in diabetic rats and subsequent raise after the treatment with *Senna alata* is also another supporting evidence for the antioxidant potency of *Senna alata*. In the result of a previous study[40] it was suggested that the reduction of GSH level also may reduce GST enzyme. In our study the enzymatic antioxidant activities such as SOD, CAT, GPx, and GST decreased in induced rats has been raised into normal level after successful administration with our plant extract.

It has already been demonstrated[41] that the decreased antioxidants synthesis can be seen during increased oxygen metabolites. In this perspective, it was also reported a diminished activity of enzymatic antioxidants in diabetic rats.[42]

It has been proven that diabetics have low levels of nonenzymatic antioxidants and their supplementation may help to overcome diabetes. In general, these antioxidants are required to inhibit the peroxidation of membrane lipids,[43] GSH, an antioxidant needed for cellular protection, such as detoxification of ROS, and removal of toxins.[44] Depletion of tissue GSH levels confirmed among diabetic rats clearly states the elevated utilization by the hepatic cells which might be the outcome of least synthesis or higher degradation of GSH.[45] In earlier published reports, it was proven that the GSH concentration decreases in the diabetic rats.[46] It has been observed that treatment with *Senna alata* and glibenclamide significantly raised the vitamin C, vitamin E, and GSH levels in diabetic rats.

In consideration with the results we obtained and earlier study observations, it was clearly indicated that *Senna alata* L

has the tendency to prevent the liver and renal tissues from the damage caused by the oxidative stress during diabetes in STZ induced experimental rats. It has good impact on both the enzymatic (SOD, CAT, GPx, and GST) and nonenzymatic antioxidant (vitamin C, vitamin E, and GSH). It has greatly influenced the rate of protein degradation by reducing its end products (urea, uric acid, and creatinine) elimination. On the whole, *Senna alata* L has much hope for its hypoglycemic property which had been proven in its plasma glucose lowering effect, body weight gain, and plasma insulin level improvements. Therefore, further studies are necessary to elucidate the exact mechanism by which *Senna alata* L exhibits its therapeutic effects.

Conflicts of interest

We do not have any conflicts of interest.

Acknowledgments

REFERENCES

1. Mohan V, Sandeep S, Deepa R. Epidemiology of type 2 diabetes: Indian scenario. *Indian J Med Res* 2007;125:217–30.
2. Pushparaj P, Tan CH, Tan BKH. Effects of *Averrhoa bilimbi* leaf extract on blood glucose and lipids in streptozocin-diabetic rats. *J Ethnopharmacol* 2000;72:69–76.
3. Findik E, Ceylan M, Elmastas M. Isoeugenol-based novel potent antioxidants: Synthesis and reactivity. *Eur J Med Chem* 2011;46:4618–24.
4. Elgebaly M, Portik-Dobos V, Sachidanandam K. Differential effects of ETA and ETB receptor antagonism on oxidative stress in type 2 diabetes. *Vasc Pharmacol* 2007;47:125–30.
5. Szkudelski T. The mechanism of alloxan and streptozotocin action of b-cells of the rat pancreas. *Physiol Res* 2001;50:537–46.
6. Kume E, Fujimura H, Matsuki N, Ito M, Aruga C, Toriumi W, et al. Hepatic changes in the acute phase of streptozotocin (SZ)-induced diabetes in mice. *Exp Toxicol Pathol* 2004;55:467–80.
7. Maritim AC, Sandres RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 2003;17:24–39.
8. Nayak Y, Veerapur VP, Nagappa AN, Unnikrishnan MK. Perspectives in efficacy, safety clinical evaluation bioactive natural products. In: Compendium of bioactive natural products, M/S. Studium Press. LLC, USA, 2009;vol. 2, p. 1-30.
9. Siddique O, Sun Y, Lin JC, Chien YW. Facilitated transdermal transport of insulin. *J Pharm Sci* 1989;76:341–5.
10. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *J Clin Pathol* 1969;22:158–61.

11. Burgi W, Briner M, Franken N, Kessler ACH. One step sandwich enzyme immunoassay for insulin using monoclonal antibodies. *Clin Biochem* 1988;21:311–4.

12. Lowry OH, Roseobrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenols reagent. *J Biol Chem* 1957;93:265–75.

13. Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. *J Clin Pathol* 1960;13:156–9.

14. Caraway WT. Determination of uric acid in serum by carbonate method. *Am J Clin Pathol* 1955;25:840–5.

15. Tietz NW. *Fundamentals of clinical chemistry*. Philadelphia: Saunders; 1987:p638.

16. Jaffe M. Concerning the precipitate produced in normal urine by picric acid and a new reaction of creatinine. *Physiol Chem* 1886;10:400.

17. Omaye ST, Turbull TD, Sauberlich HC. Selected method for the determination of ascorbic acid in animal cells, tissues and fluids. In: McCormic DB, Wright DL, eds. *Methods enzymology*. 62. New York, USA: Academic Press; 1979:3–11.

18. Desai ED. Vitamin E analysis methods for animal tissue. *Methods Enzymol* 1984;105:138–42.

19. Moron MS, Defierre JW, Mannervak D. Levels of glutathione, glutathione reductase, and glutathione-S-transferase activities in rat lung and liver. *Biochemica et Biophysica Acta* 1979;582:67–8.

20. Marklund SL, Marklund G. Simple assay for super oxide dismutase using autooxidation of pyrogallol. *Eur J Biochem* 1974;47:469–74.

21. Sinha AK. Colorimetric assay of catalase. *Anal Biochem* 1972;47:389–94.

22. Rotruck JT, Pope AL, Gasther HE, Hafeman DG, Hoekstra WG. Selenium biochemical role as a component of glutathione peroxidase. *Science* 1973;179:588–90.

23. Habig WH, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974;249:7130–9.

24. Loew D, Kaszkin M. Approaching the problem of bioequivalence of herbal medicinal products. *Phytother Res* 2002;16:705–11.

25. Jaiswal D, Rai PK, Watal G. Antidiabetic effect of withania coagulans in experimental rats. *Indian J Clin Biochem* 2009;24:88–93.

26. Jang SM, Kim MJ, Choi MS, Kwon EY, Lee MK. Inhibitory effects of ursolic acid on hepatic polyol pathway and glucose production in streptozotocin-induced diabetic mice. *Metab Clin Exp* 2010;59:512–9.

27. Shimazu T. Neuronal regulation of hepatic glucose metabolism in mammals. *Diabetes Metab Res Rev* 1987;3:185–206.

28. Chen V, Ianuzzo CD. Dosage effect of streptozotocin on rat tissue enzyme activities and glycogen concentration. *Can J Physiol Pharmacol* 1982;60:1251–6.

29. Murray RR, Granner DK, Mayes PA, Rodwell VW. *Harper's biochemistry, gluconeogenesis and the control of blood glucose*. Stanford, CT: Appleton and Lange; 2003:153–62.

30. Resmi CR, Venukumar MR, Latha MS. Antioxidant activity of Albizzia Lebbeck (LINN.) Benth. in: alloxan diabetic rats. *Indian J Physiol Pharmacol* 2006;50:297–302.

31. Wolff SP. The potential role of oxidative stress in diabetes and its complication novel implications for theory and therapy. In: Crabbes MJC, editor. *Diabetic complications—scientific and clinical aspects..* Edinburgh: Churchill Livingstone; 1987:167–220.

32. Prince PSM, Venugopal menon P. Antioxidant activity of *Tinospora cordifolia* roots in experimental diabetes. *J Ethnopharmacol* 1999;65:277–81.

33. Uday B, Dipak D, Ranajit Banerjee K. Reactive oxygen species: oxidative damage and pathogenesis. *Curr Sci* 1999;77(5):658–65.

34. Manna P, Ghosh J, Das J, Si PC. Streptozotocin induced activation of oxidative stress responsive splenic cell signaling pathways: Protective role of arjunolic acid. *Toxicol Appl Pharm* 2010;244:114–29.

35. McCord JM, Keele BB, Fridovich Jr I. An enzyme based theory of obligate anaerobiosis: The physiological functions of superoxide dismutase. *Proc Natl Acad Sci* 1976;68:1024–7.

36. Maritim AC, Sanders RA, Watkins JB. Effects of alipoic acid on biomarkers of oxidative stress in streptozotocininduced diabetic rats. *J Nutr Biochem* 2003;14:288–94.

37. Rajasekaran S, Sivagnanam K, Subramanian S. Antioxidant effect of Aloe vera gel extract in streptozotocin induced diabetic rats. *Pharmacol Rep* 2005;57:90–6.

38. Ewis SA, Abdel-Rahman MS. Effect of metformin on glutathione and magnesium in normal and Streptozotocin induced diabetic rats. *J Appl Toxicol* 1995;15:387–90.

39. Zhang XF, Tan BK. Antihyperglycemic and antioxidant properties of *Andrographis paniculata* in normal and diabetic rats. *Clin Exp Pharmacol Physiol* 2000;27:358–63.

40. Al-Wabel NA, Mousa HM, Omer OH, Abdel-Salam AM. Biological evaluation of aqueous herbal extracts and stirred yoghurt filtrate mixture against alloxan-induced oxidative stress and diabetes in rats. *Int J Pharm* 2008;4:135–9.

41. Schettler V, Wieland E, Verwiebe R, Schuff-Werner P, Scheler F, Oellerich M. Plasma lipids are not oxidized during hemodialysis. *Nephron* 1994;67:42–7.

42. Karthikesan K, Pari L, Menon VP. Protective effect of tetrahydrocurcumin and chlorogenic acid against streptozotocin–nicotinamide generated oxidative stress induced diabetes. *J Funct Foods* 2010;2:134–42.

43. Opara EC. Oxidative stress, micronutrients, diabetes mellitus, and its complications. *J R Soc Promot Health* 2002;122:28–34.

44. Brown LA, Harris FL, Ping XD, Gauthier TW. Chronic ethanol ingestion and the risk of acute lung injury: A role for glutathione availability. *Alcohol* 2004;33:191–7.

45. Furfaro AL, Nitti M, Marengo B, Domenicotti C, Cottalasso D, Marinari UM, et al. Impaired synthesis contributes to diabetes-induced decrease in liver glutathione. *Int J Mol Med* 2012;29:899–905.

46. Sayed AA. Ferulsinaic acid modulates SOD, GSH, and antioxidant enzymes in diabetic kidney. *Evid Based Complement Alternat Med* 2012;58:1–9.