Section A-Research paper
ISSN 2063-5346

Evaluation of modulatory effects of plant extracts on antioxidant and transaminases in liver and pancreas of streptozotocin induced diabetic rats.

V. Sravani[1], S. Payani[2], C. Chandraprakash[2] and M. Bhaskar[1,2]*

Affiliations:

1: Division of Bioinformatics, Department of Zoology, Sri Venkateshwara University, Tirupati, India, 517502.

2: Division of Animal Biotechnology, Department of Zoology, Sri Venkateshwara University, Tirupati, India, 517502.

Email addresses of authors

V. Sravani: v.sravanireddy3@gmail.com

S. Payani: s.payani63@gmail.com

C. Chandraprakash: chandraprakashchinta@gmail.com

M. Bhaskar: matchabhaskar@gmail.com

Corresponding author Address:

Prof M. BHASKAR
Department of Zoology,
S.V.U. College of Sciences,
Sri Venkateshwara University,
Tirupati517502, AP, India.
9959911927;
E.mail: matchabhaskar@gmail.com.

Abstract:

Background: Consumption of Mango (*Mangifera indica*) and *Aloe vera* have shown bioactive properties against Important diseases like diabetes, therefore, we evaluated how a mango pulp and *Aloe vera* supplemented diet affects metabolic pathways in diabetic rats.

Methods: The present study was taken up to evaluate the antioxidant and total protein activity in mango pulp and *Aloe vera* supplementation of streptozotocin (STZ) induced diabetic rats. After experimental period of 21 days the liver and pancreatic tissues were collected from 48 male wistar rats divided into 8 groups and total protein and antioxidative enzymes activity was evaluated.

Result: Mango pulp and *Aloe vera* supplementation maintained significant antioxidant and transaminase effects after treatment and total proteins in the liver and pancreas of diabetic rats, likely at normal level due to its phenolic compounds, like mangiferin and its metabolites.

Key words: Mangiferin, *Aloe vera* , streptozotocin

INTRODUCTION:

Diabetes mellitus (DM) is most common and epidemic in nature and is growing rapidly across the world and is now recognized as one of the key threats to human health **(Khawandanah *et al.,* 2019).**DM is a group of metabolic disorders characterized by hyperglycemia, where alterations in the carbohydrate, fat and protein metabolisms accompanied by absolute or relative deficiencies in insulin secretion and /or its action. Moreover, basal hyperglycemia occurs irrespective of whether insulin deficiency or insulin resistance is the dominant defect **(Rajasekaran *et al.,* 2005).**

Persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), in tissues from glucose auto-oxidation and protein glycosylation **(Gerstein *et al.,* 2007; Baynes *et al.,* 2010; Giacco *et al.,* 2010).** Several reports suggested that increased free-radical mediated oxidative stress is involved in diabetic complications **(Boudrea *et al.,* 2006),** which includes primarily the ROS generated due to the increased free fatty acids (FFA) levels in the cells **(Ajabnoor, 1990; McGarry, 2002; Gerstein *et al.,* 2007; Radha Madhavi *et al.,* 2012).** Increased levels of FFAs are positively correlated with both insulin resistance (**Ajabnoor, 1990; McGarry, 2001)** and the deterioration of cell function in the context of concomitant hyperglycemia (**Gerstein *et al.,* 2007; Radha Madhavi *et al.,* 2012).** The metabolic abnormalities of diabetes cause mitochondrial superoxide overproduction in endothelial cells of both large and small vessels, as well as in the myocardium. Therefore, oxidative stress plays a major role in the development of diabetes complications, both micro vascular and cardiovascular complications **(Ajabnoor, 1990; Rajasekaran *et al.,* 2005).**

The liver is the important insulin-dependent organ, which plays a vital role in glucose and lipid homeostasis and it is severely affected during diabetes **(Giacco *et al.,* 2010).** Pancreatic β cells are the source of insulin production and are extremely susceptible to oxidative stress (**Jingjing Wang *et al.,* 2010**). oxidative stress has also been proposedto play a role in the pathogenesis of hepatic tissues damage **(Ha *et al.,* 2001; Amarapurkar *et al.,* 2002; Kashihara *et al.,* 2010).** However, during pathological conditions, the decline free radical production and the protective antioxidant defense system may cause ROS-induced tissue damage including hepatic injury **(Gerstein *et al.,* 2007; Giacco *et al.,* 2010; Baynes *et al.,* 2010).**

Earlier, hormone therapy using insulin is one of the classical approaches to treat diabetes. Though it is promising, the ineffectiveness of oral insulin therapy against diabetes limits the insulin therapy, moreover, the insulin formulations including biguanides, sulfonylureas, glinides and glitazones **(Lorenzi *et al.,* 2011; Singh *et al.,* 2011)** were causes side effects like hematological and gastrointestinal reactions, brain damage and disturbances of liver and pancreas functions **(Takamoto *et al.,* 2011).**

Recent approaches suggest that treatment of diabetes should not only focus on insulin secretion but also on antioxidant protection of the b-cell. This may facilitate the repair of b-cells undergoing damage by oxidative stress secondary to hyperglycemia. World Health Organization has recommended that the evaluation of traditional medicinal plants treatment for diabetes were

effective, non-toxic, with less or no side effects and is considered being excellent candidates for oral therapy **(Liu *et al.,* 2013).**

Nature provides us many plants to treat many diseases among them we selected natural miracle medicinal plant *Aloe vera* and pride fruit of India the mango (*mangifer Indica)*to treat Diabetes mellitus. *Aloe vera* is a wonderful succulent plant widely used in traditional medicine. It contains 75 active compounds like anthraquinones, saponins, carbohydrates, chromones, hormones, minerals, vitamins, enzymes, lignin, salicylic acids, and amino acids and it has many biological activities **(Hamman, 2008; Sharrif Moghaddasi *et al*., 2011).** Many studies suggest that it has anti diabetic properties by controlling the blood glucose level, biochemical parameters and antioxidant stress enzymes in an alloxan or streptozotocin induced diabetic animal models **(Guru Sekhar *et al.,* 2010; Sadak Basha *et al*., 2010; Radha Madhavi *et al*., 2012).**

Mangifera indica belongs to family Anacardiaceae and usually known as mango. It is used in traditional medicine for the cure of various ailments due to presence of more bioactive constituents like carotenoids, tocopherols, ascorbic acid, dietary fiber, and the phenolic compounds mangiferin, gallic acid and quercetin **(USDA, 2005)**.The stem bark and leaves aqueous extract of mango was reported in lowering of blood glucose in streptozotocin-induced diabetic rats **(Muruganandan, *et al*., 2005)** and glucose-induced hyperglycemia in rats and mice. High-fat diet fed mice showed positive effect on body composition, blood glucose and lipid profile on supply of freeze-dried mango pulp. Recent studies on *Aloe vera* fresh juices and Mango pulp showed a hypoglycemic effect by decreasing glucose levels and retaining serum enzymes **(Chandraprakash *et al.,* 2020).** Based on these facts, this research is designed to correlate antioxidant and antidiabetic effects of *Aloe vera* and mango pulp in experimentally induced diabetes *in vivo* in rats.

2. MATERIALS AND METHODS

2.1 Plant Material Preparation:

2.1.1*Aloe vera* extract:

Aloe vera solid translucent gel in the center of the leaf was collected and homogenized result in mucilaginous, thick and straw colored homogenate was obtained then filtered with filter paper to avoid fibrous particles in gel. *Aloe vera* gel was extracted freshly every day before dosing (300mg/kg body weight)throughout the experimental period and administered to rats daily by oral gavage.

2.1.2 Mango pulp:

Mango (*Mangifera indica*) pulp was purchased from the Srini food park Pvt. Ltd, Chittoor, Bangarupalem. Mango pulp was stored in refrigerator, 5 gms of mango pulp was mixed in 10ml of distilled water daily and administrated orally(300 mg/kg BW) to the rats.

2.2 Chemicals:

Chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and plastics from Nunclon (Roskilde, Denmark).

2.3 Animals:

ISSN 2063-5346

Male Wistar albino rats 48 in number weighing around 180 ± 200g of 3 months old obtained from animal house of Bangalore were used for this study. Animals were group housed in clean polypropylene cages contains 6 rats/cage, they were maintained at a room temperature of 22 ± 30C, humidity of 30-70% with 12h light/dark cycle and feed with standard rat pellet supplied by Hindustan Lever Ltd., Bangalore, India and water was supplied ad libitum through plastic bottle provided with nipples. Animals were identified with tail marking using permanent marker. This study was carried out according to guidelines for the care and use of laboratory animals and approved by the Institutional Animal Ethical Committee at Sri Venkateswara university, Tirupati, India.

2.3.2 Induction of Diabetes:

After fasting for 18 hours, Group IV,V,VI,VII, and VIII rats were injected intraperitonially with a single dose of Streptozotocin (STZ) (40 mg/kg), which is prepared freshly by dissolving in 0.1 M cold sodium citrate buffer, (pH 4.5). After injection, they had a free access to food and water, later given 5% glucose solution to drink overnight to counter hypoglycemic shock. After 96 h of streptozotocin injection, rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the experiment.

2.3.1 Experimental Design:

The animals were randomized and grouped into eight groups. Out of VIII groups, Group I, II, III were selected as control group and group IV, V, VI, VII and VIII as experimental groups. Group I act as control which is fed with standard diet. Group II rats were nourished with standard diet and supplemented with 300 mg/kg BW of mango pulp. Group III rats were nourished with standard diet and supplemented with 300 mg/kg BW of *Aloe vera* gel . Group IV rats were nourished with standard diet and STZ induced diabetic group. Group V rats were STZ induced diabetic group and treated with 300 mg/kg BW of *Aloe vera* gel. Group VI rats were STZ induced diabetic group and treated with 300 mg/kg BW of mango pulp. Group VII rats were STZ induced diabetic group and treated with *Aloe vera* gel and mango pulp in 1:1 (300+300 mg/kg) ratio. Group VIII rats were STZ induced diabetic group and treated with 600 µg/kg BW of Glibenclamide (Standard drug).

The animals were sacrificed after 24 hrs of the last treatment (21 day) by cervical dislocation and the liver and pancreatic tissues were isolated. The tissues were washed with ice-cold saline, and immediately stored in deep freeze at -80^{0}C for biochemical analysis and enzymatic assays (Table 1).

Lipid peroxidation and Anti-oxidative enzymes:

The 5% homogenate (w/v) of liver and pancreas tissues were prepared in 50 mM iced up phosphate buffer (pH 7.0) contain 0.1 mM EDTA. Centrifugation was carried out at 10,000 rpm for 10 min at 4 °C. The supernatant was separated and used for the enzyme assays of SOD and CAT.

Superoxide dismutase (SOD - EC: 1.15.1.1)

Superoxide dismutase activity was calculated by measuring the optical density at 480 nm for 4 min in Hitachi U-2000 spectrophotometer according to the process of Misra and Fridovich, (1972). The oxidation of epinephrine was inhibited by the enzyme was equal to one unit of SOD activity. The assay mixture contains supernatant, carbonate buffer (0.05 M, pH 10.2, containing 0.1 mM EDTA) and to it 30 mM epinephrine (in 0.05% acetic acid) was added.

Catalase (CAT - EC: 1.11.1.6)

The catalase activity was considered by decreasing the velocity at 240 nm in UV - spectrophotometer according to the method of **Hugo Aebi 1984**. Ethyl alcohol was added to supernatant and kept in an ice bath for half an hour. Then Triton X-100 RS was added. The reaction mixture containing 200 µl of phosphate buffer and 50 µl of supernatant to it 250 µl of 0.066 M H2O2 (in phosphate buffer) was added. One unit of activity is equal to the moles of H2O2 degraded / mg protein / min.

Lipid peroxidation:

The TBARS levels, measured as an index of malondialdehyde production and hence lipid peroxidation, were assessed in the tissues by the method of **Sato, et al., 1979** as previously described. In brief, tissue supernatant (0.2 ml) was added to test tubes containing 4.0 ml of 0.042 M sulphuric acid (H2 SO4). To this 0.6 ml of phosphotungstic acid (10% solution) was added and the tubes were vortexed and centrifuged at 1088 g for 10 min. The supernatant was discarded and 2.0 ml of H2 SO4and 0.3 ml of phosphotungstic acid were added to the pellet. The tubes were vortexed, centrifuged again and supernatant was discarded. The pellet was suspended in 4.0 ml of distilled water and 1.0 ml of thiobarbituric acid reagent (mixture of equal volumes of 0.67% thiobarbituric acid aqueous solution and glacial acetic acid, pH 3.4). The reaction mixture was heated at 95 °C for 60 min. After cooling, 5.0 ml of n-butanol was added to each tube, and the tubes were vortexed for 20 s and then centrifuged at 1088 g for 15 min. The n-butanol layer was used for fluorometric measurement at 553 nm emission and 515 nm excitation. Tetraethoxypropane was used as a standard and the results were expressed as nmol TBARS}mg protein.

Aspartate aminotransferase (AAT) (E.C.2.6.1.1)

Aspartate aminotransferase activity in the mitochondrial fraction of rat liver and pancreas was assayed by using the method adopted as described by **Bergmayer and Bruns 1965**. The reaction mixture contained100 µM phosphate buffer (pH : 7.4), α-ketoglutaric acid (20 µM), L - aspartic acid (20µM), Enzyme source and distilled water were added and incubated at 37^{0}C for 30 min and then 2,4-dinitrophenyl hydrazine (0.01 M) added and allowed to stand at 20 min and then yellow colour complex formed on addition of NaOH (0.4 N). The reaction was measured at 545 nm in a UV-VIS spectrophotometer against NaOH blank. The enzyme activity was expressed as µ moles of pyruvate formed / mg protein /min.

Alanine aminotransferase activity (ALAT) (E.C.2.6.1.2)

Alanine aminotransferase activity in the mitochondrial fraction of rat liver and pancreas was assayed at 545 nm in a UV- spectrophotometer by the method of **Reitman and frankel**

1957 as described by **Bergmayer and Bruns 1965**. The reaction mixture contains phosphate buffer (100 µM) pH : 7.4 α-ketoglutaric acid (20 µM), Alanine (100µM). Enzyme and distilled water were added and allowed it to stand for 20 min and then NaOH (0.4 N) was added. Yellow colour was observed. The enzyme activity was expressed as µ moles of pyruvate formed / mg protein /min.

Estimation of protein in enzyme source

Protein content in the enzyme source was estimated by the Lowry method (**Sharrif Moghaddasi *et al.*, 2011**)using bovine serum albumin as standard.

Statistical analysis

The data were statistically analyzed using One-way Analysis of Variance (ANOVA) followed by Dunnet's t-test and 'p' value <0.05 was considered significant. The data were presented as mean ± S.D. and analysis was carried out by using SPSS 16.0.1 program.

3. RESULTS:

The results of this investigation is summarized and presented in tables. Present changes all groups comparisons were made and against control group without treatment.

Total protein

Total protein content (Table 2) in untreated control rats liver and pancreas found to be 140.24g and 136.54g respectively. No significant change observed in total protein content of liver and pancreas tissues of control rats treated with *Aloe vera* and mango pulp when compared with untreated control groups. Significant reduction observed in protein content of liver and pancreas of diabetic rats. Recovery in total protein content of liver and pancreas was evident in treatment groups (Group V, VI and VII) and also recovery in treated group with *Aloe vera* and mango pulp in combination is comparable with known standard drug values.

Antioxidative Enzymes Activity

The activity of SOD (Table 3 and 4) in control rats was found to be 19.14 and 2.08 mg protein / min in liver and pancreatic tissue respectively. In the Group-II and III, where rats were control but treated with Aleo vera gel and mango pulp respectively the SOD activity of liver and pancreas is comparable with control. In Group-IV, STZ treated animals the activity was found significantly decrease in both liver and pancreas tissue. Group-V and VI had showed increased activity when compared to Group-III and activity showed by group VII animals is comparable with effect showed by standard drug.

The Catalase (CAT) activity (Table 3 and 4) is significantly reduced in liver and pancreatic tissue of diabetic rats when compared with control animals. In Group-II and III, the activity was slightly increased with no significance. Group- V, VI and VII animals showed decreased in catalase activity when compared to control rats whereas they had showed increased activity when compared to group-IV rats and activity showed by group VII animals is comparably higher than the effect showed by standard drug.

The activity of AAT (Table 3 and 4) in control animals was found to be 6.80 and 1.90 mg protein/min in liver and pancreas tissues respectively. Diabetic rats were showed significant reduction in AAT activity of liver and pancreas tissues. The control groups animals treated with

Aloe vera and mango plup were showed slight increase in activity of AAT in both liver and pancreas tissues. Group- V,VI and VII animals showed decreased in AAT activity when compared to control rats whereas they had showed increased activity when compared with diabetic rats. Animals treated in combination of *Aloe vera* and mango pulp were showed slight increase in AAT activity when compared with animals treated with *Aloe vera* and mango pulp individually and the values were comparable with the values of standard drug treated animals.

The activity of ALAT (Table 3 and 4) in control rats was found to be 3.67 and 2.08 mg protein/min in liver and pancreas respectively. In Group-II and III, where rats were control but treated with *Aloe vera* gel and mango pulp were showed slightly increase activity in Liver and pancreas. Diabetic rats shows significant reduction in activity in both liver and pancreas. Group-V, VI and VII rats showed increased levels of activity when compared to diabetic rats. Animals treated in combination of *Aloe vera* and mango pulp were showed slight increase in ALAT activity when compared with animals treated with *Aloe vera* and mango pulp individually and the values were comparable with the values of standard drug treated animals.

Lipid Peroxidation

As per results mentioned in table 5, statistically significant increase observed in lipid peroxidation of diabetic rat liver and pancreas tissue. Lipid peroxidation activity in Control group animals treated with *aloe vera* and mango pulp found to be comparable with control animals without treatment. Significantly reduced lipid peroxidation was observed in diabetic animals liver and pancreatic tissue treated with *aloe vera* and mango pulp individually and in combination when compared with diabetic animals. lipid peroxidation in liver and pancreas tissues of diabetic animals treated with *aloe vera* and mango pulp in combination is comparable with the changes noted in standard drug treated diabetic animals.

Discussion

In our study, a drastic increase in the concentration of TBARS was observed in the pancreatic and liver tissue of diabetic rats. Higher levels of lipid peroxides and low SOD and CAT activity indicate an oxidative stress condition. Treatment of diabetic rats with the combination of *Aleo vera* and mango pulp significantly increased the levels of nonprotein thiols in liver as well as in pancreatic tissues of rats as compared to pathogenic diabetic rats. Compared to all the experimental rat's group-VII rats are showed best therapeutic results. Many experimental studies suggest that mango pulp treated diabetic rats might protect the prevention of liver and kidney tissue dysfunction against the cytotoxic action of STZ **(Liu *et al*., 2013). Muruganandan *et al*., 2005** investigated the effect of Mangifera indica on hyperglycemia, atherogenicity, and oxidative damage to cardiac and renal tissue in streptozotocin- induced diabetic rats. The reported pharmacological activities of mango pulp include antioxidant, antidiabetic, radioprotective, antitumor, anti-inflammatory, which may support the numerous traditional therapeutic uses of the plant **(Severi *et al*., 2009).** Streptozotocin (STZ) exerts selective toxicity related to pancreatic β cells. STZ administration causes reduction in the number of β-cells and induces hyperglycemia **(Kaleem *et al*., 2006)**. The STZ-induced diabetes is associated with the chronic hyperglycemia be able to cause oxidative stress **(Gondi *et al*.,**

2015), which lead to the cellular tissue damage and excess production of reactive oxygen species (ROS) especially to cytotoxicity in β-cells, thus decreasing the synthesis and release of insulin and also affecting the pancreas. **Kakkar *et al*.,1984** reported that streptozotocin induced diabetes rats was being reduced SOD and GSH activity observed in the liver and kidney with progression of diabetes may be due to non-enzymatic glycosylation of the enzyme, which happens in a diabetic state.

Mango pulp has a high antioxidant activity and contains flavonoids and phenols, respectively, which higher than that found in *aloe vera*gel. Mango is regarded as the king of all fruits, having a rich dietary source, antioxidants like vitamin E, C, A, and phenolic compounds. The Aloe-mango juice blends the antioxidant activity of mango juice increased as the concentration of gel in the mixtures increased as the gel contains a variety of active compounds, i.e., phenolic compounds, vitamins, flavonoids and tannins **(Nejatzadeh-Barandozi 2013; Radha *et al*.,2015; Taukoorah *et al*.,2016).** Both animal and human studies suggest that mango pulp maintains some glucose-lowering properties. The possible mechanism mango pulp hypoglycemic action may be through potentiating the plasma insulin effect by increasing either pancreatic secretion of insulin from regenerated β-cells. Moreover, it has decreased from bound insulin response in type 2 diabetic patients also suggests that mango is well tolerated in diabetics **(Corrales Bernal, *et al*., 2014).** Mango pulp has been decreased the levels of the pancreas thiobarbituric acid reactive substance (TBARS) could be due to the increase in free radicals and decrease in non-enzymatic antioxidants.

Over the past 10 years, researchers and food manufacturers have been increasingly interested in the potential of polyphenols mainly because of their antioxidant properties, their abundance in our diet, and their role in the prevention of various oxidative stress-related diseases such as cancer, cardiovascular disease and neurodegeneration **(Choudhary *et al*., 2009).** The same pattern in the content of phenols was observed in the juice for the flavonoids, and thus the highest flavonoid content (6.2 mg/100ml) was reported for 25 percent of Aloe supplemented mango juice. Dietary intake of flavonoids was associated with a lower risk of both heart disease and cancer, probably associated with the antioxidant activity of these compounds. While mango pulp has a high nutritional value, it can be recommended to complement *Aloe vera* gel to produce high-quality functional mango juice rich in some bioactive compounds. The functional food consists of conventional foods containing naturally occurring bioactive substances and bioactive enriched food (e.g., probiotics, antioxidants) **(Goldberg 1994)** Phytochemicals and functional foods constituents have been associated with the prevention and/or treatment of at least four of the leading causes of death in the U.S.A. cancer, diabetes, cardiovascular disease, and hypertension, along with the prevention and/or treatment of other medical diseases including neural tube defects **(Esterbauer *et al*., 1990)**.

AAT and ALAT are essential hepatic metabolic enzymes that indicate liver damage by xenobiotics or other causes; when liver damage occurs, these enzymes are released from the liver

into the blood serum. Consequently, the activities of AAT and ALAT are considered to indicate the degree of liver damage. Data in Table 1 revealed significant differences in the levels of AAT and ALAT in all groups. Group VII showed an highest level of increase in the activities of AAT and ALAT as compared to those observed in other groups. During diabetes, there was a significant elevation of lipid peroxidation (MDA) compared to the normal group. Aloe gel and mango pulp treatment (group VII) prevented MDA changes that indicated the beneficial effect of Aloe gel and its protective efficacy against oxidative stress. The present study differs from others in that we specifically measured the indices of oxidative stress (TBARS and antioxidant enzymes) during the early development of diabetes. We have shown that there is an increase in the levels of MDA and the activity of antioxidant enzymes in the liver and pancreas during the progression of diabetes. MDA were measured as an index of malondialdehyde production. Oxygen free radicals are suggested to increase lipid peroxidation **(Behrens *et al*., 1991).** The increase observed in MDA levels of liver and pancreas could be due to increased levels of OFRs despite an increase in the activity of antioxidant enzymes which we have shown. The increase could also be due to a decrease in the non-enzymic antioxidant **(Parinandi *et al*, 1990).** The increase in MDA after the experimental period diabetes induced rats (Group-IV) liver and pancreas suggests that lipid peroxidation occurs well after induction of hyperglycaemia. The magnitude of increase in MDA levels in liver was lower compared with pancreas suggesting that other factors may be responsible in the liver for resistance against oxidative stress. A similar observation in the heart was made by **Parinandi *et al*, 1990.**The accumulation of MDA during progression of diabetes may play a role in pancreatic and hepatocyte damage associated with diabetes. In fact, it has been shown that exposure of pancreas to OFRs in vitro causes increased lipid peroxidation correlated with structural damage and loss of cellular integrity **(Misra and Fridovich.,1972; Thaete *et al*., 1985).** Our study showed that after supplementation of *Aleo vera* and mango pulp there are increasing pancreatic β cell activity and decrease insulin resistance, and hypolipidemic effect.

Conclusion:

The current study showed that induction of STZ leads to Antioxidant, oxidative imbalance on the albino rat lipid peroxidation had direct correlation with STZ induced rats in Group-IV. Hence, we conclude that the combination of *Aloe vera* and mango pulp offers protection of β-cells against reactive oxygen species-mediated damage by enhancing cellular antioxidant defense and reducing hyperglycaemia in chemically induced diabetes.

References

1. Ajabnoor, M. A. (1990). Effect of aloes on blood glucose levels in normal and alloxan diabetic mice. *Journal of ethnopharmacology*. *28*(2): 215-220.
2. Amarapurkar, D., Das, H.S. (2002). Chronic liver disease in diabetes mellitus. *Trop Gastroenterol*. 23:3-5.

3. Baynes, J.W., Thorpe, S.R. (1999). Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes*. 48:1–9.
4. Behrens, W. A., Madere, R. (1991). Vitamin C and vitamin E status in the spontaneously diabetic BB rat before the onset of diabetes. *Metabolism*. *40*: 72-76.
5. Boudreau, M.D., Beland, F.A. (2006). An evaluation of the biological and toxicological properties of *Aloe barbadensis* (Miller), *Aloe vera*. *J Environ Sci Health Part C*.24:103–54.
6. Bergmeyer, H. U., Bruns, F. H. (1965). Fructose-1, 6-diphosphate aldolase. In Methods of enzymatic analysis. *Academic Press*: 724-731.
7. Chandraprakash C., Mamatha C., Payani S., Sujatha B., Bhaskar M. (2020). Beneficial Effect of Aloe Vera Gel And Mango Pulp on Streptozotocin induced Diabetic Rats and Its Regulation On Oxidative Enzymes Of Heart And Kidney. *Journal of Emerging Technologies and Innovative Research*, volume 7; 1059 to 1068
8. Choudhary, R., Tandon, R. V. (2009). Consumption of functional food and our health concerns. *Pakistan Journal of Physiology*. *5:* 76-83
9. Corrales-Bernal, A., Urango, L. A., Rojano, B., Maldonado, M. E. (2014). In vitro and in vivo effects of mango pulp (Mangifera indica cv. Azucar) in colon carcinogenesis. *Archivos Latinoamericanos de Nutrición*. *64*(1): 16-23.
10. Esterbauer, H., Cheeseman, K. H. (1990). Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. In *Methods in enzymology* London: *Academic Press.* 186:407-421.
11. Gerstein, H.C., Santaguida, P., Raina, P., Morrison, K.M., Balion, C.,Hunt, D. (2007). Annual incidence and relative risk of diabetes in people with various categories of dysglycemia: a systematic overview and meta-analysis of prospective studies. *Diabetes Res Clin Pract*. 78:305–12.
12. Giacco, F., Brownlee, M.Z. (2010). Oxidative stress and diabetic complications. *Circulation Res.* 29(107):1058–70.
13. Goldberg, I. (1994) Reducing risk of cardiovascular disease. In: Functional Foods, Designer Foods, Pharmafoods, Nutraceuticals. 1 st ed. Duthie, G.G., Brown, K.M., Eds.; Chapman and Hall, New York, NY: 19- 38.
14. Gondi, M., Prasada Rao, U. J. S. (2015). Ethanol extract of mango (*Mangifera indica* L.) peel inhibits α-amylase and α-glucosidase activities, and ameliorates diabetes related biochemical parameters in streptozotocin (STZ)-induced diabetic rats. *Journal of food science and technology. 52*: 7883-7893.
15. Guru Sekhar, M., Sadak Basha S., Radha Madhavi,Y.R., Rama Krishna, S., Mannur Ismail,S., Bhaskar, M. (2010). The effects of *Curcuma longa* and *Trigonella foenum graecum* on antioxidant enzymes is kidney of alloxan induced type-I diabetic male rats. *Adv. Phrmacol. Toxicol.* 11: 95-105.
16. Ha, H., Lee, H.B. (2001).Oxidative stress in diabetic nephropathy: Basic and clinical information. *Curr Diab Rep*. 1:282-7.

17. Hamman, J. H. (2008). Composition and applications of *Aloe vera* leaf gel. *Molecules*. *13*: 1599-1616.
18. Hugo Aebi (1984). Chapter 13 catalase *in vitro,* Methods in Enzymology. *Academic press*. 105: 121-126
19. Kakkar, P., Das, B., Viswanathan, P.N., (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian J, BiochemBiophys*. 21: 130-132.
20. Kaleem, M., Asif, M., Ahmed, Q. U., Bano, B. (2006). Antidiabetic and antioxidant activity of Annona squamosa extract in streptozotocin-induced diabetic rats. *Singapore medical journal*. 47(8): 670.
21. Kashihara, N., Haruna, Y., Kondeti, V.K., Kanwar, Y.S. (2010). Phytochemical Composition of Kalanchoe pinnata and Bidens pilosa Leaves Associated with Management of Diabetes. *Curr Med Chem*. 17:4256-69.
22. Khawandanah, J. (2019). Double or hybrid diabetes: A systematic review on disease prevalence, characteristics and risk factors. *Nutrition and Diabetes*. 9: 1-9.
23. Liu, Y.W., Zhu, X., Zhang, L., Lu, Q., Wang, J.Y., Zhang, F., Guo, H., Yin, J.L., Yin, X.X.(2013). Up-regulation of glyoxalase 1 by mangiferin prevents diabetic nephropathy progression in streptozotocin-induced diabetic rats. *Eur. J. Pharmacol*. 721: 355-364.
24. Lorenzi, R., Andrades, M.E., Bortolin, R.C., Nagai, R., Dal-Pizzol, F., Moreira, J.C.F. (2011). Oxidative damage in the liver of rats treated with glycolaldehyde. *Int J Toxicol*. **30:**253-8.
25. McGarry, J. D. (2002). Dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes*. 51(1): 7-18.
26. Misra, H. P., Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological chemistry*. *247*: 3170-3175.
27. Muruganandan, S., Srinivasan, K., Gupta, P.K., Lal, J. (2005). Effect of mangiferin on hypoglycemia and atherogenicity in STZ-diabetic rats. *Ethnopharmacol*. 97: 497-501
28. Nejatzadeh-Barandozi, F. (2013). Antibacterial activities and antioxidant capacity of *Aloe vera. Organic and medicinal chemistry letters*. 3: 1-8.
29. Parinandi, N. L., Thompson, E. W., Schmid, H. H. (1990). Diabetic heart and kidney exhibit increased resistance to lipid peroxidation. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism*. *1047*: 63-69.
30. Radha Madhavi, Y.R., SwapnaRekha, S., Pradeep Kiran, J., Ismail, S.M., Maduri, E., and Bhaskar, M. (2012).Protective role of ethanolic extract of *Aloe vera* antioxidant properties on liver and kidney of streptozotocin - induced diabetic rats. *Digest Journal of Nanomaterials and Biostructures.* 7(1): 175-184.
31. Radha, M. H., Laxmipriya, N. P. (2015). Evaluation of biological properties and clinical effectiveness of Aloe vera: A systematic review. *Journal of traditional and complementary medicine*. *5*: 21-26.

32. Rajasekaran, S., Sivagnaman, K., Subramanian, S. (2005).Modulatory effects of *Aloe vera* leaf gel extract on oxidative stress in rats treated with streptozotocin. *J Pharm Pharmacol*. 57:241–6.
33. Reitman, S., Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*. 28(1): 56-63.
34. Robertson, R.P. (2010). Antioxidant drugs for treating beta-cell oxidative stress in type 2 diabetes: glucose-centric versus insulin-centric therapy. *Discov Med*. 9(45):132–7.
35. Sadak Basha, S., Guru Sekhar, M., Radha Madhavi, Y.R, Mannur Ismail, S., Bhaskar, M. (2010). Combination of *Trigonella foenum graecum* and *Curcuma longa* treatment to prevent histopathological abnormalities in liver tissue of aloxan induced type-I diabetes male albino rats. *Int. J. Pharmocol. Biol. Sci.* 4: 93-102.
36. Sato, Y., Hotta, N., Sakamoto, N., Matsuoka, S., Ohishi, N., Yagi, K. (1979). Lipid peroxide level in plasma of diabetic patients. *Biochemical medicine.* 21(1): 104-107.
37. Severi, J. A., Lima, Z. P., Kushima, H., Monteiro Souza Brito, A. R., Campaner dos Santos, L., Vilegas, W., Hiruma-Lima, C. A. (2009). Polyphenols with antiulcerogenic action from aqueous decoction of mango leaves (*Mangifera indica* L.). *Molecules*: *14:* 1098-1110.
38. Sharrif Moghaddasi, M., Res, M. (2011). *Aloe vera* their chemicals composition and applications: A review. *Int J Biol Med Res*. *2*: 466-71.
39. Singh, D.K., Winocour, P., Farrington, K. (2011). Oxidative stress in early diabetic nephropathy: *fueling the fireNat Rev Endocrinol*. **7**:176-184.
40. Takamoto. I., Kadowaki, T. (2011). Treatment of diabetes mellitus with oral hypoglycemic agents. *Nihon rinsho. Japanese Journal of Clinical Medicine.* 69:563-72.
41. Taukoorah, U., Mahomoodally, M. F. (2016). Crude *Aloe vera* gel shows antioxidant propensities and inhibits pancreatic lipase and glucose movement in vitro. *Advances in pharmacological sciences. 2016:* 1-9
42. Thaete, L. G., Crouch, R. K., Buse, M. G., Spicer, S. S. (1985). The protective role of copper-zinc superoxide dismutase against alloxan-induced diabetes: morphological aspects. *Diabetologia*. *28*: 677-682.
43. U.S. Department of Agriculture & Agricultural Research Service (2005) USDA National nutrient database for standard reference, Release 18. http://www.ars.usda.gov/ba/bhnrc/ndl.
44. Wang, J., Wang, H. (2017). Oxidative stress in pancreatic beta cell regeneration. *Oxidative medicine and cellular longevity*, *2017*. Article ID 1930261

Tables

Table 1: Tabular Representation of Experimental Design.

Group No.	Treatment condition	Treatment	Dose (mg/kg)	No.of animals/group
I	-	-	0	6
II	-	Mango pulp	300	6
III	-	*Aleo vera* Gel	300	6
IV	STZ	-	40	6
V	STZ	Mango pulp	300	6
VI	STZ	*Aleo vera* Gel	300	6
VII	STZ	Mango pulp + *Aleo vera* Gel	300+300	6
VIII	STZ	Glibenclamide	600 µg/kg	6

Table 2: The Level of Total Protein in Control and Treated Animals

Tissue	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Liver	140.24 ±14.06 -	141.83 ±14.53 1.13 NS	142.03 ±14.83 1.28 NS	99.84 ±9.66 -28.81 P<0.0001	137.92 ±12.95 -1.65 NS	132.63 ±12.73 -5.43 NS	139.63 ±13.13 -0.43 NS	134.35 ±12.08 -4.20 NS
Pancreas	136.54 ±13.54 -	133.07 ±13.01 -2.54 NS	137.36 ±13.35 0.60 NS	73.81 ±7.24 -45.94 P<0.0001	124.24 ±11.85 -9.01 NS	116.35 ±9.54 -14.79 0.0276	131 ±12.31 -4.06 NS	129.29 ±11.76 -5.31 NS

Values are mean, ± S.D. of 6 individual Rats
NS indicates No significant change. P <0.0001 statistically significant

Table 3: The levels of Antioxidant in Liver Tissue of Control and Experimental Rats

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
SOD superoxide anion reduced/ mg protein/min.	19.14 ±1.42 -	19.16 ±1.43 0.10 NS	19.19 ±1.41 0.26 NS	4.88 ±0.31 -74.50 P<0.0001	14.22 ±1.33 -25.71 P<0.0001	13.29 ±1.32 -30.56 P<0.0001	18.91 ±1.58 -1.20 NS	18.66 ±1.74 -2.51 NS
CAT (µmoles of H2O2 metabolized/mg protein/min)	34.14 ±2.42 -	34.16 ±2.43 0.06 NS	34.19 ±2.41 0.15 NS	9.88 ±1.31 -71.06 P<0.0001	28.22 ±2.33 -17.34 P 0.0003	22.29 ±2.32 -34.71 P<0.0001	33.14 ±2.38 -2.93 NS	31.62 ±2.08 -7.38 NS
AAT (µ moles of pyruvate formed/ mg protein / min)	6.8 ±0.61 -	6.84 ±0.62 0.59 NS	6.86 ±0.63 0.88 NS	5.19 ±0.44 -23.68 P 0.0001	6.76 ±0.52 -0.59 NS	6.74 ±0.59 -0.88 NS	6.85 ±0.60 0.74 NS	6.01 ±0.523 -11.62 NS
ALAT (µ moles of pyruvate formed/ mg protein / min)	3.67 ±0.32 -	3.68 ±0.33 0.27 NS	3.72 ±0.34 1.36 NS	2.02 ±0.20 -44.96 P<0.0001	3.35 ±0.24 -8.72 NS	3.13 ±0.26 -14.71 0.014	3.59 ±0.28 -2.18 NS	3.64 ±0.310 -0.82 NS

Values are mean , ± S.D. of 6 individual rats
NS indicates No significant change. P<0.05, P <0.0001 statistically significant

Table 4: The levels of Antioxidant in Pancreas tissue of Control and Experimental rats

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
SOD superoxide anion reduced/ mg protein/min.	2.08 ±0.20 -	2.10 ±0.20 0.96 NS	2.11 ±0.21 1.44 NS	0.91 ±0.01 -56.25 P<0.0001	1.91 ±0.16 -8.17 NS	1.89 ±0.14 -9.13 NS	2.01 ±0.20 -3.37 NS	1.94 ±0.13 -6.73 NS

CAT (µmoles of H2O2 metabolized/mg protein/min)	9.80 ±0.90 -	9.81 ±0.92 0.10 NS	9.83 ±0.93 0.31 NS	1.65 ±0.02 -83.16 P<0.0001	7.97 ±0.72 -18.67 P 0.0011	6.04 ±0.62 -38.37 P <0.0001	8.94 ±0.83 -8.78 NS	7.98 ±0.75 -18.57 P 0.0012
AAT (µ moles of pyruvate formed/ mg protein / min)	1.90 ±0.02 -	1.99 ±0.02 4.74 NS	2.00 ±0.02 5.26 P 0.0033	1.11 ±0.01 -41.58 P <0.0001	1.85 ±0.02 -2.63 NS	1.76 ±0.02 -7.37 P <0.0001	1.96 ±0.02 3.16 NS	1.80 ±0.12 -5.26 P 0.0033
ALAT (µ moles of pyruvate formed/ mg protein / min)	2.08 ±0.20 -	2.10 ±0.20 0.96 NS	2.11 ±0.21 1.44 NS	0.91 ±0.01 -56.25 P <0.0001	1.91 ±0.16 -8.17 NS	1.89 ±0.01 -9.13 NS	2.01 ±0.20 -3.37 NS	1.97 ±0.14 -5.29 NS

Values are mean , ± S.D. of 6 individual rats
NS indicates No significant change. P<0.05, P <0.0001 statistically significant

Table 5: The levels of Lipid peroxidation in Liver and Pancreas tissue.

Tissue	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Liver	43.21 ±4.43 -	43.08 ±4.23 -0.30 NS	44.02 ±4.07 1.87 NS	99.02 ±4.09 129.16 P <0.0001	48.92 ±3.98 13.21 NS	53.21 ±4.42 23.14 P 0.0006	44.21 ±3.23 2.31 NS	45.62 ±3.41 5.58 NS
Pancreas	13.21 ±1.43 -	13.18 ±1.23 -0.23 NS	13.12 ±1.07 -0.68 NS	39.02 ±4.09 195.38 P <0.0001	15.92 ±1.98 20.51 NS	17.21 ±1.52 30.28 P 0.0072	13.82 ±1.47 4.62 NS	16.89 ±1.34 27.86 0.0153

Values are mean, ± S.D. of 6 individual rats
NS indicates No significant change. P<0.05, P <0.0001 statistically significant

Ethics committee approval:
(No 10(i)/a/CPCSEA/IAEC//SVU/Zoo/MBR/dt.08.07.2012)