Chapter-IV

Antidiabetic activity of
Aloe vera gel
INTRODUCTION

Poorly controlled diabetes mellitus can cause devastating consequences, including microvascular and macrovascular disease (The American Diabetes Asso.). It is stated that in diabetes mellitus, uncontrolled hyperglycemia may be responsible for all the diabetic complications. Hyperglycemia generates abnormally high levels of free radicals by autoxidation of glucose and protein glycation. The goal of treatment is glycemic control and oxidative stress reduction to control the risk of complications (Okhubo et al., 1995; UKPDS group., 1998). It is well known that the major reason for mortality occurring in diabetic patients is cardiovascular disease, because reactive oxygen species (ROS) are increased in various tissues and are involved in the development of diabetic complications (Dandona et al., 1996; Baynes and Trope, 1999). Heart, liver and pancreatic β cells emerged as targets of oxidative stress mediated tissue damage.

In modern medicine, no satisfactory effective therapy is available to cure diabetes mellitus (Sumana and Suryawanshi, 2001). It can be managed by exercise, diet and chemotherapy. However, the pharmaceutical drugs either too expensive or have undesirable side effects or contraindication (Berger, 1985; Hupponen, 1978). Therefore, the search for more effective and safer hypoglycemic agents has continued to an area of active research (Pari and Uma, 1999; Pari and Venkateswran, 2000).

_Aloe vera_ is one of the oldest known medicinal plants gifted by nature, _Aloe vera_ often called Miracle plant known by many names, there are over 200 types of _Aloe vera_ of these only 4 or 5 are commonly used in medicines. It is perennial, succulent plant with stiff fleshy leaves.
Medicinal species

*Aloe vera*, *A. barbadensis* (Curacao or Barbados Aloe), *A. vulgaris*, *A. arborescens*, *A. ferox* (cape Aloe), *A. perryi* (scoatrine or Zanzibat Aloe). The different species have somewhat different concentration of active ingredient (Yagi et al., 1998). The most widely used variety of *Aloe vera* is *Barbadensis Millar*.

Common names

*Aloe*, *Aloe capenis*, *Aloe spicata*, *Aloe vera*, Babados Aloe, Cape Aloe, Chirukatto (India), Curacao Aloe, Ghai kunwar (India), Gjikumar (India), Indian *Aloes*, Kumari (Sanskrit) Lalo (Haiti), Lohoi (Vietnam). Sabilla (Cuba) (Kapoor, 1990; Ross, 1999). The name *Aloe* is derived from the Arabic word *alloeh* meaning a shining bitter substance (Robbes et al., 1996).

Botanical family: Liliaceae

Plant description

Plant is succulent, almost sessile perennial herb; leaves 30-50 cm long and 10 cm broad at the base that have spikes along the edges; peagreen in color; bright yellow to reddish tubular flowers 25-35 cm in length arranged in slender loose spike; stamens frequently project beyond the Perianth tube (Youngken, 1950). The fresh parenchymal gel from the center of the leaf is clear. The sticky latex liquid is derived from the yellowish green pericyclic tubules that line the leaf (rid); this is the part that yields laxative andheraquinge (Murry, 1995; Schulz, 1997).

Geographical distribution

*Aloe vera* is probably native to North Africa along the upper Nile in the Sudan, most of the tropics and warmer areas of the world, including Asia, the Bahamas, Central America, Mexico, the southern United States of America, Mexico, the southern United States of America (Grindly and...
Reynolds, 1986). In the US Aloe is commercially cultivated in southern Texas (Foster, 1999).

Chemical Constituents

The gel obtained from the flesh of the leaf contains quite different compounds. Aloe gel is 99% water with pH of 4.5 (Klein and Penneys, 1988). A review by Vogler and Ernst (1999) lists 75 potentially active constituents, including vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids, this list was modified from a larger list of ingredients reported by other investigators (Shelton, 1991 Townsend, 1998; Atherton, 1998). A report from the same year described 39 chemical constituents, including the essential amino acids; numerous monosaccharides and enzymes such as acid phosphatase, alkaline phosphatase, amylase, lactase dehydrogenase, and lipase; and numerous organic compounds such as aloin, barbaloin, and emodin (Hayes, 1999). Reynolds and Dweck (1999) listed 16 different polysaccharides that have been extracted from the Aloe vera leaf gel. Yamaguchi et al., in 1993 reported the presence of aluminium, boron, barium, calcium, iron, magnesium, sodium, phosphorous, silicon, and strontium in Aloe vera gel.

Acemannan, the major carbohydrate fraction in the gel, is a watersoluble long chain mannose polymer which accelerates wound healing, modulates immune function (particularly macrophage activation and production of cytokines) and demonstrates antineoplastic and antiviral effects (Peng et al., 1991; Zhang et al., 1996; Ramamoorthy et al., 1996). The gel also contains bradykininase, an anti-inflammatory (Yagi et al., 1982), magnesium lactate, which helps prevent itching, and salicylic acid and other antiprostaglandin compounds which relieve inflammation.
Pharmacological Properties

*Aloe vera* gel has been widely promoted and used by patients for the treatment of a range of inflammatory digestive and skin disease (Langmead et al., 2004). The moisturizing effect of *Aloe vera* gel appears to be due to the mix of water and polysaccharide components, creating a jelly-like consistency that holds the water within the mix and minimizes its evaporation, providing a sustained moist environment when applied to drying tissue and humectant properties that promote retention of moisture in tissue (Meadows, 1980).

Countless studies have demonstrated the healing power of *Aloe vera* gel. A study reported that a high molecular weight polypeptide constituent from the gel demonstrated a healing effect on excisional wounds in rats (Heggers et al., 1996). Yagi et al. (1997), reported that *Aloe vera* gel contains a glycoprotein with cell proliferating-promoting activity, while Davis et al in 1989, noted that *Aloe vera* gel improved wound healing by increasing blood supply, which increased oxygenation as a result. In 1991, Thompson reported that topical application of the *Aloe vera*- derived allantoin gel stimulated fibroblast activity and collagen proliferation. Angiogenesis is the growth of new blood capillaries and is a part of tissue regeneration. A 1993 study showed that topical application of *Aloe vera* gel re-established vascularity of burn tissue for a guinea pig, although no specific constituents were identified (Heggars et al., 1993). It is reported that the low molecular weight component of freeze-dried *Aloe vera* gel stimulated blood vessel formation in a chick chorioallantoic membrane; in addition, a methanol-soluble fraction of the gel stimulated proliferation of artery endothelial cells in an *in vitro* assay and induced them to invade a collage substrate (Lee et al., 1995). The *Aloe vera* gel polysaccharide acemannan was shown to activate macrophages, an effect that improved wound healing in a rat model (Maxwell et al., 1996; Tizard et al., 1994). A mannose-6-phosphate
component of the gel has been credited with a wound healing effect (Davis et al., 1994).

Hanley et al. (1982) reported that an Aloe vera extract (described as 5.0% leaf homogenate) decreased inflammation by 48% in a rat adjuvant-induced arthritic inflammatory model (Davis et al., 1991). More recently, the peptidase bradykinase was isolated from Aloe and shown to break down the bradykinin, an inflammatory substance that induces pain (Ito et al., 1993). The leaf lining (latex, resin or sap) contains anthraquinone glycosides (aloin, aloe-emodin and barbaloin) that are potent stimulant laxatives. These water soluble glycosides are split by intestinal bacteria into aglycones which effect the laxative action. The laxative effect from Aloe is stronger than from any other herb, including senna, cascara or rhubarb root; it also has more severe side effects such as cramping, diarrhea, and nausea (Schilcher, 1997). For medicinal use, the leaf lining is dried and the residue is used as an herbal laxative. The products are usually taken at bedtime they are poorly absorbed after oral administration, but moderately well absorbed after bacterial hydrolysis. Residue is eliminated in the urine, bile, feces and breast milk, it turns alkaline urine red (Bissett et al., 1994).

Most herbalists recommend that they be avoided during pregnancy due to the risk of stimulating uterine contractions and also avoided during lactation due to the risk of excretion in breast milk (Hoffman, 1996). Aloe is seldom recommended as a first choice among laxative preparations due to the severe cramping and nausea associated with its use.

Many has demonstrated its activity against feline leukemia virus and solid tumors (Peng et al., 1991; Corsi, 1998; Harris et al., 1991; Sheets et al., 1991; King et al., 1995; Yates et al., 1992; Desai et al., 1996). Among cats with feline leukemia, a virally-induced disease with a mortality rate of 70%-100%, a six-week treatment series with acemannan injections (2 mg/kg per weekly dose) resulted in a 71% survival rate (Sheets et al., 1991). Peng
et al. (1996) reported that mice implanted with malignant sarcoma cells, treated with intraperitoneal injections of acemannan, showed 40% survival and signs of tumor necrosis and regression. In rats, concurrent treatment with Aloe extracts inhibited hepatic tumor induction (Tsuda et al., 1993; Shamaan et al., 1998). In a study of dogs and cats with fibrosarcomas treated with daily injections of acemannan in combination with surgery and radiation therapy, significant shrinkage of tumors and increase in necrosis and inflammation were observed (King et al., 1995). In another study of 46 dogs and cats with spontaneous tumors who were treated with acemannan injections, 26 had histopathologic evidence of tumor necrosis and 12 exhibited significant clinical improvement; soft tissue sarcomas appeared to be particularly susceptible to treatment (Harris et al., 1991).

Based on findings from animal studies, Aloe research in human cancer patients is currently in progress. At the University of Texas-Houston Medical School and Herman Hospital, a Phase I study with injectable Aloe for cancer patients is being conducted. In a preliminary study of 50 patients suffering from lung cancer, gastrointestinal tract tumors, brain stem gliomas or breast cancer who were treated with melatonin alone or melatonin plus Aloe. Those in the combination therapy group had significantly better one-year survival (Lissoni et al., 1998).

Several reports are available showing the glucose lowering and antioxidant activity of Aloe vera gel. Okyar et al. (2001) reported hypoglycemic activity of gel on IDDM and NIDDM rats. It is reported that alcoholic extract of Aloe vera gel at the concentration of 200 and 300 mg/kg maintain the glucose homeostasis by controlling the carbohydrate metabolizing enzyme in oral glucose-loaded rats (Rajasekaran et al., 2004). It is found that oral administration of ethanolic extract of Aloe vera leaf gel in dose of 300 mg/kg for 21 day in STZ diabetic rats significantly restored altered membrane bound phosphatase and lysosomal hydrolases' activity to near normal (Rajasekaran et al., 2007). In contrast to these
studies Koo (2006), reported that pretreatment with *Aloe vera* gel in alloxan-injected rats showed twice plasma glucose level as compared to controls. These findings do not support the claimed efficacy of the gel in treating ulceration and diabetes mellitus. Rajasekaran (2005), reported that ethanolic extract of *Aloe vera* gel showed an antioxidant effect on tissue due to reduction in blood glucose level in diabetic rats, which prevent excessive formation of free radicals through various biochemical pathways and also reduces the potential glycation of the enzyme, this is the current interest.

If the inhibitory function of *Aloe* on the elevation of blood glucose has been established, effective utilization of *Aloe* by people who feels insecurity for diabetes mellitus would be expected since part of herb components of *Aloe* and like is are widely utilized as a food product (Ghannam et al., 1986; Alarcon–Aguilara et al., 1998).

We conducted animal experimentation to clarify the mechanism of antidiabetic and cardioprotective effects of *Aloe* along with its antioxidant activity. In this study the effect of *Aloe* as dietary supplementation was evaluated, using 30% *Aloe vera* gel directly.

**MATERIAL AND METHODS**

STZ was procured from Sigma chemicals (St Louis, MO, USA), and dissolved in 0.1 M citrate buffer. STZ was given i.v. through tail vein once at the beginning of study. *Aloe vera* gel was resuspended in normal saline for making 30% suspension and given orally for the period of 20 days. Male wistar rats were sacrificed on 21\textsuperscript{st} day of study and various parameters were studied.
Animals

Animals were divided into seven groups consisting of six animals in each group; treatment was given from 5th day of STZ administration, for 20 days.

I  Rats given normal saline (1 ml/kg, p.o.) once daily.

II Streptozotocin (40 mg/kg, i.v.) + Normal saline (1 ml/kg, p.o.) once daily.

III Streptozotocin (40 mg/kg, i.v.) + 30% Aloe vera gel (100 mg/kg, p.o.).

IV Streptozotocin (40 mg/kg, i.v.) + 30% Aloe vera gel (200 mg/kg, p.o.).

V Streptozotocin (40 mg/kg, i.v.) + Gliclazide (25 mg/kg, p.o.).

VI Rats given 30% Aloe vera gel (200 mg/kg) p.o.

VII Rats given gliclazide (25 mg/kg), p.o.

On 21st day rats were anesthetized with ether for the collection of blood and sacrificed. Liver, heart and pancreas were dissected out for biochemical estimations and also for histopathology.

Blood glucose, glycosylated haemoglobin, serum lactate dehydrogenase (LDH) and Serum creatine kinase (CK) were evaluated by using various kits.

For blood GSH 0.2 ml Fresh blood was collected from animal and 1.8 ml distilled water was added to it, 3.0 ml of precipitating solution was added to above mixture. 2.0 ml of filtrate was added to 8.0 ml of phosphate solution (Beutler et al., 1963). The OD was measured at 412 nm against blank. Lipid peroxidation was studied out by measuring the formation of thiobarbituric acid reactive substance (TBARS) according to method of Okhawa (1979) 1 ml of suspension medium was taken from the
supernatants of the 10 % tissue homogenate and centrifuged at 10,000 rpm 0.5 ml of 30 % TCA followed by addition of 0.8 % TBA. The tubes were kept in shaking water bath for 30 minutes at 80° C then in ice for 10 min. They were then centrifuged at 3000 rpm for 15 minutes. The OD of supernatants was read at 540 nm at room temperature against appropriate blank.

Tissue GSH was estimated by method of sedlek and Lindsay (1968) by using Ellman reagent. In brief, tissue was homogenized in 0.02 M EDTA, after centrifugation 2 ml of supernatant was mixed with 0.4 M tris buffer and 0.01 M DTNB was added to it, absorbance was recorded at 410nm against appropriate blank.

For catalase estimation tissues (10%) were homogenized in PBS, 50 µl of supernatant was added in curette containing 2.95ml of 19mM solution of H$_2$O$_2$. Disappearance of H$_2$O$_2$ was monitored at 240nm for 3 min at interval of 1 min (Clairborne et al., 1985). SOD estimation was done by method of Marklund (1985), in which cytosolic supernatent was added to Tris buffer (pH 8.5) at last pyrogallol was added and inhibition of pyrogallol was observed in presence of SOD at 420nm.

Protein in tissues was estimated by Lwory's method (Lwory et al., 1959) with Folin's Ciocalteum phenol reagent.

For histopathological examination, tissues were dissected out and pruned in to small pieces. These tissues were fixed in 10% natural buffered formalin solution. After that tissue was processed for dehydration, clearing with chloroform, impregnation in paraffin wax, then sections of 5-6 µ in thickness were cut with the help of rotary microtome. From each tissue 5 slides were prepared having 2 sections at each slide, out of 10 sections 3 sections were evaluated for histological findings.
LD<sub>50</sub> was assessed by using single dose of 250 mg/kg, 500 mg/kg, 1 g/kg of Aloe vera gel and rats were observed for gross behaviour, neurologic, autonomic, and toxic effects continuously for 14 days (Gupta et al., 2005).

RESULTS AND OBSERVATIONS

1. Haemodynamic parameters

Figure 4.1 represents the effect of Aloe vera gel on hemodynamic parameters of diabetic rat. The heart rate of rats fed on normal diet was 395.0± 2.2 beats/min, conversely, in the STZ treated groups (Pathogenic group) there was a no significant change was observed in heart rate. Treatment with Aloe vera gel (100 mg/kg and 200 mg/kg) in STZ treated animal also did not produce any significant alteration in heart rate; Aloe vera per se also did not show any significant alteration in heart rate.

The mean blood pressure of healthy control group was 137.0 ± 6.5 mmHg. STZ treatment resulted in significant increase in mean blood pressure as compared to normal healthy group. Treatment with 30% Aloe vera gel (100 mg/kg and 200 mg/kg) in STZ treated animals maintained the mean blood pressure level to normal levels. Gliclazide treatment also maintained the mean blood pressure in diabetic animals Figure 4.1.

2. Biochemical studies

2.1 Biochemical studies in blood

Table 4.1 represents the blood glucose, % HbA1c and blood GSH level in diabetic and drug treated rats. The mean blood glucose level in normal healthy rats was 69±0.8 mg/dl and stable throughout the study. STZ treated animals showed a significant rise in blood glucose level compared to normal healthy control group. Aloe vera treatment in the both the doses in STZ treated rats for 20 days, significantly reduced the blood glucose level and tends it towards normal.
The blood HbA1c level in normal rats was found to be 5.05±0.2 %, whereas pathogenic group showed a three fold rise in glycosylated hemoglobin level. Treatment with 30 % Aloe vera gel in both the doses as well as gliclazide significantly reduced the level of glycated hemoglobin in pathogenic rats.

The blood GSH in normal healthy rats was found to be 3.2±0.03 mg/dl. STZ treatment significantly reduced the level of GSH in blood. Treatment with 30% Aloe vera gel recover the GSH level of pathogenic rats and bring it toward normal. Gliclazide treatment for 20 days also showed a significant recovery of GSH as compared to pathogenic group rats.

2.2 Biochemical studies in serum

Figure 4.2 shows the effect of Aloe vera gel on serum enzyme marker in diabetic rats. The LDH levels in normal rats group was found to be 159.2±2.7 IU/l, whereas single i.v. administration of STZ increased the LDH level up to 4 folds, compared to control group. Pathogenic rats treated with 30% Aloe vera gel showed a significant reduction in elevated LDH. Gliclazide treatment also reduced LDH level and brings that toward the normal level.

The serum CK level in healthy rats was found to be 82.2±1.4 IU/l. Pathogenic rats showed a significant increase in serum CK level compared to normal rats. Treatment with 30% Aloe vera gel maintains the level of CK toward the normal significantly. The results are comparable to gliclazide treated animals.

2.3 Biochemical studies in tissues (heart, liver and pancreas)

Table 4.2 represents the protective effect of Aloe vera gel on tissue MDA level in diabetic rats. The lipid peroxide levels (TBARS) in the heart homogenate of normal healthy rats were found to be 0.52 nmmols MDA/mg protein. STZ administration through iv increased this level significantly and
raised this level up to three fold as compared to normal rats. The 30% Aloe vera treatment in dose of 100 mg/kg and 200 mg/kg produced a significant reduction in level of TBARS level compared to pathogenic rats. Gliclazide treatment also significantly reduced the TBARS level compared to diabetic rats. Treatment of Aloe vera gel and gliclazide in healthy rats did not showed any alteration in TBARS level compared to healthy ones. The lipid peroxide level in the liver of normal rats was found to be 0.47±0.02 nmoles MDA/mg protein. STZ treated rats showed approximately five-fold rise compared to normal one. Treatment with both the doses (100 &200 mg/kg) of 30% Aloe vera gel showed a significant reduction in lipid peroxidation level compared to pathogenic group. Gicalzide treated rats also showed a significant reduction in lipid peroxide level of liver. Treatment with Aloe vera alone did not showed any significant change in lipid peroxidation level. The lipid peroxidation level in pancreas of healthy rats was 0.46 ± 0.04 nmoles MDA/ mg protein. Pathogenic control group rats showed about 7-fold rise in lipid peroxide level as compared to healthy control rats. The 30 % Aloe vera gel treatment in both the doses significantly reduces the lipid peroxide level of pancreatic tissue and brought it towards normal. Gicalzide treatment also showed significant reduction in lipid peroxide level of pancreatic tissue compared to pathogenic rats.

Table 4.3 shows the protective effect of Aloe vera gel on tissue GSH level in diabetic rats. The mean glutathione level in heart of the normal rats was found to be 99.6±6.0 pg/mg protein. STZ treatment reduced the level of GSH up to 60% in rats, this reduction is significant compared to normal healthy control rats. Administration of 30% Aloe vera gel in STZ diabetic rats for 20 days significantly increased GSH level, compared to pathogenic group rats. Gliclazide treatment did not show any significant protection of heart by means of glutathione. The mean glutathione level in liver of the normal rats was found to be 68.3±2.2 µg/mg protein. Pathogenic group showed significant reduction in level of
glutathione compared to normal healthy control rats. *Aloe vera* gel treatment for 20 days significantly regain the GSH level and tent it toward the normal rats compared to pathogenic rats. Gliclazide was found to be incapable in maintaining the level of GSH in liver tissue of rats. The mean glutathione level of GSH in pancreatic tissue of normal healthy control rats was found to be 45.2±2.9 µg/mg protein. Single dose administration of STZ significantly reduced the level of GSH in pancreatic tissue compared to healthy rats group. 30% *Aloe vera* gel administration in diabetic rats for the period of 20 days showed a significant recovery of GSH in both the doses, when compared to pathogenic rats. GSH was significantly increased on treatment with gliclazide in comparison with pathogenic rats. Administration of *Aloe vera* gel and gliclazide in normal rats did not show any significant alteration in tissue GSH level compared to healthy rats.

Table 4.4 shows the protective effect of *Aloe vera* gel on tissue catalase level in diabetic rats. The mean catalase level of normal healthy control rats showed 1.9±0.4 nmoles of H$_2$O$_2$/min/mg protein. In pathogenic rats this level raised significantly, compared to healthy control rats. Treatment with *Aloe vera* gel in both the doses significantly reduced the level of catalase compared to pathogenic rats group. Gliclazide treatment also showed a significant rise in catalase level compared to toxic group. The level of catalase in liver of healthy rats was found to be 2.9 ± 0.6 nmoles of H$_2$O$_2$/min/mg protein. Conversely, in pathogenic group, there was a rise in level of catalase was observed. 30 % gel treatment in dose of 100 mg/kg and gliclazide showed a significant reduction in catalase level of liver compared to pathogenic rats. The catalase level in pancreatic tissue of normal rats was found to be 0.92 ± 0.01 nmoles of H$_2$O$_2$/min /mg protein. Pathogenic rats showed significant increased level of catalase in pancreatic tissue of rats. Treatment with 30% *Aloe vera* gel in diabetic rats showed a significant recovery of pancreatic catalase level compared to pathogenic rats, results are comparable to gliclazide also.
Table 4.5 represents the effect of Aloe vera gel on tissue SOD levels in diabetic rats. The mean SOD level of normal heart was found 1.61 ± 0.03 U/mg protein, STZ administration showed a significantly decrease the SOD levels in comparison of normal healthy rats. Aloe vera gel administration recovers the SOD level significantly as compared to pathogenic rats. Treatment with gliclazide in diabetic rat did not show any significant recovery of SOD in heart tissue of diabetic rats. The 30% Aloe vera gel administration and gliclazide administration alone in normal rats did not produce any marked difference in SOD level of normal rats. The STZ administration significantly reduced the level of SOD in the liver tissue of rats when compared to normal healthy control rats (1.73 ± 0.13 U/ mg protein). Administration of 30 % Aloe vera at dose of 100 mg/kg did not show any significant change in liver SOD level compared to diabetic rats. Treatment with 200 mg/kg of Aloe vera gel and gliclazide in diabetic rats showed a significant recovery in comparison of diabetic rats. The mean SOD level in pancreatic homogenate of normal rats was 0.88 U/ mg protein. STZ administration reduced this significantly as compared to healthy rats group. Administration of 30% Aloe vera gel in both the doses recover the level of SOD significantly in pancreatic tissue of diabetic rats, compared to normal rats. Gliclazide also showed a significant rise in pancreatic SOD level compared to pathogenic rats.

3. Anthropometrics Measurement

No significant change in food and water intake was observed in diabetic and drug treated rats.

4. Histopathological observations

Heart section of healthy control rats showed a normal cardiac muscle bundles and normal myocardium, while diabetic rat showed marked congestion and hemorrhages. Treatment with 30% Aloe vera gel (100 mg/kg) in diabetic rats' heart section showed congested blood vessels with
mild hemorrhage, Administration of 30% gel in dose of 200 mg/kg in diabetic rats, heart section showed very mild congestion. Gliclazide treated diabetic rats heart section showed congestion with patchily hemorrhage. Administration of 30% gel (200 mg/kg) and gliclazide (25 mg/kg) in normal healthy rats did not produce any toxic manifestations in heart (Figure 4.3).

The histopathological section of liver tissue of normal rats showed normal hepatocytes arranged in chords with portal triads and central vein with normal sinusoid. STZ administration produced toxicity in liver, section showed feathery/ hydropic degeneration of hepatocytes, hypertrophy, clumping of cytoplasm. Diabetic rats followed by 30% Aloe vera gel administration, rats' heart section showed hydropic degeneration at the dose of 100 mg/kg and mild atrophy. Treatment with 200 mg/kg of Aloe vera gel in diabetic rats liver section showed mild hemorrhage and necrosis with almost normal hepatocytes with portal triads. Gliclazide treatment in diabetic rats liver section showing normal hepatocytes Administration of 30% Aloe vera gel (200 mg/kg) and gliclazide (25 mg/kg) in normal rats did not produce any liver toxicity (Figure 4.4).

Pancreatic section of normal rat showed globules of acini with islet cells, while diabetic rats’ pancreatic section showed eosinophilic infiltrate, atrophy of pancreatic cells with necrosis and reduction in pancreatic cells was also observed. Treatment with 30% Aloe vera gel in diabetic rats for 20 days showed mild protection at the dose of 100 mg/kg and good protection at the dose of 200 mg/kg. Gliclazide also provide good protection against the STZ induced toxicity in pancreatic cells (Figure 4.5).

5. LD$_{50}$

The behaviour of the treated rats appeared normal upto dose of 1g/kg, no toxic effect and death was observed at given doses. The consumption of food was increased by 10% with in 2 hrs and became normal afterwards, body weight was also normal.
Figure 4.1: Effect of Aloe vera gel on mean blood pressure and heart rate in diabetic rats (Mean ±SEM)

- P<0.01, as compared to group I (ANOVA followed by Dunnett's t test)
- P<0.01, as compared to group II (ANOVA followed by Dunnett's t test)
- P<0.05, as compared to group II (ANOVA followed by Dunnett's t test)
Figure 4.2: Effect of Aloe vera gel on serum lactate dehydrogenase (LDH) and creatine kinase (CK) activity in diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH (IU/L)</th>
<th>CK (IU/L)</th>
</tr>
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<tbody>
<tr>
<td>CON</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STZ+AG-100</td>
<td></td>
<td></td>
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<tr>
<td>STZ+AG-200</td>
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<tr>
<td>STZ+GLI</td>
<td></td>
<td></td>
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<tr>
<td>AG-200</td>
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<tr>
<td>GLI</td>
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</table>

a P<0.01, as compared to group I (ANOVA followed by Dunnett’s t test)
b P<0.01, as compared to group II (ANOVA followed by Dunnett’s t test)
c P<0.05, as compared to group II (ANOVA followed by Dunnett’s t test)
Table 4.1: Effect of 30% Aloe vera gel (AG) on blood glucose, blood glycosylated haemoglobin (HbA1c), blood glutathione (GSH), levels in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters (mean ± SE)</th>
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<tbody>
<tr>
<td></td>
<td>Blood Glucose (mg/dl)</td>
<td>Blood HbA1c (%)</td>
</tr>
<tr>
<td>I (Normal healthy Control)</td>
<td>69.3±0.78</td>
<td>5.05±0.2</td>
</tr>
<tr>
<td>II (STZ treated i.e. pathogenic control)</td>
<td>324.1±11.3</td>
<td>10.5±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III (STZ + AG-100mg/kg)</td>
<td>104.7±1.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.9±0.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV (STZ + AG-200mg/kg)</td>
<td>81.7±1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.2±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V (STZ + Gli-25 mg/kg)</td>
<td>79.0±1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.9±0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VI (AG-200 mg/kg)</td>
<td>72.5±2.1</td>
<td>5.1±0.2</td>
</tr>
<tr>
<td>VII (Gli-25mg/kg)</td>
<td>71.0±1.0</td>
<td>4.9±0.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>P<0.01, as compared to group I (ANOVA followed by Dunnett's t test)
<sup>b</sup>P<0.01, as compared to group II (ANOVA followed by Dunnett's t test)
<sup>c</sup>P<0.05, as compared to group II (ANOVA followed by Dunnett's t test)
Table 4.2: Effect of 30% Aloe vera gel (AG) on lipid peroxides (TBARS) levels in heart, liver and pancreatic tissue of diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmoles MDA/mg protein)</th>
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<tbody>
<tr>
<td></td>
<td>Heart</td>
<td>Liver</td>
<td>Pancreas</td>
<td></td>
</tr>
<tr>
<td>I (Normal healthy Control)</td>
<td>0.52±0.01</td>
<td>0.47±0.03</td>
<td>0.46±0.04</td>
<td></td>
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<tr>
<td>II (STZ treated i.e. pathogenic control)</td>
<td>1.73±0.05(^a)</td>
<td>2.71±0.02(^a)</td>
<td>3.80±0.89(^a)</td>
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<tr>
<td>III (STZ +AG-100mg/kg)</td>
<td>0.95±0.23(^c)</td>
<td>1.05±0.33(^c)</td>
<td>1.64±0.03(^b)</td>
<td></td>
</tr>
<tr>
<td>IV (STZ +AG-200mg/kg)</td>
<td>0.41±0.02(^b)</td>
<td>0.69±0.01(^b)</td>
<td>0.82±0.06(^b)</td>
<td></td>
</tr>
<tr>
<td>V (STZ + Gli-25 mg/kg)</td>
<td>0.89±0.12(^c)</td>
<td>2.15±0.01(^c)</td>
<td>1.35±0.40(^b)</td>
<td></td>
</tr>
<tr>
<td>VI (AG-200 mg/kg)</td>
<td>0.65±0.10</td>
<td>0.74±0.12</td>
<td>0.51±0.10</td>
<td></td>
</tr>
<tr>
<td>VII (Gli-25mg/kg)</td>
<td>0.59±0.06</td>
<td>0.63±0.02</td>
<td>0.71±0.07</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) P<0.01, as compared to group I (ANOVA followed by Dunnett’s t test)
\(^b\) P<0.01, as compared to group II (ANOVA followed by Dunnett’s t test)
\(^c\) P<0.05, as compared to group II (ANOVA followed by Dunnett’s t test)
Table 4.3: Effect of 30% Aloe vera gel (AG) on GSH level in heart, liver and pancreatic tissues of diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart</td>
</tr>
<tr>
<td>I</td>
<td>Normal healthy Control</td>
</tr>
<tr>
<td></td>
<td>99.6±6.0</td>
</tr>
<tr>
<td>II</td>
<td>STZ treated i.e. pathogenic control</td>
</tr>
<tr>
<td></td>
<td>34.5±3.3</td>
</tr>
<tr>
<td>III</td>
<td>STZ + AG-100mg/kg + ISO</td>
</tr>
<tr>
<td></td>
<td>61.1±4.1(^b)</td>
</tr>
<tr>
<td>IV</td>
<td>STZ + AG-200mg/kg + ISO</td>
</tr>
<tr>
<td></td>
<td>79.8±3.9(^b)</td>
</tr>
<tr>
<td>V</td>
<td>STZ + Gli-25 mg/kg + ISO</td>
</tr>
<tr>
<td></td>
<td>40.5±3.1</td>
</tr>
<tr>
<td>VI</td>
<td>AG-200mg/kg</td>
</tr>
<tr>
<td></td>
<td>101.1±4.9</td>
</tr>
<tr>
<td>VII</td>
<td>Gli-25mg/kg</td>
</tr>
<tr>
<td></td>
<td>98.7±2.8</td>
</tr>
</tbody>
</table>

\(^a\) P<0.01, as compared to group I (ANOVA followed by Dunnett’s t test)
\(^b\) P<0.01, as compared to group II (ANOVA followed by Dunnett’s t test)
\(^c\) P<0.05, as compared to group II (ANOVA followed by Dunnett’s t test)
Table 4.4: Effect of 30% *Aloe vera* gel (AG) on catalase levels in heart, liver and pancreatic tissue of diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase (nmol H$_2$O$_2$–consumed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart</td>
</tr>
<tr>
<td>I (Normal healthy</td>
<td>2.1±0.4</td>
</tr>
<tr>
<td>Control)</td>
<td></td>
</tr>
<tr>
<td>II (STZ treated i.e.</td>
<td>134.9±6.3$^{a}$</td>
</tr>
<tr>
<td>pathogenic control)</td>
<td></td>
</tr>
<tr>
<td>III (STZ +AG-100mg/kg)</td>
<td>5.5±0.5$^{b}$</td>
</tr>
<tr>
<td>IV (STZ + AG-200mg/kg)</td>
<td>4.9±0.6$^{b}$</td>
</tr>
<tr>
<td>V (STZ + Gli-25 mg/kg)</td>
<td>16.9±0.5$^{b}$</td>
</tr>
<tr>
<td>VI (AG-200mg/kg)</td>
<td>2.0±0.8</td>
</tr>
<tr>
<td>VII (Gli-25mg/kg)</td>
<td>1.8±0.5</td>
</tr>
</tbody>
</table>

$^{a}$ P<0.01, as compared to group I (ANOVA followed by Dunnett’s t test)

$^{b}$ P<0.01, as compared to group II (ANOVA followed by Dunnett’s t test)

$^{c}$ P<0.05, as compared to group II (ANOVA followed by Dunnett’s t test)
Table 4.5: Effect of 30% *Aloe vera* gel (AG) on SOD levels in heart, liver and pancreatic tissues of diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (IU/mg protein) (Mean± SE)</th>
<th>Heart</th>
<th>Liver</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal healthy Control)</td>
<td></td>
<td>1.61±0.03</td>
<td>1.73±0.13</td>
<td>0.88±0.14</td>
</tr>
<tr>
<td>II (STZ treated i.e. pathogenic control)</td>
<td>0.79±0.06(^a)</td>
<td>0.46±0.01(^a)</td>
<td>0.16±0.03(^a)</td>
<td></td>
</tr>
<tr>
<td>III (STZ + AG-100mg/kg)</td>
<td>1.04±0.04(^b)</td>
<td>1.04±0.05</td>
<td>0.61±0.06(^c)</td>
<td></td>
</tr>
<tr>
<td>IV (STZ + AG-200mg/kg)</td>
<td>1.71±0.21(^b)</td>
<td>1.54±0.07(^b)</td>
<td>0.79±0.08(^b)</td>
<td></td>
</tr>
<tr>
<td>V (STZ + Gli-25 mg/kg)</td>
<td>0.89±0.10</td>
<td>1.23±0.03(^b)</td>
<td>0.68±0.07(^c)</td>
<td></td>
</tr>
<tr>
<td>VI (AG-200mg/kg)</td>
<td>2.01±0.19</td>
<td>1.63±0.10</td>
<td>1.02±0.23</td>
<td></td>
</tr>
<tr>
<td>VII (Gli-25mg/kg)</td>
<td>1.89±0.10</td>
<td>2.18±0.10</td>
<td>1.09±0.10</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) P<0.01, as compared to group I (ANOVA followed by Dunnett’s t test)
\(^b\) P<0.01, as compared to group II (ANOVA followed by Dunnett’s t test)
\(^c\) P<0.05, as compared to group II (ANOVA followed by Dunnett’s t test)
Figure 4.3 (A-G): Photomicrograph of control, diabetic rats heart and protection by Aloe vera gel: (H&E 20x): (A) Control (B) Diabetic control (C) STZ treated rat following with AG -100mg/kg (D) STZ treated rat following with AG -200mg/kg (E) STZ treated rat following with Gli 25 mg/kg (F) AG-200mg/kg (G) Gli-25 mg/kg.
Figure 4.4 (A-G): Photomicrograph of control, diabetic rats liver and protection by Aloe vera gel: (H&E 20x): (A) Control (B) Diabetic control (C) STZ treated rat following with AG-100mg/kg (D) STZ treated rat following with AG-200mg/kg (E) STZ treated rat following with Gli 25 mg/kg (F) AG-200mg/kg (G) Gli-25 mg/kg.
Figure 4.5 (A-G): Photomicrograph of control, diabetic rats pancreas and protection by Aloe vera gel; (H&E 20x): (A) Control (B) Diabetic control (C) STZ treated rat following with AG -100mg/kg (D) STZ treated rat following with AG -200mg/kg (E) STZ treated rat following with Gli 25 mg/kg (F) AG-200mg/kg (G) Gli-25 mg/kg.
DISCUSSION

Diabetes mellitus is one of the most common chronic diseases and is associated with hyperglycemia and co-morbidities such as obesity and hypertension. Hyperglycemia, defining established diabetes, can induce oxidative stress by free radical generation or by overproduction of superoxide anion. Hyperglycemia can simply inactivate existing enzymes by glycating their protein, also leads to DNA cleavage (Wiernsperger, 2003). It is reported that hyperglycemia and insulin resistance both seem to have important roles in the pathogenesis of diabetic induced macrovascular complications. Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism (Ayber et al., 1990). The use of lower dose of STZ (40 mg/kg) produced a partial destruction of β-cells even though rats become permanently diabetic (Ayber et al., 2001). From ancient age many plants have been used for the regulation of blood glucose level in diabetes mellitus in Indian system of medicine and world. Out of these, only few have been evaluated as per modern system of medicine. Poorly controlled diabetes can cause devastating consequences, including microvascular and macrovascular disease.

Yeh et al. (2003) cited Aloe among the five kinds of herb that showed a favorable activity in controlling blood glucose levels in around 5000 diabetic patients who had been using either one of 108 kinds of dietary supplements including 36 kinds of herb.

Hypertension is generally believed to be more prevalent among diabetic than non-diabetic subjects and is known that hypertension is an independent risk factor for cardiovascular mortality in patients with diabetes (Dubrey et al., 1994; Tomlinson et al., 1992; Rodrigues 1986; Litwin et al., 1990).

It has been considered that alteration in the reactivity of blood vessels to neurotransmitters and circulating hormones are responsible for
the cardiovascular complications associated with diabetes (Christlieb et al., 1976; Weidmann, 1985). The induction of STZ diabetes in the normotensive animals raised blood pressure values, but appeared to reduce heart rate. In the hypertensive rats, however, both blood pressure and heart rate were significantly reduced by the induction of STZ diabetes. Others (Somani et al., 1979; Agrawal et al., 1987) also observed that the induction of lowering of blood pressure values, possibly as a result of hypovolaemia, provoked by osmotic diuresis. Other studies performed in STZ-diabetic normotensive rats show inconsistent results regarding blood pressure values (Tomlinson et al., 1992). Although the reason for these discrepancies is unknown, they appeared to be unrelated to differences in the dose of STZ time course after treatment, or the strain of rat used.

In diabetes, it seems that increased oxidative stress due to hyperglycemia and or vascular tissue leads to reduced availability of NO that leads to hypertension. Consequently treatment which can reduce oxidative stress and or treatment with L-arginine may be effective in preventing hypertension observed in diabetes (Ozcelikay et al., 2000).

In our study we recorded a significant increase in mean blood pressure and found normal heart rate after 20 days of STZ administration, these results agree with the conclusion of studies which had used the direct technique for measurement of BP (Maeda et al., 1995; Brooks et al., 1989; Dai et al., 1994). It is reported that for longer duration of study STZ administration in normotensive rats show increase in heart rate and blood pressure due to oxidative stress and reduction in the availability of NO for maintaining the heart rate. Various studies along with our finding demonstrated the anti-oxidant activity of Aloe vera gel, thus it provide protection against the BP due to its antioxidant and hypoglycemic activity in diabetic rats. Gliclazide is also reported to reduce the blood pressure in diabetic rats in longer duration (De Mattia et al., 2003).
It is well known that the chronic hyperglycemic state can have adverse effects on various organs; good control of the glycemic state, therefore, should be the aim of the treatment in diabetic patients. The use of lower dose of STZ (40 mg/kg) selectively destroys the pancreatic insulin secreting beta cells, leaving the less active cells thus resulting in a diabetic state (Kamtchoung et al., 1998; Szkudelski, 2001, Ayber et al., 2001). It make this as a most suitable model for type 2 diabetes. In our study hyperglycemia remain maintained through out the study after administration of STZ in pathogenic control rats showing selection of proper dose of STZ.

The increased level of blood glucose in STZ-induced diabetic rats was lowered by both the doses (100 mg/kg and 200 mg/kg) of 30% Aloe vera gel. Aloe have long been used all over the world for their various medicinal plants. Various reports are available regarding the antidiabetic activity of different extracts of Aloe vera gel. Okyar et al (2001), reported the hypoglycemic activity of alcoholic extract of Aloe vera leaf pulp and gel on type 1 and type 2 diabetic rats; they also reported that both the extracts were not having any hypoglycemic activity in normal rats. In our study also we did not found any significant alteration in blood glucose level after 20 days treatment of Aloe vera gel (200 mg/kg) in normal rats. Our finding also matches with the finding of Can et al (2004) who reported the antidiabetic, antioxidant and hepatoprotective activity of Aloe vera leaf pulp and gel extract in type 2 diabetic rats.

In our study we have directly used 30% gel to reduce the bitterness of gel and consistency that make gel easy to eat and swallow by oral route.

Based on our results there are few possible explanations for our finding. Aloe vera gel may exert its effect by preventing the STZ induced pancreatic-beta cell death and/or second, it may permit recovery of partially destroyed beta cells like Momordica charantina (Ahmed et al., 1998). Third possibility of resulting hypoglycemic action in diabetic rats is due to
potentiation of insulin releases from existing pancreatic beta cells of the islet of langerhans (Alhussain, 2002). Some plants possess' hypoglycemic action due to presence of insulin- like substances (Collier et al., 1987; gray and Flatt, 1999).

In our study histopathological finding showed (Figure 4.5) normal islets with increase in number when diabetic rats were treated with 200 mg/kg of Aloe vera gel, whereas section of pancreatic tissue of gliclazide treated diabetic rats showed degenerative changes. Similar results in STZ treated diabetic animals were found with pancreas tonic (Rao et al., 1998), ephedrine (Xiu et al., 2001), Gymnema sylvestera leaf extract (Shanmugasundaram et al., 1990) and TerminaLla calappa fruit (Nagappa et al., 2003).

As STZ degenerate pancreatic beta cells though alkylating action and form reactive oxygen species, few reports are available demonstrating the antioxidant action of some constituents of Aloe vera gel (Hu et al., 2003). This could also contribute to reduction in STZ induced pancreatic damage in rats.

Aloe vera gel administration also significantly maintain the blood GSH levels in diabetic rats compared to untreated one and provide protection against STZ induced free radicals. Beppu et al (2003a) reported that kidachi Aloe leaf skin has an inhibitory effect on the distruction caused by methyl (CH₃⁻) radical on isolated sells of pancreatic islets. The low molecular weight omponents of Aloe vera containing anthrone derivatives such as iso berbaloin (IBL), barbaloin (BL) and Aloe emodin are reported to have anti-inflammatory (Yamamoto et al., 1991) and antitumor activity (shimpo et al., 2003). These low molecular main components also have chronone derivatives (CAS and FAS) that are more potent radical scavenger (Yagi et al., 2002; Shimpoo et al., 2003b; Beppu et al 2003). From these finding it is presumable that antrone or chronome derivatives
could be the involving components to protect beta cells of the pancreatic islet from damaged caused by free radical derived from STZ administration (Beppu et al., 2006).

The other possible mechanism of action of Aloe vera gel may be mediated through liver and affecting gluconeogenesis, glycogenesis or glycogenolysis. Some flavonoids have been found to inhibit glucose transport in the intestine, decrease the expression of genes that control gluconeogenesis, increase the storage of the glucose in the liver (up-regulated glycogeneogenesis) and reduce glycogen breakdown (down regulation of glycogeneogenesis), they also reduce the glycogen breakdown (Sarkhail et al., 2007).

It is mentioned that measurement of blood levels of glycosylated hemoglobin is one of the best indicators used to monitor the diabetes (Kuroki et al., 1990). In uncontrolled or poorly control diabetes, there is an increased glycosylation of number of proteins including haemoglobin and beta-crystalline of lense (Alberti and Press, 1982). Glycosylated Hb was found to increase in patients with DM to approximately 16% and this rise in level also depends on severity of the disease (Koeing et al., 1976): During diabetes the excess glucose present in blood reacts with hemoglobin. Therefore, the total hemoglobin level is decreased in alloxan diabetic rats (Sheela and Augusti, 1992).

Glycosylated-hemoglobin measure the percentage of Hb bound to glucose. Hemoglobin is the protein found in every RBC. As hemoglobin and glucose together in the RBC, the glucose gradually binds to hemoglobin A at its NH₂ terminus and the process called glyosylation, thus glycosylate hemoglobin (HbA1c) is a measurement of mean blood glucose level, over the previous 6 to 8 weeks, during the life span of red blood cells (Ghacha et al., 2001). The values show the past 30 days level as 50% of A1c, the
preceding 60 day giving about 25% of the value and preceding 90 days giving about 25% of the value (Ghacha et al., 2001).

Our study is short term study but still we found a significant rise in level of HbA1c level in a diabetic control rats, Fuji and Nomoto (1984) also reported a significant change in HbA1c level after 2 week of STZ administration in rats. Treatment with Aloe vera gel significantly reduced the HbA1c level thus maintaining the blood glucose level. It is reported that each percent reduction in HbA1c level resulted in a 35% reduction in the risk of microvascular complications including MI in patients with type 2 diabetes (UKPDS, 1998; Krapek et al., 2004). Administration of Boerhaavia diffusa extract for four weeks in diabetic rats also reduce the level of HbA1c level significantly compared to diabetic control rats (Pari and Satheesh, 2004).Reports are available with the same result given by various herbs including Cynodon dactylon (Mahesh and Brahatheeswaran, 2007) vitamin C (Forghani et al., 2003).

In diabetics uncontrolled elevation in blood sugar level give rise to formation of oxygen free radicals (OFR). There are many ways by which hyperglycemia may increase the generation of OFRs, such as glycooxidation, polyol pathway, prostanoid biosynthesis, and protein glycation (Armstrong and Young, 1996). In animal studies we observed a significant increase in blood glucose level and a reduction in blood GSH level could be due to destruction of pancreatic beta cells by STZ reinforcing the view that STZ induce diabetes through NO production which give rise to generation of OFR (Wohaieb and Godin, 1987). GSH is an important inhibitor of free radical mediated lipid peroxidation and it protect the cellular system against the toxic effects of lipid peroxidation (Garg and Bansal, 1996). Hence, drug that could prevent the generation of OFRs or increase the free radical scavenging enzymes maybe effective in STZ induced diabetes (Paolisso et al., 1992). It is reported that ethanolic extract of Aloe vera gel posses antioxidant property in our study also
administration of 30% *Aloe vera* gel in both the doses maintain the blood GSH level. This indicates that the extract may be helpful in the prevention of damage caused by oxygen free radical. Gliclazide also reduced the generation of free radicals and thus providing the antioxidant as well as hypoglycemic effect.

The cytotoxicity of a xenobiotic can evaluated using the serum activities of marker enzyme. One such enzyme is serum lactate dehydrogenase (LDH) which, though distributed throughout the body, posses isoenzymes recognized as marker for liver and muscle lesion (Aldrich, 2003). We have observed the antihyperglycemic activity of *Aloe vera* gel and this drug also reduce the level of LDH in serum of diabetic rats indicating protection of liver and muscles from damage caused by OFR produced by glycation of protein and fat. Our data matches with the findings of others (Stanely et al., 2000; Mansour et al., 2002).

Contradictory reports are available in literature on the relationship between diabetes and serum creatine kinase (CK) activity, which has been variously described as increasing or decreasing in the diabetes (Pepato et al., 2004; Scott et al., 1984; Lazarov et al., 1990; Zhao et al., 1999; Al-Shabanah et al., 2000). The quantity of enzyme released from the damaged tissue is a measure of the number of the necrotic cells. In our previous study (Bhandari et al., 2007) we found a significant rise in serum CK level, this time also we get the same result in diabetic rats. Treatment with 30% *Aloe vera* gel reduce the leakage of CK from tissue bed to serum thus showing muscle integrity and reduction in cytotoxicity in diabetic rats. *Aloe vera* gel showed reduction in the leakage of these enzymes in serum, thus provide the protection against STZ induced muscular damage.

As mentioned above that hyperglycemia induced oxidative stress has been proposed as a cause of many complications of diabetes (Okhubo et al., 1995; Thornalley et al., 1996). STZ diabetic animals exhibit most of
the diabetic complications like myocardial, gastrointestinal, nervous, vas deferens, kidney and urinary bladder dysfunction though reactive oxygen species (ROS) formed due to hyperglycemia.

It has been shown that elevated extra and intra cellular glucose concentrations result in oxidation stress, which is defined not only due to ROS formation and imbalance between prooxidant and antioxidants (Sakurai et al., 1988; Hunt et al., 1991). Several other mechanisms seem to be involved on the genesis of this oxidative stress. Some of them are glucose auto-oxidation, protein glycosylation and formation of advance glycation end product (AGE), polyol pathway, formation of peroxide and impaired antioxidant enzyme (West, 2000; Vincent et al, 2004; Baynes, 1999; Pari and Latha, 2005). The susceptibility of given organ or system to oxidative stress is a function of the balance between prooxidant factor and those scavenging them.

Various studies has been shown that DM is associated with oxidative stress and leading to an increased production of reactive oxygen species (ROS), including superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH$^-$) or reduction in antioxidant defense system (Sarkhail et al., 2007; Rahmani et al, 2005; Vincent et al., 2004). All these factors promote dysfunction of tissue mitochondria. All these intermediate reduction products of oxygen metabolism resulting in ROS are controlled by various cellular defense mechanisms consisting of enzymatic superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase [(GSH-PX)/ oxidized glutathione reductase (GSSG RD) system] and non enzymatic (glutathione, ascorbic acid and vitamin E) scavenger components. Oxidative stress can therefore be the consequence of raised free radical production, insufficient antioxidant potential or both (Zhang et al. 2000; Lin et al., 2005). It is reported that oxidative stress that leads to an increased production of reactive oxygen species and finally cellular lipid
peroxidation, has been found to play an important role in DM induced complications (Baynes, 1999).

Lipid peroxidation (LPO) is a key marker of oxidative stress. The lipid peroxidation resulting from the oxidative degradation of polyunsaturated fatty acids of the cells membrane may produce tissue damage and finally causes various diabetes induced complications (Tatsuki et al., 1997; Pari and Latha, 2005). In vivo assessment of reactive free radicals is difficult because of their high reactivity, shorter half life and very low concentration. Thus, the indirect methods usually used for measurement of secondary products of oxidative stress, such as TBARS (Thiobarbituric acid reactive substance), a convenient assay for lipid hydroperoxidation is employed (Giugliano et al., 1996).

In experimental diabetes the main organ for STZ, a potent methylating agent and act as NO donor, induced ROS is beta cells of pancreas because it has low level of free radical scavenging enzymes (Lukic, 1998; Spinas, 1999; Singh, 2001). Srinivasan and Menon (2003) reported the antioxidant drug bis-o-hydroxycinnamoyl methane provide protection of beta cells against STZ induced ROS mediated damage by enhancing antioxidant activity and by reducing persistent hyperglycemia.

LPO may bring about protein damage and inactivation of membrane bound enzymes either through direct attack by free radicals or through chemical modification of its end product, such as MDA. Results of our study regarding the protection of beta cell by 30 % Aloe vera gel consistent with the results of Wolf., 1993; El Missiry et al., 2000; Coskum et al., 2005).

Like and Rosani (1976) reported that STZ may not exert any direct effect on the activity on antioxidant enzymes, since it had a half life of 15 min only and is readily metabolized. Thus the alteration in antioxidant enzyme activity may, primarily be due to the diabetic state and secondarily
due to the effect of STZ (Tiedge et al., 1997; Rabinovitch and Suarez., 1998; Toyokuni. 1999; Ihara et al., 1999). STZ, a beta cytotoxin, induces "chemical diabetes" in a wide variety of animals by damaging the insulin secreting pancreatic beta cells, resulting in endogenous insulin release, which paves the ways for decrease utilization of glucose by tissue (Saravanan and Pari., 2005). It is also reported that even after the moderate decrease of blood glucose levels was achieved in the antioxidant treated diabetic mice, the blood glucose level in those mice remained high enough to cause glucose toxicity, and that the antioxidant kept protecting beta cells against it by neutralizing the toxic effects of oxidative stress provoked due to hyperglycemia (Kaneto et al., 1999).

Apart from glucose regulation insulin also plays an important role in the metabolism of lipid. Insulin is potent inhibitor of lipid. Insulin is a potent inhibitor of lipolysis. Since it inhibit the activity of the hormone sensitive lipase in adipose tissue and suppress the release of free fatty acids (Loci et al., 1994). During diabetes, enhanced activity of this enzyme increases lipolysis and release more free fatty acids in to the circulation (Agardh et al., 1999). Increased free fatty acids due to lipolysis give rise to produce more acetyl CoA and cholesterol during diabetes. This increased concentration of cholesterol could result in a decrease in membrane fluidity (Dario et al., 1996).

Liver is the organ which have higher concentration of easily peroxidizable fatty acids, these fatty acids in liver causes activation of NADPH dependent microsomal lipid per oxidation and give rise to hepatic MDA level. Liver and kidney phospholipids were increased in diabetic rats. Phospholipids are vital part of biomembrane rich in PUFA, which are susceptible substrate for free radicals such as O$_2^-$ and OH$^-$ radicals (Ahemad et al., 2001). Increased phospholipids level in tissue was reported in STZ treated animals (Venkateswaranan et al., 2002; Pari and satheesh, 2004). Lipid peroxidation was also found to be increased in the liver of
diabetic rats, STZ rats have increased level of hepatic MDA in other studies also (Jang et al., 2000; Yadav et al., 1997).

Heart is also a vital organ for diabetes induced secondary complications like diabetes cardiomyopathy, which generally occurs due to hyperglycemia and OFRs. Several reports are available stating that cardiomyopathy have been resulted due to accumulation of myocardial collagen which has related to early diastolic and systolic dysfunction (Modrak et al., 1980; Shimizu et al., 1993; Sipro and Crowley et al., 1993; Tschope et al., 2004). This accumulation of collagen results due to nonenzymatic glycosylation of collagens, down regulation or reduced activity of collagen degrading matrix metalloproteinase (MMP), up regulation in collagen mRNA expression (Sipro and Crowley et al., 1993). All these factors impaired endothelium regulation due to formation of OFR (Rosen et al., 1995).

Direct evidence that free radicals and superoxide anion can mediate endothelial dysfunction and quench NO which produce vasodilatation has already been presented (Diederich et al., 1994; Pipper et al., 1993; Langenstreor and Pipper, 1992; Gryglewsk et al., 1986; Rosen et al., 1995). It is assumed that oxygen free radicals affect the vascular system by reacting with released NO to produce peroxynitrite which can decay to nitrogen dioxide and hydroxy radicals (Langenstreor and Pipper, 1992; Rosen et al., 1995), this generated free radicals are enhanced in diabetes supported by considerable evidence (Rosen and Tschope, 1991; Lyons et al., 1991) The source of these oxygen derived radicals is unclear and has not been identified but; the autoxidation of glucose (Wolf and Dean, 1987), lipid peroxidation (Rosen and Tschope, 1991; Lyons et al., 1991) as well as glycated proteins might lead to an increased release of oxygen derived radicals and thereby exert an increased oxidative stress on vasculature of diabetics (Rosen et al., 1995; Schrauwen and Hesselink, 2004).
Elevated blood glucose levels contribute to the glycation of protein and lipids, resulting in the formation of advanced glycation end product (AGE) (Basta et al., 2004). Receptors for AGEs (RAGE) are expressed in many different tissues and cell types, including endothelial cells, vascular smooth muscle cells and macrophages (Neeper et al., 1992; Brett et al., 1993). The binding of AGEs to RAGE leads to the intracellular generation of ROS (Yan et al., 1994) which further activate NF-kB. This NF-kB activation further express variety of cytokines, including TNF-alfa and TNF-beta, interleukins (IL)1, 6, 8 and 18 interferon-γ (Basta et al., 2002). These entire factors affect vascular function and increased the expression of cellular adhesion molecules on the endothelial cell surface. These molecules attach to circulating leucocyte, leading to the formation of the artherosclerotic plaque. Inhibition of lipid peroxidation, prostanoid synthesis, and platelet activity were shown to be effective in the prevention of tissue injury and diabetic angiopathy (Sensaki et al., 1985; Somova et al., 1989).

The significant extent of LPO, measured as TBARS has reported in diabetes (Pari and Latha, 2005; Rajesekaran et al., 2005). In our results also, we found the significant rise in heart, liver and pancreas MDA level in diabetic rats and treatment with 30% Aloe vera gel reduced the OFR generation by various mechanisms including; by maintaining blood glucose level through out the study, it also posses antioxidant property (Beppu et al., 2003; Yagi et al., 2003), it is reported component of Aloe vera gel, Aloe sin inhibit COX-2, various interleukins, thromboxaneA₂ activity and posses anti-inflammatory activity by which it protect heart by endothelial dysfunction (Yamamoto et al., 1991). Can et al. also reported that Aloe pulp and gel extract reduce liver damage caused by STZ. Interestingly gliclazide, a second generation sulfonylurea derivative by controlling blood glucose level showed same results against oxidative stress induced due to persistent hyperglycemia (Saravanan et al., 2005). Similar results have
been reported with other compounds like N-acetyl-L-cystein, Vit C, Vit E and a herbal formulation Diasulin (Stoffers et al., 1997; Kaetno et al., 1997; Saravanan and Pari, 2005).

It has been generally reported that diabetic patients with vascular lesion have higher TBARs level then their healthy counterparts. TBARS and hydroperoxides significantly increased in diabetic rats (Ananthan et al., 2003). Lipid peroxidation may bring about protein damage and interaction of membrane bound enzymes either through direct attack by free radicals or through chemical modifications of its end product such as MDA (Coskun et al., 2005).

Cooperative defense systems that protect the body from free radical damage include the antioxidant (GSH, vit C, vit E) nutrients and enzymes (SOD, CAT, GPx). Oxidative stress in diabetes coexists with a reduction in the antioxidant capacity, which can increase the deleterious effects of stress generated free radical.

Glutathione (GSH) is the first line of defense against prooxidant status (Ananthan et al., 2003). It is a tripeptide normally present in millimolar concentration intracellularly, and constitutes the major reducing capacity of cytoplasm (Lu, 1999). Glutathione is known to protect the cellular system against toxic effect of lipid peroxidation (Anuradha and Selvam, 1993).

ROS intermediate such as superoxide (O$_2^-$) and hydrogen peroxide formed due to oxidative stress leads to further protection of toxic oxygen radical that cause formation of LPO and cell injury. The endogenously produced hydrogen peroxide is reduced by GSH (Lu, 1990). It has multifaceted role in antioxidant defense (Soon and Tan, 2002). It is a direct scavenger of free radicals as well as cosubstrate for peroxide detoxification by glutathione peroxidase (Meister and Anderson, 1983). Loven et al. (1986) suggested that the decrease in hepatic GSH in diabetics could be
due to decreased synthesis, or increased degradation of GSH by oxidative stress. Consequently, tissue antioxidant status is suggested to be an important factor in the development of diabetic complications (Vankateswaran and Pari, 2000). H$_2$O$_2$ can also be reduced by catalase, which is present only in the peroxisome. In the mitochondria only GSH is particularly important because there is no catalase present (Deleve et al., 1991). It is well known that oxidative stress is an imbalance between free radical production and lipid peroxidation on the one hand and the antioxidant defense system on the other hand.

In this study decreased levels of GSH in the liver, heart and pancreas during diabetes represent its utilization due to oxidative stress. It appears that generation of oxygen radicals by increased levels of glucose causes utilization of GSH and thus lowers GSH level in the various tissues (Babu and Prince, 2004).

Administration of 30% Aloe vera gel showed a significant elevation in GSH level of various organs, this indicates that the extract can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects, our finding matches with the findings of Rajesekaran et al., 2005. Aloe vera gel showed antihyperglycemic activity, i.e. it is also maintaining LPO level by maintaining blood sugar levels. Tissue GSH level of pancreas is very less that's why this organ is prone to oxidative damage induced to STZ administration. Aloe vera gel in dose of 200 mg/kg/day showed a better protection by maintaining tissue as well as blood GSH levels in diabetic rats.

It is reported that diabetes induces alterations in the activity of antioxidant enzymes in various tissues, and theses alterations are quite heterogeneous with respect to tissue (Oberlcy et al., 1988). This finding was also demonstrated in the present study using STZ. However, it is not
clearly known how these changes are involved in organ-specific complications (Kakkar et al., 1998). As we mentioned previously that STZ do not exert any direct effect on antioxidant enzyme due to its short half life, this effect on SOD, CAT and GPx may primarily be due to diabetic state and secondly due to STZ induced ROS (Jang et al., 2000).

SOD is principally an antioxidant enzyme that scavenges superoxide anion (O \(^{-}\)) formed as intermediate product of \(\text{H}_2\text{O}_2\) breakdown (Kaleem et al., 2006). However, there is variation as to the status of this enzyme in the diabetic state. Some studies have reported decrease in SOD activity in vital organs during diabetes (Kedizora, 2003; Obrosova et al., 2000) or increased in activity (Rauscher et al., 2001) or no change in the enzyme (Meckinova et al., 1995; Maritim et al., 2003). SOD plays a vital role in cardioprotection and muscular integrity during diabetes. It is reported that impairment of endothelium-dependent vasodilatation by the coronary bed could be abolish by perfusion with SOD and persistent with alfa-tocopherol (Rosen et al., 1995).

In our study administration of STZ decreases the activity of SOD in liver heart and pancreas, same finding are reported by others also (Kedizora, 2003). The observed decrease in SOD activity could result from inactivation of SOD by \(\text{H}_2\text{O}_2\) or by glycation of the enzyme, which have been reported to occur in diabetes (Sozmen et al., 2001; Soon and Tan, 2002; Ravi et al., 2004).

Same as SOD, catalase is a hemeprotein, which catalyzed reduction of \(\text{H}_2\text{O}_2\) and protect the tissues from highly reactive hydroxyl radical (Chance et al., 1952; Uchigata et al., 1982). Likes SOD, variation in the activity of CAT is reported in diabetic rat organs. Some studies has reported decrease in CAT levels in all the vital organ including pancreas while some have reported sharp rise in CAT activity in heart only. (Otsyula et al., 2003), some observed no change in activity (Godin et al., 1988).
Bukan et al. (2004), reported decreased activity of SOD and increased activity of CAT in diabetic rat heart, in contrast to these Babu et al (2006), have reported a significant rise in SOD, CAT and GPx activity in diabetic heart. Kono and Fridovich (1982) reported that an increase in the SOD activity may protect CAT against enzyme inactivation by superoxide radicals.

In our study we observed decreased activity of CAT and SOD in pancreatic tissue; this is because of weak defense system that make pancreas more vulnerable to oxidative stress. This inhibition in activity of both the enzyme may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Rajasekaran et al., 2005).

Treatment of diabetic rats with 30% Aloe vera gel for 20 days increased the level of non-enzymatic antioxidants such as GSH in heart, liver, pancreas and blood. This treatment also retains the activity of SOD in all these organs. Thus, this antioxidant activity of Aloe vera gel could be either due to inhibition of glycation of this antioxidant enzyme or it protect this by protecting β-cells from STZ induced damage and prohibit further generation of free radicals to forms new attacking moiety. Glucose which forms shiff's base with protein has been reported to have affinity for proteins especially those containing transition metal ions (Taniguchi et al., 1996), this increased level of glucose is maintained by Aloe vera gel thus reduce the glycation of enzyme. The elevated level of SOD in diabetic rats after treatment with Aloe vera gel also predict that Aloe vera gel contain the free radical scavenging activity, which could exert a beneficial action against the pathological alteration caused by the presence of $O_2^{-}$ and $^{1}\text{OH}$ (Pushparaj and Tan, 2000).
In this study we also observed a sharp peak in heart and liver CAT activity in diabetic rats, which matches with the findings of others also (Kakkar et al., 1996, Noyan et al., 2005; Wohaieb et al., 1987; Tatsuki et al., 1997; Matkovics, 1997/98; Stefek et al., 2000). This result suggests a compensatory response to oxidative stress due to an increase in endogenous $\text{H}_2\text{O}_2$ production.

The number of functionally intact $\beta$-cells in the islet and various organ integrity are of decisive importance for the development course and outcome of diabetes mellitus. To measure the extent of tissue damage by STZ, protection by Aloe vera gel and to correlate the biochemical finding with organ specific damage we have carried out the histological studies of heart, liver and pancreas.

The pancreatic section of diabetic rats showed reduction beta cells of islet, tissue necrosis and destruction in shape. The pancreatic section of diabetic rat received 30% Aloe vera gel showed increase volume density of islets, percent of $\beta$-cell and size of islet, which may be sign of regeneration, photomicrograph shows that this regeneration is more with treatment of 200 mg/kg of 30% Aloe vera gel. Increased percent of $\beta$-cell without change of volume density of $\beta$-cell in islet may be due to increase number or size of other types of pancreatic cells especially alfa- cells (Sudha Rastogi et al., 1990). In biochemical finding also we observed reduction in blood-glucose level in drug treated rats, this could be due to regeneration of pancreatic beta cell; this also result in reduction of oxidative stress due to pancreatic cell death. The relative distribution of pancreatic islets was similar to control rats. Similar results are reported with ethanolic etxtract of Aloe vera leaf pulp (Noor et al., 2008), Sign of regeneration of $\beta$-cell has been reported following consumption of other plants such as a flavonoid fraction extracted from pterocarpus marsupium (Chakravarthy et al., 1980) and also with Nigella sativa. Gliclazide also act on same principal but results are more to words normal with Aloe vera gel.
In liver section diabetic rats shrunken nuclei granular cytoplasm, dilatation in the sinusoids and inflammation were noticed. These changes were reduced with the treatment of 30% Aloe vera gel. This may be due to beneficial and protective effect of gel on liver and it could also be due to anti-inflammatory activity of Aloe vera gel constituents (Yamamoto et al., 1991). Liver is reported to be as major organ for antioxidant enzymes. Our finding suggest that Aloe vera reduce the destruction of these enzymes either due reduced glycation of enzyme or due to reduction in generation of free radicals by STZ induced oxidative stress and thus maintain the normal cellularity of this organ (Armstrong and Young, 1996).

Aloe vera treatment in diabetic rat also protects the heart tissue from STZ induced cardiomyopathy. It provides protection by preventing heart muscle from STZ induced ROS and hyperglycemia induced tissue damage. Tissue section of Aloe vera gel treated rats heart shows normal myofibrils with branched appearance. At the dose of 200 mg/kg heart section showed features comparable to normal heart section.