**Advances in Bioresearch**
Volume 3 [3] September 2012: 67 - 73
ISSN 0976 – 4585
© Society of Education, INDIA

ABR

www.soeagra.com/abr/abr.htm

**ORIGINAL ARTICLE**

# Protective effect of Aloe Vera Leaves Extract on Hepatic Tissue Injury in Streptozotocin-induced Diabetic Rats

**Bahram Amouoghli Tabrizi[1]*, Daryoush Mohajeri[2]**
1-Department of Clinical Sciences, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2-Department of Pathobiology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[1]Corresponding author: E-mail: Email: b_tabrizi@iaut.ac.ir, daryoushmohajeri@yahoo.com;
Tel: +98 914 1144 560.

**ABSTRACT**
*The negative impact of diabetes on the liver is well recognized. This study was designed to evaluate the hepatoprotective properties of Aloe vera (Aloe barbadensis Miller) leaves extract in streptozotocin-induced diabetes in rats. Male Wistar rats were made diabetic with a single injection of STZ (65 mg/kg i.p.). Rats were randomly allocated into four groups, 10 animals each: Group 1, healthy control rats; Group 2 non-diabetic rats treated with 50 mg/kg b.w./day i.p. injection of Aloe vera extract; Group 3, diabetic rats; Group 4, diabetic rats treated with the extract (50 mg/kg b.w./day, i.p.) for 8 weeks. Finally, serum AST, ALT, ALP and albumin levels as well as liver MDA, GSH contents and activities of GSH-Px, SOD and CAT were measured to assess hepatic injury. Liver MDA content and serum ALT, AST, AP and bilirubin levels in Groups 3 were found to be significantly increased as compared to Group 1 (P<0.05) and these parameters in Group 4 were significantly decreased as compared to Group 3 (P<0.05). Liver GSH, SOD, CAT and GSH-Px contents and serum albumin level in Group 3 was significantly decreased as compared to Group 1 (P<0.05) and were found to be significantly increased in Group 4 as compared to Groups 3 (P<0.05). Histopathological changes were in agreement with biochemical findings. This study showed that Aloe vera extract have hepatoprotective effects in experimentally induced diabetic rats.*
***Keywords**: Aloe vera, Diabetes mellitus, Liver, Protective effect, Rat*

## INTRODUCTION

The detrimental effects of diabetes mellitus on the liver, yet little is known and [13]. Diabetes is the most important cause of liver disease in the world and liver insufficiency is one of the important causes of death in type II diabetes [33]. Patients with diabetes have a high incidence of liver disease and patients with liver disease have a high prevalence of diabetes. Almost, most of liver disease is seen in patients with type 2 diabetes [19]. Liver is the main effective organ for maintaining blood glucose levels within normal limits. Hyperglycemia can generate a redox imbalance inside the cells, especially in the liver [12]. Lipid peroxidation and antioxidant status of hepatic tissue were studied by Feillet-Coudray et al., in experimental diabetes [7]. However, when the liver fails, there is no equivalent form of management, such as hemodialysis or retinal photocoagulation. Therefore, although diabetic hepatopathy is potentially less common, it may be appropriate for addition to the list of target organ conditions related to diabetes. Antidiabetic drugs have been shown to decrease the levels of serum biomarkers of liver injury [9]; but these agents can produce serious side effects [25]. Medicinal plants are safe alternative treatment for hyperglycemia and liver injury. Herbal drugs or their extracts are prescribed widely, although their biological active compounds are unknown [36]. Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety. Herbal medicines are being increasingly used to treat a wide variety of disease. Herbal remedies with a concomitant antioxidant effect would be more useful in the management of diabetes mellitus. Many compounds are known, which lower blood glucose levels and exhibit antioxidative properties could also alleviate the oxidative damage in diabetic tissues. In this basis, Aloe vera can be suggested as a plant of considerable interest. Aloe vera (Aloe barbadensis Miller) is a cactus-like plant that grows in hot, dry climates [38]. It is used in Ayurveda for managing painful conditions and is also mentioned in folk medicine of Arabian Peninsula for management of diabetes [11]. Aloe vera has been reported to possess antioxidant, immunomodulatory, antiinflammatory, UV protective, antiprotozoal, antifungal, hypoglycemic, gastroprotective and wound and burn healing properties [4,38]. The effects of Aloe vera gel were studied on the blood glucose levels of alloxan-diabetic mice and were found to reduce plasma glucose levels [23]. Hu et al (2005) showed that compared to the commercial antioxidants, Aloe vera extracts have powerful antioxidant capacity than α-tocopherol and the activities [32].

Considering the antihyperglycemic properties and antioxidant activities of Aloe vera extract, this study was designed to evaluate the hepatoprotective effects of Aloe vera extract in streptozotocin-induced diabetes of rats.

## MATERIALS AND METHODS

*Chemicals*

Streptozotocin was from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade. All chemicals used in this study were of analytical grade and obtained from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

*Plant*

Aloe vera leaves were collected from a single garden plant, to obtain a fresh extract. Identification of the plant was done by Department of Cultivation and Development of Institute of Medicinal Plants, Islamic Azad University, Iran.

Preparation of the extract: Some 300 g of the clean fresh plant leave material was ground using an electrical grinder. The extraction was carried out using 70% ethanol. The mixture was agitated over the mechanical shaker for 12 h. The resulting mixture was filtered and the filtrate concentrated into a residue over water bath [20]. The yield was 11.5% (w/w). Consequently the residue from the extract was dissolved in saline and used in the study.

*Induction of diabetes Mellitus*

Diabetes was induced by intravenous injection of streptozotocin (Sigma, St. Louis, Mo, USA) into the tail vein at a dose of 65 mg/kg body weight. STZ was extemporaneously dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. After 18 h, animals with fasting blood glucose levels greater than 120-250 mg/dl were considered diabetic and then included in this study [36]. Fasting blood glucose was estimated by using one touch glucometer (Accu-chek sensor) of Roche Diagnostics, Germany.

*Animal treatment*

Forty healthy male Wistar rats (about 180–200 g body weight) were used for this study. All animals were conditioned at room temperature at a natural photoperiod for 1 week before experiment execution. A commercial balanced diet and tap water ad libitum were provided. The duration of experiment was 8 weeks. The rats were randomly divided into 4 groups (10 rats each) as the following: Group 1, healthy control rats received isotonic saline solution (ISS, 10 ml/kg1) intraperitoneally; Group 2 non-diabetic rats were treated with 50 mg/kg b.w. /day intraperitoneal (i.p.) injection of Aloe vera leaves extract; in Group 3, diabetic rats administered by ISS (10 ml/kg1) was given through Intraperitoneal (IP) route; Group 4, diabetic rats were treated with Aloe vera leaves extract (50 mg/kg b.w. /day, i.p.) for a period of 8 weeks. The animals of different groups were sacrificed under light anesthesia (diethyl ether) 1 day after the end of the treatment. This experimental study was carried out in Islamic Azad University Research Center and all procedures and works on animals was conducted under Animal Rights Monitoring Committee of Islamic Azad University Research Center.

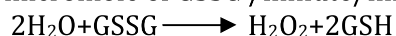
Biochemical factors evaluation: At the end of experiment, blood samples were collected from the retro-orbital plexus and the sera prepared through centrifuging at 2500 ×g for 15 minutes at 30˚C. Serum biomarkers of liver function including ALT, AST [37], ALP [31], albumin [28], and total bilirubin [16] were measured using commercially available kits.

*Microscopic studies*

The animals of different groups were sacrificed under light anesthesia (diethyl ether) 1 day after the end of the treatment. A small piece of hepatic tissue from the anterior portion of the left lateral lobe was removed for histological analysis. The sample was fixed by immersing it in 10% neutral-buffered formalin. The sample was then embedded in paraffin, sliced into 5 m sections, and stained with hematoxylin-eosin for blinded histological assessment. The degree of liver tissue injury was evaluated semi quantitatively according to the method reported by Jamshidzadeh et al. [2]. The stained 5 m sections were graded as follows: 0, absent; 1, minimal; 2, mild; 3, modest; 4, severe. The histological changes were evaluated in nonconsecutive, randomly chosen ×200 histological fields using light microscope, NIKON ECLIPSE E200.

*Measurement of antioxidant activity*

The rat's Liver were removed immediately and washed in normal saline and homogenate 10% prepared in 1.15% w/v of potassium chloride. The homogenate was centrifuged in 7000 ×g for 10 minutes at 4°C and supernatant were used for measurement of oxidative stress by estimation of reduced glutathione (GSH) and determination of malondialdehyde (MDA) as well as antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). GSH, MDA, SOD, CAT and GSH-Px were measured by using commercially available kits according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).
Reduced glutathione (GSH) content was measured according to Sedlak J, Lindsay method [17]. GSH reacts with 5,5′-dithiobis-2-nitrobenzoic acid, and the absorbance spectra of the product have a maximum absorbance at 410 nm. The results were expressed as mol/gwt. Liver homogenate MDA levels were expressed as nmol MDA per mg protein and antioxidant activity was expressed as arbitrary units per mg protein. Degree of lipid peroxidation in liver tissue homogenates was determined in terms of thiobarbituric acid reactive substances (TBARSs) formation by following the protocol of Esterbauer and Cheesman [14]. SOD activity was measured by Nishikimi method [32] and was modified by Kakkar method [29]. Each unit of SOD activity was determined as required enzyme concentration for prohibition of creation color at 1 minute, under study conditions. CAT activity was determined by Claiborne method [1] and was based on hydrogen peroxide breakdown. GSH-Px activity was measured by Rotruck method [18] and was stated as micromole of GSSG /minute/milligram of protein, based on blew reaction:



$$2H_2O + GSSG \longrightarrow H_2O_2 + 2GSH$$

*Statistical analysis*

All data are presented as mean ± SEM. The data obtained were tested by ANOVA followed by Tukey's post-hoc test using SPSS software. The Kruskal-Wallis test, followed by Mann–Whitney U post-test, was used for the analysis of degree of histopathological liver injury. P<0.05 was considered statistically significant.

## RESULTS

Results of the effect of daily treatment of Aloe vera leaves extract at a dose of 50 mg/kg for 8 weeks on blood glucose levels of experimental rats are presented in Table 1. The Aloe vera leaves extract produced significant hypoglycemic effect in normal (P<0.05) and diabetic (P<0.01) rats after 8 weeks of administration. Table 1 shows the effects of Aloe vera leaves extract on the serum levels of markers of liver injury (ALT, AST, ALP and bilirubin) in diabetic rats. ALT, AST, ALP and bilirubin serum contents in Groups 3 was found to be significantly increased as compared to Group 1 (P<0.05) and these parameters in Group 4 were significantly decreased as compared to Group 3 (P<0.05). The albumin serum level in Group 3 was significantly decreased as compared to Groups 1 (P<0.05) and this parameter was significantly increased in Group 4 as compared to Group 3 (P<0.05).

**Table 1:** Comparison of the effect of Aloe vera leaves extract on blood glucose levels and serum markers of liver tissue injury among the experimental groups (mean±SEM)

| Groups | | Biochemical parameters | | | | |
|---|---|---|---|---|---|---|
| | Blood glucose level (mg/dL) | Alanine aminotransferase (U/L) | Aspartate aminotransferase (U/L) | Alkaline phosphatase (U/L) | Total serum bilirubin (Mg/dl) | Albumin (g/dl) |
| **Halthy control rats** | 84.81±4.23 | 54.82±2.36 | 68.6±2.35 | 195.77±6.9 | 0.81±0.03 | 4.38±0.32 |
| **Nondiabetic rats+ Aloe vera extract** | 55.94±2.37 | 55.45±2.64 | 69.71±3.24 | 198.75±8.65 | 0.87±0.06 | 4.35±0.66 |
| **Diabetic rats** | 152.25±6.44 | 75.43±3.86[a] | 102.15±4.85[a] | 275.34±10.22[a] | 1.21±0.08[a] | 3.14±0.23[a] |
| **Diabetic rats+ Aloe vera extract** | **87.11±4.71** | **52.11±2.33[b]** | **67.59±3.41[b]** | **205.73±9.9[b]** | **0.89±0.04[b]** | **4.31±0.42[b]** |

*p<0.05; [a]compared to Group 1, [b]compared to Group 3.

 © Society of Education, INDIA

Table 2 shows the effects of Aloe vera leaves extract on antioxidative activity in liver tissue of diabetic rats. MDA contents of the liver tissue in Groups 3 was found to be significantly increased as compared to Group 1 ($P<0.05$) and liver MDA level in Group 4 were significantly decreased as compared to Group 3 ($P<0.05$). The GSH, SOD, CAT and GSH-Px contents of the liver in Group 3 were significantly decreased as compared to Groups 1 ($P<0.05$) and GSH, SOD, CAT and GSH-Px activity were increased in Group 4 as compared to Group 3 ($P<0.05$). Pathologically, liver histological structure was normal in healthy control group (Fig. 1, A). In Group 2 also there were no pathological changes so that hepatic lobular structure seemed quite normal (Fig. 1, B). In group 3, Diabetic rats showed fatty changes in centrilobular portions of the livers (Fig. 1, C).

**Table 2:** Comparison of the effect of Aloe vera leaves extract on liver MDA and GSH contents and antioxidant enzymes activities among the experimental groups (mean±SEM)

| Groups | Biochemical parameters | | | | |
|---|---|---|---|---|---|
| | GSH (µg/mg protein) | MDA (nmol/g protein) | SOD (U/mg protein) | CAT (U/mg protein) | GPX (U/mg protein) |
| **Healthy control rats** | 8.95±0.64 | 3.59±0.16 | 13.55±0.56 | 62.17±3.42 | 21.84±1.22 |
| **Nondiabetic rats+ Aloe vera extract** | 8.87±0.35 | 3.25±0.21 | 13.75±0.83 | 63.52±2.35 | 22.68±1.32 |
| **Diabetic rats** | 5.89±0.46[a] | 5.43±0.23[a] | 8.67±0.70[a] | 49.84±1.81[a] | 17.47±0.63[a] |
| **Diabetic rats+ Aloe vera extract** | **7.60±0.57[b]** | **4.35±0.36[b]** | **11.95±0.59[b]** | **59.77±4.21[b]** | **20.75±1.12[b]** |

*p<0.05; [a]compared to Group 1, [b]compared to Group 3.

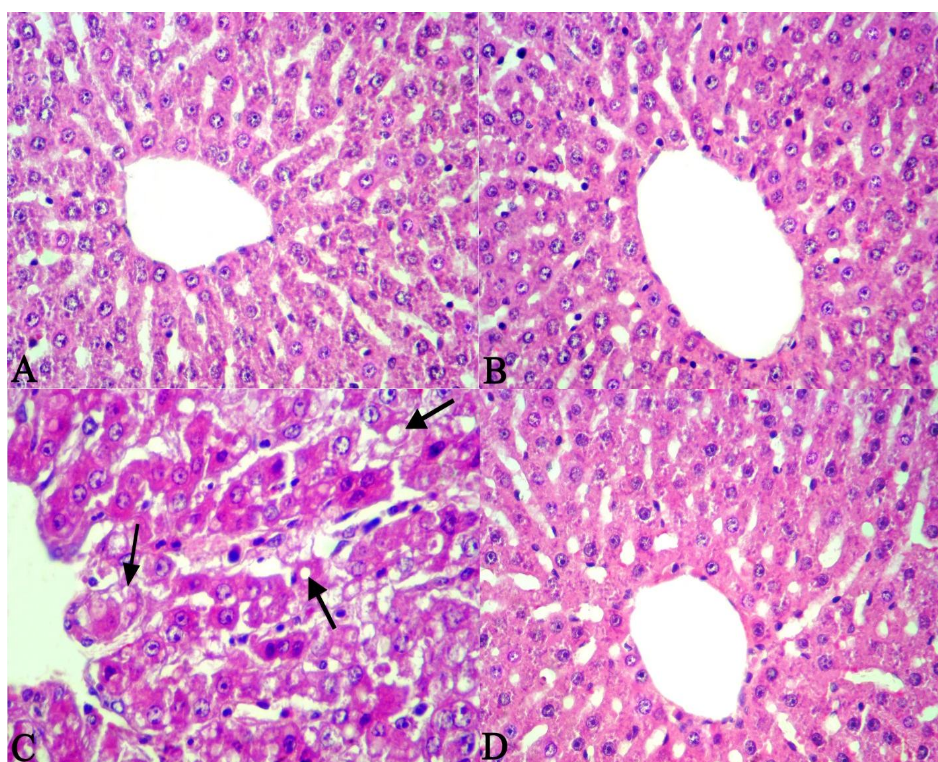



**Fig 1:** Microscopic appearance from liver tissues of the experimental rats (H&E, 100×). A- Healthy control rat liver showing normal hepatocytes. B- non-diabetic+ Aloe vera extract treated rat liver shows normal appearance. C- Diabetic rat liver showing microvesicular fatty change (arrows) in centrilobular portions. D- Diabetic+ Aloe vera extract treated rat liver showing no fatty change.

Finally, in group 4, Aloe vera leaves extract prevented the pathologic changes and no considerable fatty change was observed (Fig. 1, D). Quantitative microscopic results of experimental rats are presented in Table 3.

**Table 3:** Effect of Aloe vera extract on hepatic injuries of diabetic rats (mean±SEM)

| Groups | Treatment | Degree of liver tissue injury | The Kruskal-Wallis test |
|---|---|---|---|
| **1** | healthy control rats | 0.0±0.0 | p<0.001 |
| **2** | non-diabetic rats+ Aloe vera extract | 0.0±0.0 | |
| **3** | diabetic rats | 2.42±0.23[a] | |
| **4** | diabetic rats+ Aloe vera extract | 0.51±0.11[b] | |

0=without injury, 1= minimum injury, 2= mild injury, 3= moderate injury, 4= sever injury (n=10), [a]compared to Group 1, [b]compared to Group 3

## DISCUSSION

Intraperitoneal injection of Aloe vera leaves extract caused significant reductions in blood glucose levels of healthy normal rats. Aloe vera leaves extract, also caused significant hypoglycemic effect in diabetic rats. Hypoglycemic response with Aloe vera leaves extract has already reported [11]. It has been shown that, hypoglycemic effect of Aloe vera is mediated through stimulation of synthesis and/or release of insulin [23]. In our study, results of biochemical and histopathological assessments, reflects liver injuries in rats with streptozotocin-induced diabetes. Significant alterations in the markers of liver in diabetic rats were observed in comparison with normal healthy rats. These results are consistent with the findings reported by Ramesh et al [6]. Patients with type 2 diabetes have a higher incidence of liver biomarkers abnormalities than healthy peoples [9]. This finding is also in line with our results. The data of our study also revealed that daily treatment of Aloe vera extract markedly improves biochemical and histopathological status of rats with streptozotocin induced diabetes. Increase in serum levels of AST shows hepatic injuries similar to viral hepatitis, infarction and muscular damages. ALT is specific for liver and is a suitable indicator of hepatic injuries. Increased levels of these enzymes are an indicator of disturbance of hepatocytes membranes [35]. ALP is membrane bound and its alteration affects the membrane permeability and produce derangement in the transport of metabolites [8]. Bilirubin and albumin values are associated with the function of hepatic cells [30]. Return of the above enzymes to normal serum values following Aloe vera extract treatment may be duo to prevention of intracellular enzyme leakage resulting from cell membrane stability or cellular regeneration [24]. Impressive control of bilirubin and albumin indicates improvement of functional and secretory mechanism of hepatic cells. In this study, histopathological evaluation of liver tissues showed fatty changes in centrilobular regions of the livers in diabetic rats. With Aloe vera treatment in diabetic rats no considerable fatty change were observed demonstrating the protective effect of Aloe vera extract against hepatic complications of diabetes. In any way, pathologic findings were matched with biochemical results. In our study significant reduction in GSH level and antioxidant enzymes activity and augmented lipid peroxidation in the liver tissue of rats shows oxidative stress of the liver in experimental diabetes. These results are in line with the findings reported by Feillet-Coudray et al [7]. The data of our study also indicated that daily treatment of Aloe vera extract considerably improves antioxidant status of liver tissue of rats with streptozotocin-induced diabetes. Oxidative stress leads to an increased production of ROS (reactive oxygen species), as well as lipid peroxidation is increased in diabetes [3]. Elevation in MDA level and reduction in GSH stores of liver tissue of diabetic rats suggest that oxidative stress due to free-radical damage is one of the possible mechanisms in diabetic hepatopathy. By administration of Aloe vera extract, the MDA levels have decreased and the GSH levels have increased. This indicates that in the presence of Aloe vera extract there is an improvement in the oxidative stress. Increased oxidative stress in the tissues of streptozotocin diabetic rats was similarly reported [34]. They observed that GSH administration reverses these effects [10]. SOD, CAT and GPx constitute a defense against ROS. In our study, reduction in the activities of these enzymes in liver tissue of diabetic animals and recovery in Aloe vera extract treated rats; indicate oxidative stress in hepatic tissue of diabetic rats had been inactivated by the effect of the extract. This finding is completely in agreement with those

of Parihar et al who demonstrated hypoglycaemic and antioxidant activity of Aloe vera extract in streptozotocin induced diabetic mice [26]. Liver is one of the most important organs that maintains blood glucose levels within normal limits thus enhancement of blood glucose leads to imbalance of oxidation-reduction reactions in hepatocytes, so that, hyperglycemia through increasing in advanced glycation end products (AGEs) facilities free radicals production through disturbance in ROS production [27]. Therefore, it reveals that diabetic hepatic damage is not controllable only by inhibition of hyperglycemia [15]. In other words, in early stages of diabetes, tissues injuries are in association with hyperglycemia but its progress is not related to hyperglycemia. Therefore, monitoring of blood glucose levels solely is not sufficient in retarding diabetes complications. Thus, a suitable drug must have both antioxidant and blood glucose decreasing properties [5].

## CONCLUSION

We observed that Aloe vera extract improved serum biomarkers of liver tissue injury and histopathologic properties of this organ. It is presumed that Aloe vera extract prevents diabetic complications and ameliorates diabetic hepatopathy through its antioxidant potential.

## ACKNOWLEDGEMENTS

This work was supported by Research vice-chancellor of Tabriz Branch, Islamic Azad University.

## REFERENCES

1. A.Claiborne (1985). Catalase activity. In: Handbook of methods for oxygen radical research, Boca Raton F.L., Ed. Florida: CRC Press, Boca Raton. pp: 542.
2. A. Jamshidzadeh, M. Baghban, N. Azarpira, A. Mohammadi Bardbori, H. Niknahad (2008). Effects of tomato extract on oxidative stress induced toxicity in different organs of rats, *Food Chem. Toxicol*. **46**, 3612-3615.
3. A. Kamal, G.J. Biessels, S.E. Duis, W.H. Gispen (2000). Learning and plasticity in diabetes and aging, *Diabetologia*. **43**, 500-506.
4. A.M. Gressner (1991). Liver fibrosis: Perspectives in pathobiochemical research and clinical outlook, *Eur. J. Clin. Chem. Clin. Biochem.* **29**, 293-311.
5. B. Ramesh, K.V. Pugalendi (2006). Impact of umbelliferone (7-hydroxycoumarin) on hepatic marker enzymes in streptozotocin diabetic rats, *Indian J. Pharmacol*. **38,** 209-210.
6. B. Ramesh, P. Viswanathan, K.V. Pugalendi (2007). Protective effect of Umbelliferone on membranous fatty acid composition in streptozotocin-induced diabetic rats, *Euro. J. Pharmacol*. **566**, 231-239.
7. C. Feillet-Coudray, E. Rock, C. Coudray, K. Grzelkowska, V. Azais-Braesco, D. Dardevet, A. Mazur (1999). Lipid peroxidation and antioxidant status in experimental diabetes, *Clin. Chim. Acta*. **284**, 31-43.
8. E.E. Mehana, A.R. Meki, K.M. Fazili (2012). Ameliorated effects of green tea extract on lead induced liver toxicity in rats, *Exp. Toxicol. Pathol*. **64**, 291-295.
9. E.H. Harris (2005). Elevated Liver Function Tests in Type 2 Diabetes, *Clin. diabetes*. **23**, 115-119.
10. F. Curcio, I. Pegoraro, P. Dello Russo, E. Falleti, G. Perrella, A. Ceriello (1995). SOD and GSH inhibit the high glucose induced oxidative damage and the PGDF increased secretion in cultured human endothelial cells, *Thromb Haemost.* **74**, 969-973.
11. F.M. Al-Awadi, K.A. Gumaa (1987). Studies on the activity of individual plants of an antidiabetic plant mixture, *Acta Diabetol. Lat.* **24**, 37-41.
12. G. Gallou, A. Ruelland, B. Legras, D. Maugendre, H. Allannic, L. Cloarec (1993). Plasma MDA in type 1 and type 2 diabetes, *Clin. Chim. Acta.* **214**, 227-234.
13. G. Orasanu, J. Plutzky (2009). The pathologic continuum of diabetic vascular disease, *J. Am. Coll. Cardiol*. **53**, S35-42.
14. H. Esterbauer, K.H. Cheeseman (1990). Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal, *Methods Enzymol*. **186**, 407-421.
15. H.R. Liu, X.Y. Tang, D.Z. Dai, Y. Dai (2008). Ethanol extracts of Rehmannia complex (Di Huang) containing no corni fructus improve early diabetic nephropathy by combining suppression on the ET-ROS axis with modulate hypoglycemic effect in rats, *J. Ethnopharmacol*. **118,** 466-472.
16. H.T. Malloy, K.A. Evelyn (1937). The determination of bilirubin level with the photoelectric colorimeter, *J. Biol. Chem.* **119**, 481-484.
17. J. Sedlak, R.H. Lindsay (1968). Estimation of total, protein bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent, *Anal. Biochem*. **25**, 192-205.
18. J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, W.G. Hoekstra (1973). Selenium: biochemical role as a component of glutathione peroxidase, *Science*. **179**, 588-590.
19. K.G. Tolman, V. Fonseca, A. Dalpiaz, M.H. Tan (2007). Spectrum of Liver Disease in Type 2 Diabetes and Management of Patients with Diabetes and Liver Disease, *Diabetes care.* **30**, 734-743.
20. K.R. Brian, T.D. Turner (1975). The practical evaluation of phytopharmaceutical, *Wright Scentechica, Bristol*. pp: 90.
21. L.J. Marnett (2000). Oxyradicals and DNA damage, *Carcinogenesis*. **21**, 361-370.
22. M. Nishikimi, N. Appaji, K. Yagi (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen, *Biochem. Biophys. Res. Commun*. **46**, 849-854.

23. M.A. Ajabnoor (1990). Effect of aloes on blood glucose levels in normal and alloxan diabetic mice, *J. Ethnopharmacol*. **28**, 215-220.
24. M.I. Thabrew, P.D. Joice, W. Rajatissa (1987). A comparative study of the efficacy of Pavetta indica and Osbeckia octanda in the treatment of liver dysfunction, *Planta Med*. **53**, 239-241.
25. M.S. Akhtar, J. Iqbal (1991). Evaluation of the hypoglycemic effect of achyranthes aspera in normal and alloxan diabetic rabbits, *J. Ethnopharmacol*. **31**, 49-57.
26. M.S. Parihar, M. Chaudhary, R. Shetty, T. Hemnani (2004). Susceptibility of hippocampus and cerebral cortex to oxidative damage in streptozotocin treated mice: prevention by extracts of Withania somnifera and Aloe vera, *J. Clin. Neuro. Sci.* **11**, 397-402.
27. N.E. Cameron, T.M. Gibson, M.R. Nangle, M.A. Cotter (2005). Inhibitors of advanced glycation end product formation and neurovascular dysfunction in experimental diabetes, *Ann. N. Y. Acad. Sci.* **1043**, 784-92.
28. O.H. Lowry, N.J. Rosebrough, A.L. Farr (1951). Protein measurement with the folin phenol reagent, *J. Biol. Chem.* **193**, 265-275.
29. P. Kakkar, B. Das, P.N. Viswanathan (1984). A modified spectrophotometric assay of superoxide dismutase, *Indian J. Biochem. Biophys.* **21**, 130-132.
30. P. Muriel, T. Garciapiña, V. Perez-Alvarez, M. Mourelle (1992). Silymarin protects against paracetamol-induced lipid peroxidation and liver damage, *J. Applied Toxicol*. **12**, 439-442.
31. P.R. Kind, E.J. King (1954). Estimation of plasma phosphates by determination of hydrolyzed phenol with antipyrin, *J. Clin. Pathol*. **7**, 322-6.
32. Q. Hu, Y. Hu, J. Xu (2005). Free radical-scavenging activity of Aloe vera (Aloe barbadensis Miller) extracts by supercritical carbon dioxide extraction, *Food Chem*. **91**, 85-90.
33. R. de Marco, F. Locatelli, G. Zoppini, G. Verlato, E. Bonora, M. Muggeo (1999). Cause-specific mortality in type 2 diabetes: The Verona Diabetes Study, *Diabetes Care.* **22**, 756-761.
34. R. Kakkar, J. Kalra, S.V. Mantha, K. Prasad (1995). Lipid peroxidation and activity of antioxidant enzymes in diabetic rats, *Mol. Cell Biochem*. **151**, 113-119.
35. R.B. Drotman, G.T. Lawhorn (1978). Serum enzymes are indications of chemical induced liver damage, *Drug Chem. Toxicol.* **1**, 163-71.
36. R.K. Gupta, A.N. Kesari, P.S. Murthy, R. Chandra, V. Tandon, G. Watal (2005). Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of Annona squamosa L. in experimental animals, *J. Ethnopharmacol*. **99**, 75-81.
37. S. Reitman, S. Frankel (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase, *Am. J. Clin. Pathol*. **28**, 56-63.
38. T. Reynolds, A.C. Dweck (1999). Aloe vera leaf gel: a review update, *J. Ethnopharmacol*. **68**, 3-37.

 © Society of Education, INDIA