5.0 DISCUSSION

5.1. Phytochemical characterization of *Acalypha indica*

5.1.1. Moisture Content

The moisture content of aerial parts of *A. indica* was measured as 51.3% (Table 1). Moisture content (loss on drying) determines the water drying off from the drug. Drug containing excess moisture will lead to the activation of enzymes and gives suitable condition for the proliferation of living microorganisms. Higher water content indicates the presence of large amount of mucilage or starch and paves way for more chances for microbial degradation and if the value is not too high, it indicates less chances of microbial degradation (Trease and Evans, 1983).

5.1.2. Ash content

The presence of ash content in plant material was determined as total ash, acid insoluble ash, water soluble ash and sulphated ash (Table 1). The determination of ash value is useful for detecting exhausted drugs and excess of sandy and earthy matter. The total ash usually consist of carbonates, phosphates and silicates of silica. Ash value determination is a good index of quality and is useful in the detection of adulteration. An increase in the ash value when compared with standard value is an indication of contamination or adulteration (Trease and Evans, 1983).

In the present study, the plant sample contains total ash value of 9.6%, acid insoluble ash value of 12.9%, water soluble ash value of 34.8% and sulphated ash value of 10.2%. Acid insoluble ash value can be used to determine the silica impurities mixed with the drug during collection. Water soluble ash value helps in determining the added mineral matter and quality of the powdered drug (Pruthi, 1980).

5.1.3. Extractive values and yield of extracts

In the present study, the extractive values are depicted in Table 2. The percentage of extractive value was maximum in ethanol (16%) followed by water (13%), chloroform (8%) and petroleum ether (5%).
The determination of extractive value refers to the amount of constituents present in given amount of raw material extracted with suitable solvents. These values provide an indication of the extraction of polar and non-polar components present in the sample and are useful in the evaluation of plant drugs (Miller, 1973).

5.1.4. Qualitative phytochemical screening

Medicinal plant based drugs have shown the added advantage of being simple, effective, free from side effects and offer a broad spectrum of activity with great emphasis on preventive action of chronic and degenerative diseases (Chin et al., 2006). Medicinal plants are the richest bio-resource of drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube et al., 2008; Nirmala et al., 2011 a,b). The medicinal plants have chemical substances called phytochemicals that produce various physiological action on the human body. Phytochemical screening is an essential step towards the discovery of new drugs as it provides the information regarding the presence of particular primary and secondary metabolites in the plant extract of clinical significance. Phytochemicals derived from the plant sources are used for prevention and treatment of diabetes mellitus, cancer, heart diseases and high blood pressure (Waltner-Law et al., 2002). The therapeutic effect of several medicinal plants have been attributed to the presence of phenolic compounds such as flavonoids, phenolic acid, proanthocyanidins, diterpenes and tannins (Pourmorad et al., 2006).

In the present study, the qualitative phytochemical analysis of ethanolic extract of A.indica revealed the presence of alkaloids, glycosides, flavonoids, saponins, phenols and tannins (Table 3). The positive response of the above mentioned compounds in the ethanolic extract may be due to the dissolution capacity of phytochemicals in the organic solvent. Earlier, similar studies were carried out in Strumpfia maritima (Hsu et al., 1981), Uncaria species (Heitzman et al., 2005), Mitracarpus scaber (Abere et al., 2007) and Teucrium stocksianum (Rahim et al., 2012).

5.1.5. Quantitative analysis of phytochemicals

Natural products have played an important role in the development of drugs for various diseases. Until 1990’s scientists thought that most of the compounds produced by
the plants were useless waste products. These waste compounds are called as secondary metabolites. But later it was found that these compounds may perform a huge array of functions. Many of these compounds cannot be synthesized economically on commercial basis. The secondary metabolites have complex stereo structure with many chiral centres which may be essential for various biological activities (Farnsworth and Morris, 1976). The secondary metabolites from natural sources are good products for drug development because being elaborated within the living systems, they are perceived to exhibit more similarities to drugs and show more biological friendliness than synthetic drugs (Shoeb, 2006). Plants produce a diverse array of bioactive molecules, making them a rich source of diverse type of medicines. Plants with natural products exhibit pharmacological and biological activities and play an important role in life-threatening conditions (Onocha et al., 2011).

Flavonoids are present in all vascular plants and have been reported to exert multiple biological effects including anti-inflammatory, anti-ulcerogenic, anti-allergic, anti-viral and anti-cancerous activities (Harborne, 1973; Kumar and Mathai, 2010). Tannins have been reported in the leaves of Pomegranate, Tambolan and Guava and medicinally tannins are used in anti-diarrhoeal and anti-haemorrhoidal preparations (Nascimento et al., 2000; Edeoga et al., 2005). Saponins are glycosides of steroids, steroid alkaloids found in plants, especially in plant skins where they form a waxy protective coating. They are useful in lowering cholesterol, as antioxidants and anti-inflammatory agents. Terpenoids are diverse class of naturally occurring organic chemicals found in all classes of living organisms, which may exhibit antibacterial properties (Nostro et al., 2000; Edeoga et al., 2005). Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia and are found as secondary metabolites in several plants like Digitalis, Convallaris and Euphorbia species (Edeoga et al., 2005).

5.1.5.1. Total Alkaloids content

Alkaloids are the largest class of secondary plant substances present in higher plants of some families viz., Papaveraceae, Liliaceae, Solanaceae, Rubiaceae, Rutaceae, Boraginaceae and Asclepiadaceae and are reported to possess defensive effects (Mothes et al., 1985). Alkaloids are stored predominantly in actively growing young
tissues, roots, stem barks, flowers and seedlings. In the present study, the ethanolic extract of A. indica was reported to contain 13.6 mg 100 g$^{-1}$ of alkaloids (Table 4). Presence of alkaloids was reported in Cephaelis species (Nagalkura et al., 1993), Anthocephalus cadamba (Niranjan et al., 2000) and Cinchona sps. (Daniel, 2008).

5.1.5.2. Total Phenolics content

Phenolics are the most important secondary metabolites present in plant kingdom. These diverse group of compounds serve as potential natural antioxidant in terms of the ability to act as both efficient radical scavenger and as metal chelator. It has been reported that the antioxidant activity of phenols is mainly due to their ability to act as hydrogen donor, singlet oxygen quencher and their redox property (Narayanasamy and Ragavan, 2012). In the present study, the total phenolics content of ethanolic extract of A. indica was estimated as 72.1 mg TAE g$^{-1}$ extract (Table 4).

5.1.5.3. Tannin content

Tannins are water soluble plant polyphenolics which cause protein precipitation from aqueous solutions, located in vacuoles (Dey and Harborne, 1997). This group of compound has received a great deal of attention in recent years, since it was suggested that the consumption of tannin containing beverages especially green teas and red wines can cure or prevent a variety of illness. Tannins are complex moieties produced by most of the plants as protective agent with wide pharmacological activities. They have been shown to possess astringent, anti-inflammatory, antidiarrhoeal, antioxidant and antimicrobial properties (Suresh and Harinath, 2010).

In the present study, the tannin content of ethanolic extract of A. indica was recorded as 53.5 mg TAE g$^{-1}$ (Table 4). This is in line with the report of Kannan et al. (2009) in Rubia cordifolia.

5.1.5.4. Total Flavonoid content

Flavonoids are well known phytochemicals having the biological effects such as free radical scavenging, modulation of enzymatic activity, antibiotic and anti-inflammatory activities. It is reported that flavonoids are natural products which are shown to exhibit biological properties related to antioxidant activity (Shirwaikar et al., 2004 c).
The flavonoid content of the present study plant was found to be 24.9 mg RE g\(^{-1}\) (Table 4). This correlates with the report of Lopes et al. (2004) in Chiococca braquiata.

The findings of the present study indicated that the selected plant sample was reported to contain alkaloids, phenolic compounds, tannins and flavonoids which may contribute to the antioxidant activity of the ethanolic extract of A. indica. This result correlates well with the findings of Ashafa et al. (2010), who reported that the leaf extract of Felicia muricata had shown significant antioxidant activity due to the presence of secondary metabolites such as alkaloids, flavonoids and phenols.

5.1.6. TLC analysis

TLC serves as one of the many methods in providing a chromatographic plant extract finger print (Wagner and Bladt, 1996). Gabriela (2009) suggested that the colours of the spots in TLC and their position relative to standard substances are the two important characteristics for plant extract identification. The present study showed separation of two deep violet colour spots with R\(_f\) values 0.31 and 0.72 which may represent the presence of alkaloids in the selected plant extract (Table 5; Fig.1). Similar phytochemical analysis was carried out in plant drug (John De Britto et al., 2011).

5.1.7. IR Spectral analysis

The identification of an organic compound by the infrared technique is usually carried out by examining certain regions of the spectrum in a systematic way. The absorption peaks obtained in the region of 3000 – 2850 cm\(^{-1}\) are due to the presence of aliphatic CH vibration, the carbonyl stretching vibration at 1700 cm\(^{-1}\) due to the presence of ketones, aldehydes, acids, amides and carbonates and C-O-C stretching vibration in esters and ethers are found at 700 – 800 cm\(^{-1}\) (Sharma, 1995). In the present study, the IR spectral data given in Table 6 & Fig.2 showed C-H ,\(^{-}\)C=C\(^{-}\), N-H bend, C-C ,C-H rock, O-H bend and C-Cl stretchings which may be attributed to the presence of functional groups like alcohol, alkenes, primary amines, aliphatic amines and alkyl halides.

5.1.8. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS is a method that combines the features of Gas-Liquid Chromatography (GLC) and Mass Spectrometry(MS) to identify different substances within a test sample.
The phytochemical compounds present in the selected plant extract were identified by GC-MS analysis. GC-MS chromatogram of the ethanolic extract of *A.indica* showed the presence of 13 phytochemical constituents (Table 7; Fig. 3).

The major phytochemical compounds identified were Mome inositol with peak area % of 47.29% followed by (E)-1-(tert-butyldimethylsilyl)-4,4-dimethyl-2-penten-1-one (11.57%). The occurrence of various components in GC-MS analysis and their biological activities were studied by Hanbali *et al.* (2005) in *Pulicaria odora*, Lacikova *et al.* (2007) in *Staphylea* species, Aboutab *et al.* (2010) in *Macfadyena unguis-cati*, Mothana *et al.* (2011) in *Pterocarpus marsupium*, Ramalakshmi and Muthuchelian (2011) in *Tabebuia rosea* and Sermakkani and Thangapandian (2012) in *Cassia italica*. The presence of various bioactive compounds in the ethanolic extract of *A.indica* shown by the GC-MS analysis represented the phytopharmaceutical importance of the present study plant.

5.2. **In vitro antioxidant and free radical scavenging activity of *Acalypha indica***

Numerous experimental and clinical observations have exhibited that hyperglycemia may directly or indirectly contribute to an increased formation of free radicals and consequently the onset of oxidative stress in turn lead to diabetes associated complications (Mehta *et al.*, 2006). The Reactive Oxygen Species (ROS) are the common contributory factor in the development of diabetic complications and there are many evidences showing alterations in the antioxidant parameters during diabetes induced oxidative stress (Kakkar *et al.*, 1998). Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation (Andlauer and Furst, 1998; Gulcin *et al.*, 2002a).

5.2.1. **Ferric Reducing/Antioxidant Power (FRAP) Assay**

FRAP assay is widely used in the evaluation of the antioxidant component in dietary polyphenolics (Luximon – Ramma *et al.*, 2005). The reducing capacity of a compound may serve as a significant indicator of potential antioxidant activity. However the activities of antioxidants have been attributed to certain mechanisms namely chain initiation, decomposition of peroxides, reducing capacity and radical scavenging activity (Cook and Samman, 1996). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.
The present study showed a free radical scavenging activity of $186.05 \pm 2.9$ mmol (Fe II)/mg extract by FRAP assay. Antioxidant activity is found to be linearly proportional to phenolic content of the plant. Oktay et al. (2003) reported a strong positive relationship between total phenolic contents and antioxidant activity of plants. Similar observation was reported by Huda - Faujan et al. (2009) in methanolic extract of *Cosmos caudatus*.

From the present study, it is inferred that the phenolic compounds in the ethanolic extract of *A. indica* may act as an electron donor, thereby reducing the free radical generation.

**5.2.2. DPPH (1,1 – diphenyl, 2-picryl hydrazyl) radical scavenging activity**

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. DPPH assay is a simple and acceptable method to evaluate the antioxidative activity of compounds (Chen and Ho, 1995). DPPH is a stable free radical, which when encounters proton donors such as antioxidants, the radicals get quenched, absorbance gets reduced and thus used to measure the antioxidant activity of specific compound or plant extract (Koleva et al., 2002; Qureshi et al., 2010). The DPPH radical has been widely used to test the potential of compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts. The DPPH free radical scavenging activity of plant extract may be due to neutralization of DPPH radical either by transfer of hydrogen or of an electron (Shimada et al., 1992).

The results of the present study revealed an effective free radical scavenging activity of ethanolic extract of *A. indica* in the DPPH assay as shown in Table 8. This is in correlation with an earlier report of the Molan et al. (2012) in the ethanolic extracts of 13 medicinal plants of Northern Iraq, which showed significant scavenging activity towards DPPH radical and the activity is higher in ethanolic extract compared to water extract of the same plants. Further Dutra et al. (2008) revealed that the essential oil of *Pterodon emarginatus* seeds containing phenol showed DPPH scavenging activity with $IC_{50}$ value of 163.22 and the phenolic compound may reduce the oxidative stress which causes damage to carbohydrates, lipids, proteins and nucleic acids (Pryor et al., 2006). These evidences support the present study that the phenolic compounds of ethanolic...
extract of *A. indica*, make a significant contribution to the antioxidant activity in scavenging the DPPH radical.

**5.2.3. Hydroxyl radical scavenging activity**

Hydroxyl radical is one of the potent reactive oxygen species in the biological system (Halliwell and Gutteridge, 1981). The hydroxyl radical in the cells can easily cross cell membranes and react with most of the biomolecules and cause tissue damage and cell death. Hence, removal of hydroxyl radical is very important for the protection of living system (Yang *et al.*, 2008).

In the present study, the results of hydroxyl radical scavenging activity of ethanolic extract of *A. indica* exhibited in Table 9 which inferred that the ethanolic extract of *A. indica* may be considered as a good scavenger of hydroxyl radicals. If any plant extract or drug scavenges the hydroxyl radical, they may either scavenge the radical or may chelate the Fe$^{2+}$ ion, making them unavailable for the Fenton’s reaction. Plant extracts containing polyphenolics are reported to quench oxygen derived free radicals by donating a hydrogen atom or an electron to the free radicals or neutralizing the free radicals by their chelating ability (Kahkonen *et al.*, 1999).

The results obtained in the present study is in consonance with the previous reports of Hazra *et al.* (2008) in *Spondias pinnata* methanol extract. The present investigation also revealed that the ethanolic extract of *A. indica* is shown to be significant source of natural antioxidants, which are responsible for the hydroxyl radical scavenging activity.

**5.2.4. Superoxide radical scavenging activity**

Superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, superoxide radicals have their initial effects magnified because they produce other kinds of cell damaging free radicals and oxidizing agents (Halliwell and Gutteridge, 1999). Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen (Gulcin *et al.*, 2002a). Superoxide anion radical and hydrogen peroxide radical together react to form singlet oxygen and OH$^-$ radical, which are the most reactive oxygen species among all the ROS (Chakraborti *et al.*, 1990). It has the ability to react with several biological materials by
hydrogen withdrawal, double bond addition, electron transfer and radical formation and initiates auto-oxidation, polymerization and fragmentation. Hydroxyl radical can cause sugar fragmentation, base loss and leakage of DNA strands (Liu and Nig, 2000). Furthermore, superoxide anion radical and its derivatives can cause damage in lipids, proteins and DNA. Hence, it is of great importance to scavenge superoxide anion radical (Sathish et al., 2011).

The results presented in Table 10 showed an increase of percentage scavenging activity, which indicated the consumption of superoxide anion in the reaction mixture by ethanolic extract of A. indica. Superoxide scavenging ability of plant extract might be primarily due to the presence of flavonoids (Zheng and Wang, 2001). Flavonoids are effective antioxidants mainly because they scavenge superoxide anions (Robak and Glyglewsi, 1988). Based on the above results of the percentage scavenging capacity and IC<sub>50</sub> values, it was found that the ethanolic extract of A. indica is more effective in scavenging superoxide radicals that might be due to the presence of flavonoids. This result correlates with an earlier study of Olayinka et al. (2010) in Helichrysum longifolium aqueous extract.

5.2.5. Nitric oxide radical scavenging activity

Nitric oxide, a gaseous free radical and is relatively less reactive. But its metabolic product peroxynitrite, formed after reacting with oxygen is extremely reactive and directly induce toxic reactions such as SH group oxidation, protein - tyrosine nitration, lipid peroxidation and DNA modification (Moncada et al., 1991; Yermilov et al., 1995). Nitric oxide has been demonstrated to participate in the beta cell damage during STZ-induced diabetes (Duran Reges et al., 2004). Nitric oxide plays an important role in various inflammatory processes. Sustained level of production of this radical is directly toxic to tissues and contribute to vascular collapse associated with septic shock, where as chronic exposure of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Tylor et al., 1997). The toxicity of NO increases greatly when it reacts with superoxide radical, producing the highly reactive peroxynitrite (ONOO<sup>-</sup>) anion (Huie and Padmaja, 1993).
The results presented in Table 11 expressed a potent nitrogen scavenging activity of ethanolic extract of *A. indica* with increasing concentrations. It is evident from the previous study of Balakrishnan *et al.* (2009), that the NO scavenging activity of ethanolic extract of *Acalypha indica* roots increased with increasing concentration.

From the results of *in vitro* antioxidant and free radical scavenging activity of ethanolic extract of *A. indica* obtained in the present study proved that *A. indica* has significant antioxidant and free radical scavenging activity, which might be attributed to its flavonoids, phenolic contents and other phytochemical constituents. Hence, the selected plant extract may be used as therapeutic agent in preventing oxidative stress related degenerative diseases.

**5.3. Antihyperglycemic potential of ethanolic extract of *Acalypha indica***

The functional basis of Diabetes mellitus, can be elucidated by studying diabetes induced changes in metabolic enzymes. The variation in qualitative and quantitative nature may contribute to the pathogenesis of diabetes.

**5.3.1. Body Weight**

The body weight of STZ-induced diabetic rats was found to be reduced consistently from the 1st week to 4th week of the study as shown in the Table 12. The decrease in body weight may be due to malnutrition, caused by either mal digestion or mal absorption and impaired utilization of nutrients, which leads to significant weight loss (Leiber, 2000) or due to increased muscle wasting and loss of tissue proteins (Shirwaikar *et al.*, 2004b). The decrease in body weight was restored to near normal in diabetic rats treated with ethanolic extract of *A. indica*. This may be due to the effect of plant extract in controlling muscle wasting and preventive effect on degradation of structural proteins (Veeramani *et al.*, 2007). This report is in agreement with the results of Mallick *et al.* (2006) in the seeds of *Eugenia jambolana* and roots of *Musa paradisiaca* methanolic extract.

**5.3.2. Blood glucose, glycosylated haemoglobin, serum insulin and liver glycogen**

STZ – induced diabetes is linked to the production of Reactive Nitrogen Species (RNS) and Reactive Oxygen Species (ROS), which damage DNA of pancreatic β-cells
leading to decreased secretion of insulin from beta cells manifest in the serum insulin levels and cell necrosis (Szkudelski, 2001; Nam and Hyunjuc, 2008; Nirmala et al., 2008). In the present study, the fasting blood sugar levels were elevated 72 h after the injection of STZ and reaches a high blood sugar level of 297.85±4.53 mg dl⁻¹ at the end of the study period (Table13). STZ causes pancreatic β-cell injury and lack of insulin, which may be responsible for elevated blood sugar in experimental animals. Oral administration of ethanolic extract of A. indica reduced the blood glucose level within 7 days after the study period and reached to near normal values of 132.83±2.4353 mg dl⁻¹ and 118.29±7.3953mg dl⁻¹ (Group III and Group IV). This hypoglycemic effect of ethanolic extract of A. indica may be due to its protective role against pancreatic damage in diabetic rats and potential of insulin secretion from surviving β-cells of the islets of langerhans (Subha et al., 2004). The lowering effect of blood glucose level when treated with water extract of Uraria crinita was observed in diabetic rats (Xiao-ping Liu et al., 2010). The ethanolic extract of A.indica treatment containing 500mg kg⁻¹ body weight had shown better efficacy than that of 250 mg kg⁻¹ body weight in reduction of blood glucose level.

STZ-induced diabetic rats showed elevation in blood sugar and reduction in serum insulin levels (Table 14 & Figs.4 a & c). The reduction in the serum insulin levels in the STZ treated rats might be attributed to the reduced secretion of the hormone which might be due to the damage of the beta cells of endocrine pancreas. The STZ selectively destroys the pancreatic cells and induce hyperglycemia (Jarvenin, 1995; Kurup and Bhome, 2000). Similar studies recorded earlier indicated that the levels of serum insulin significantly reduced in STZ-induced diabetic rats STZ treated rats, (Yoon and Ray, 1985; Sivaraj et al., 2009). The efficiency of ethanolic extract of A.indica to reduce, the elevated blood sugar to normal glucose level is an essential trigger for the liver to revert its normal glucose homeostasis in experimental diabetic rats. The possible mechanism by which ethanolic extract of A.indica brings about its anti- hyperglycemic action in diabetic rats may be due to increased secretion of insulin from the existing β-cells or increased release of insulin from destroyed pancreatic β-cells. This may be possible either by regenerating the partially digested pancreatic β-cells or by the release of insulin stored in the granules. The hypoglycemic action mediated by the plant extract may be due to improvement of insulin sensitivity, glucose-dependent insulin secretion and regeneration
of islets of langerhans in pancreas of STZ – induced diabetic rats (Sezik et al., 2005). The extract containing 500mg kg\(^{-1}\) body weight exhibited a better reduction in glucose level and elevation of insulin level. Earlier, similar report indicated that the medicinal plants with hypoglycemic property may affect the circulating insulin level (Patel et al., 2009).

Elevation in glycosylated haemoglobin in blood was observed in diabetic rats, when compared to control rats (Table 14 & Fig 4b). Administration of ethanolic extract of \textit{A.indica} (250mg and 500mg kg\(^{-1}\) body weight) to diabetic rats significantly decreased the level of glycosylated haemoglobin indicated that the overall blood glucose level is controlled, which may be due to improvement in insulin secretion. Glycosylated haemoglobin is formed progressively and irreversibly over a period of time and is stable till the life of RBC and is unaffected by diet, insulin or exercise on the day of testing. Therefore glycosylated haemoglobin can be used as an excellent marker of overall glycemic control. Since it is formed slowly and does not dissociate easily, it reflects the real blood glucose level (Kameswararao et al., 2003). The present report correlates well with the study of anti-diabetic activity of \textit{Eugenia singampattiana} leaves on alloxan-induced diabetic rats (Mary Jebastinkala et al. 2012), which exerted a significant decrease in glycosylated haemoglobin in diabetic rats.

Glycogen content of liver was found to be reduced in diabetic rats (Table 14 & Fig.4d) which may be due to the lack of insulin in diabetic state. In the present study, oral administration of ethanolic extract of \textit{A. indica} improved the hepatic glycogen level in diabetic rats. This inhibition of glycogen depletion in liver tissue is possibly due to an increase in glycogenesis or a decrease in glycogenolysis or due to stimulation of insulin release (Chakrabarti et al., 2003). This effect is in agreement with the result of Daisy \textit{et al.} (2009) indicated that aqueous extract of \textit{Clitoria ternatea} enhanced the hepatic glycogen level in diabetic rats.

5.3.3. Serum Total protein, Albumin, Globulin and A/G ratio

Diabetic rats showed a sharp decline in serum total protein, albumin, globulin and A/G ratio (Table 15 & Fig. 5 a, b, c and d respectively). The condition of hypoalbuminemia observed in diabetes is generally attributed in the presence of nephropathy or may be due to increased protein catabolism or may be due to increased lipid peroxidation or
decreased production of ATP in absolute or relative deficiency of insulin (Almdal and Vilstrup, 1988; Chatterjee and Rana, 1994; Prakasam et al., 2004; Sivajothi et al., 2008). Insulin generally has an anabolic effect on protein metabolism in that it stimulates protein synthesis and retards protein degradation (Murray et al., 2000).

The decrease in serum protein and albumin levels may be due to microproteinuria and albuminuria may be due to impaired tubular reabsorption or leakage of albumin due to damaged glomerular membrane, which are the important clinical markers of diabetic nephropathy (Gomes et al., 1997).

Diabetic rats treated with ethanolic extract of A. indica for 28 days enhanced the level of serum protein, albumin and globulin. It is in consonance with the previous report in alloxan-induced diabetic rats (Preetha et al., 2012) when treated with mature coconut water (Cocos nucifera). Similar result had been noticed in Glibenclamide treated diabetic rats. Glibenclamide stimulates insulin release by inhibiting carnitine palmitoyl transferase 1 activity which switches fatty acid metabolism from β-oxidation to protein kinase C-dependent insulin exocytosis (Akira et al., 2007).

A/G ratio was lower in diabetic animals. Increased protein catabolism in diabetes might have induced a direct adverse effect on the secretion and the synthesis of albumin. Diabetic rats treated with A. indica extract brought back the A/G ratio to nearing normal levels. This could be due to the effect of A. indica on the improvement of oxidative phosphorylation process in cells. Similar improvement of serum A/G ratio was previously observed in streptozotocin- induced diabetic rats upon treatment with sinapic acid (Wilson et al. 2011).

5.3.4. Blood urea and Serum Creatinine

Urea is the major nitrogen containing metabolic product of protein metabolism. Creatinine is endogenously produced and released into body fluids and its clearance is measured as an indicator of renal function (Almdal and Vilsrup, 1988). Hyperglycemia stimulates elevation of the blood urea and creatinine in serum, which are considered as significant markers of renal dysfunction.
STZ-induced diabetic rats showed a significant rise in blood urea and serum creatinine level (Table 15 Figs. 5 e & f). Elevation of blood urea and serum creatinine in diabetic rats are due to the catabolism of protein, high activities of xanthine oxidase and lipid peroxidation which results in the formation of non-protein nitrogenous compounds such as urea and creatinine (Madinov et al., 2000; Wilson et al., 2011).

Treatment of diabetic rats with ethanolic extract of A. indica exhibited a significant decrease in blood urea and serum creatinine levels, which could be due to the inhibition of protein and nucleic acid degradation by ethanolic extract of A. indica in the present investigation. This observation is in agreement with the result of Rajiv Gandhi and Sasikumar (2012) in methanol extract of Merremia emarginata in streptozotocin-induced diabetic rats.

5.3.5. DNA and RNA content in liver and kidney

In diabetes, the Reactive Oxygen Species (ROS) damages cellular DNA and RNA through induction of DNA strand breaks leading to β-cell necrosis by itself or by activation of DNA repair mechanism (Santosh and Misra, 2001). DNA is an early target for oxidative stress, which could contribute to the cascade of pathogenesis of cells (Taurna and Parihar, 1998). The diabetic rats treated with A. indica in the present study brought back the reduced level of DNA and RNA content to raised level (Table 16 & Figs.6 a & b), which may be due to the action of phytochemicals in DNA destruction process and may be useful in minimizing its damage. This result is supported by Sivalokanathan et al. (2004) in ethanolic extract of Terminalia arjuna.

5.3.6. Glycolytic enzymes—Hexokinase and Phosphoglucoisomerase

Liver functions as a ‘glucostat’ and plays a vital role in the maintenance of blood glucose level. Hence it is of interest to examine the role of ethanolic extract of A. indica on the key enzymes of carbohydrate metabolism in liver. Liver is the main site for glycolysis, a process where glucose is degraded and gluconeogenesis, where glucose is synthesized from amino acids, lactate and glycerol. These are the two important events that balance the glucose load in our body (Bhavapriya and Govindasamy, 2000). The intracellular glucose has been utilized by insulin is several ways. The increased level of insulin influences the activity of gluconeogenic enzymes that results in the initiation of
hepatic glycolysis. All types of cells contain hexokinase. It plays an important role in the maintenance of glucose homeostasis, catalyses conversion of glucose to glucose-6-phosphate, regulates glucose storage and disposal in the liver (Ghosh, 1984).

The level of liver and kidney hexokinase and phosphoglucoisomerase were found to be decreased in diabetic rats (Table 17 & Figs.7 a & b). The significant decrease in the level of hexokinase was observed in diabetic rats may be due to the direct stimulation of glycolysis in tissues with increased glucose removal from the blood (Baquer et al., 1998). Administration of ethanolic extract of A. indica for 28 days significantly reversed these values to normal. This is in correlation with the report of Kishalay et al. (2012) in the hydromethanolic extract of Caesalpinia bonducella seeds on streptozotocin – induced diabetic rats.

Phosphoglucoisomerase (PGI) is a prime enzyme in glucose utilization. The conversion of glucose–6-phosphate to fructose-6-phosphate is reversibly catalyzed by the enzyme PGI. As compared with normal control values, the PGI levels were significantly reduced in diabetic controls in the present study and is in consonance with the earlier report of Arathi and Sachdanandam (2003) in Semecarpus anacardium. Decrease in the activity of PGI may be expected to inhibit the proportion of glucose-6-phosphate metabolized in the glycolytic pathway (Ebrahim et al., 1996) or the rate of transformation of glucose-6-phosphate to fructose-6-phosphate.

Oral treatment of A.indica extract elevated the activity of PGI in liver and kidney which may be due to the normalizing rate of glycolysis. Koti et al. (2011) have reported the similar result in diabetic rats treated with alcoholic extract of Plectranthus amboinicus.

5.3.7. Gluconeogenic enzymes-Glucose-6-phosphatase and Fructose 1,6 diphosphatase

Gluconeogenesis is a biochemical process almost completely restricted to the liver (Quistorff, 1985). Gluconeogenic enzymes convert the carbohydrate as well as non-carbohydrate precursors to glucose or glycogen and clear metabolic products of the other organs. Glucose-6-phosphatase and Fructose 1, 6 diphosphatase are the two important regulatory enzymes in the gluconeogenic pathway, which get altered during uncontrolled diabetes. Glucose-6-phosphatase catalyzes the conversion of glucose-6-phosphate to
glucose and provides hydrogen, which binds with NADP+ to form NADPH and promotes lipogenesis, the synthesis of fats from carbohydrates (Krebs and Woodford, 1965).

The activity of glucose-6-phosphatase and Fructose 1,6 -diphosphatase in liver and kidney were found to be significantly elevated in diabetic rats (Table 18 & Figs. 8 a & b). The elevation in the activity of gluconeogenic enzymes in the liver of the STZ- induced diabetic rats may be due to insulin insufficiency (Leloir et al., 1959).

Oral administration of ethanolic extract of A. indica for 28 days brought back the activity of the above enzymes nearing normal level. Significant reduction in the activity of glucose–6-phosphatase and fructose 1,6–diphosphatase after the oral administration of ethanolic extract of A. indica revealed that gluconeogenesis is inhibited in diabetic rats. This is in accordance with the results of Daisy et al. (2012) in the extracts of Cassia auriculata barks.

5.3.8. TCA cycle enzymes - Succinate Dehydrogenase and Malate Dehydrogenase

The functional basis of Diabetes mellitus to certain extent, can be elucidated by studying diabetes induced changes in metabolic enzymes such as Succinate Dehydrogenase (SDH) and Malate Dehydrogenase (MDH), the enzymes directly involved in glucose metabolism.

SDH is one of the most important marker enzymes of mitochondria. Its activity is generally higher than that of the other enzymes in both developing and adult animals. SDH is directly involved in the aerobic oxidation of food stuff. As it is tightly bound to the inner mitochondrial membrane, the oxidation of succinate to fumarate in animal tissues is linked to O2 via., cytochrome and cytochrome oxidase. MDH is an important enzyme of gluconeogenesis in the cytoplasm, where it converts malate to oxaloacetate which is then converted to phosphoenol pyruvate (Seema et al., 1996).

The decreased activity of SDH in diabetic condition, affecting succinate to fumarate conversion indicates the depressed oxidative metabolism. During stress condition, diversion of phosphoenol pyruvate leads to increased formation of fumarate resulting in product inhibition of SDH (Moorthy, 1983). The decrease in the activities of
SDH in tissues of diabetic rats may be associated with enzyme dysfunction due to activation of lipid peroxidation. This may be due to excess production of free radicals to counter these toxic effects.

Oral administration of ethanolic extract of *A. indica* for 4 weeks treatment increased the activities of the above enzymes nearing normal level (Table 19 & Figs. 9 a & b). It is in corroboration with the results of Lashin Ossama *et al.* (2006), in HNE modified SDH subunit in diabetic rat. The elevation in SDH activity after treatment with plant extract may be due to the presence of phytocompounds in it (Paneerselvam and Govindasamy, 2002) or better utilization of energy yielding intermediates by TCA cycle (Singh *et al*., 2001).

In the present study, the decrease in specific activity of MDH has been noticed (Table 19 & Fig. 9 b) as a consequence of diabetes indicates decreased utilization of malate (Cederbaum and Rubin, 1976). An increase in proteolytic activity during diabetes may also be responsible for the reduced MDH activity (Ianuzzo and Armstrong, 1976). The increased production of free radicals in mitochondrial cells and decrease in oxygen consumption respiratory ratio were observed in mitochondria of diabetic rats (Puckett and Reddy, 1979). It has been suggested that diabetogenicity of STZ is dependent on the inhibition of the activities of TCA cycle enzymes like malate dehydrogenase and \( \alpha \)-ketoglutarate dehydrogenase (Boquist *et al*., 1985). In the present study, the elevation of MDH activity in diabetic rats treated with plant extract may be due to the presence of phytochemicals which may have the capacity to reduce the oxidative stress and to increase the mitochondrial enzyme such as MDH. Earlier, similar results were observed by Saddala *et al.* (2012) in *Pimpinella tirupatiensis*.

### 5.4. Serum, Liver and Kidney Marker Enzymes

#### 5.4.1. ALP, ACP, AST and ALT activities

One of the most sensitive and dramatic indicators of hepatic injury is the release of intracellular enzymes such as transaminases and serum alkaline phosphatase in the circulation after STZ-administration (Sallie *et al*., 1991). The Soluble enzymes ALT / AST are released when injury involves organelles such as mitochondria (Senthilkumar *et al*., 2003).
STZ-induced diabetic rats showed a sharp rise in the level of ALP, ACP, AST and ALT in serum, liver and kidney tissues (Table 20 & Figs.10a, b, c & d and Table 21 & Figs. 11 a, b, c & d respectively). In diabetic animals, the changes in the levels of ALP, ACP, ALT and AST are directly related to changes in metabolism in which the enzymes are involved. The increased activity of transaminases, which are active in the absence of insulin may be due to the availability of amino acids in the blood of diabetes mellitus (Batran et al., 2006; Gokce and Hasznedaroglu, 2008) and are also responsible for the increased gluconeogenesis and ketogenesis. The increase in the level of serum, liver and kidney marker enzymes in diabetes may be due to the leaking out from the tissues and migration of these enzymes into the blood stream (Prince and Menon, 2000).

The decreased level of marker enzymes observed in plant extract treated diabetic animals may be due to the suppression of hepatic gluconeogenesis and glucose output from liver (Felig et al., 1970; Ghosh and Surawanshi, 2001). The results observed in the present study correlates with hydromethanolic extract of the seeds of *Caesalpinia bonducella* on STZ – induced diabetes in male albino rats (Kishalay et al., 2012).

### 5.4.2. LDH activity

Lactate dehydrogenase (LDH) is a terminal glycolytic enzyme that plays an indispensable role in the inter conversion of pyruvate to lactate to yield energy under anaerobic conditions (Kavanagh et al., 2004) and the reaction occurs in both cytosolic and mitochondrial compartments (Tabouy et al., 1998).

Elevated level of LDH noticed in serum, liver and kidney tissues of diabetic rats in the present study (Table 22; Figs. 12 a & b) may be associated with impaired glucose stimulated insulin secretion (Ainscow et al., 2000). Singh et al. (2001) reported that, in diabetic animals, the extreme accumulation of pyruvate may result in elevated LDH activity. In the presence of LDH, excess pyruvate is converted into lactate, leading to increased LDH activity, which could be attributed to the reduced insulin level in diabetic individuals.

Treatment with ethanolic extract of *A. indica* to diabetic rats reduced the LDH activity may be due to the regulation of pyruvate and NADH proportion, thereby promoting the mitochondrial oxidation of glucose (Palsamy and Subramanian, 2009).
5.5. Lipid profile in diabetes

5.5.1. Total and free cholesterol, Triglyceride, phospholipids and free fatty acids

Diabetes is associated with hyperlipidemia (Sperling and Saunders, 2000; Qureshi et al., 2009; Rasineni et al., 2010; Singh et al., 2011). Insulin activates the enzyme lipase in adipose tissue which hydrolyzes triglycerides under normal conditions (Bhagavan, 2002). But after administration of streptozotocin, destruction of β-cells leads to depletion of plasma insulin, which in turn depletes the activity level of lipoprotein lipase results in hyperlipidemia and hypercholesterolemia, caused by derangement of lipoprotein metabolic abnormalities (Maghrani et al., 2004).

In the present investigation, STZ-induced diabetic rats found to produce increased total cholesterol, triglycerides, phospholipid levels in serum, liver and kidney, (Table 23 & Figs.13 a, b, c & d and Table 24 Figs.14 a b, c & d, respectively) which correlates with earlier report of Venkateswaran et al. (2002) who indicated similar result as concerned with the increased level of phospholipids in streptozotocin – induced diabetic rats. Phospholipids are the main components of biomembrane rich in PUFA and are susceptible substrate for free radicals such as O₂⁻ and OH⁻ radicals (Ahmed et al., 2001). During diabetes, enhanced activity of the enzyme lipase increases lipolysis and releases more free fatty acids into the blood circulation (Agardh et al., 1999). The lipid changes associated with diabetes mellitus are attributed to increased flux of free fatty acids into the liver, secondarily to insulin deficiency / resistance (Solano and Goldberg, 2005; Chahil and Ginsberg, 2006). This results in accumulation of excess fatty acid in the liver which are then converted into triglycerides (Mooradian, 2009). On contrary, in the present study decreased levels of free fatty acids and free cholesterol had been noticed in liver and kidney tissues of diabetic rats. The diabetic rats treated with ethanolic extract of A. indica significantly altered the values closer to control (Group I) (Table 25 & Figs15 a & b) which coincides with the result of Rosa et al. (2011) in chloroform leaf extract of Azadirachta indica in streptozotocin – induced diabetic rats.

5.5.2 Lipoproteins

There are four types of lipoproteins depending on the type and concentration of fat they carry. They include HDL (High Density Lipoprotein), LDL (Low Density Lipoprotein)
VLDL (Very Low Density Lipoprotein) and Chylomicrons. In STZ – induced diabetes, the increase in glucose level is usually associated with an increase in plasma cholesterol, triglycerides, LDL, VLDL and decrease in HDL. The insulin deficient state turns into the activation of Hormone –Sensitive Lipase (HSL), which further leads to enhanced release of free fatty acids from adipose tissues. Thus excess of fatty acids in the blood produced by the STZ – induced diabetes promote the conversion of excess fatty acids into phospholipids and cholesterol in the liver. These two substances, along with excess triglycerides formed in the liver, may be discharged into the blood in the form of lipoproteins (Coppack et al., 1994; Garber, 2000; Ohno et al., 2000).

STZ –induced diabetic rats showed elevated levels of LDL-C and VLDL-C and reduction in HDL-C levels (Table 26 & Fig.16). In normal condition, insulin increases the receptor mediated removal of LDL-cholesterol whereas decreased activity of insulin during diabetes causes hypercholesterolemia (Bopanna et al., 1997). LDL plays an important role in arteriosclerosis and hypercholesterolemia. Increased LDL – cholesterol may arise from glycosylation of the lysyl residues of apoprotein-B as well as from decreasing affinity for LDL receptors (Golay et al., 1986). The impaired ability of insulin inhibits free fatty acid release which leads to the raise of hepatic VLDL-cholesterol production (Frayn, 2001).The increased VLDL-cholesterol and triglyceride levels decrease the HDL-cholesterol level and increase the concentration of small dense LDL-cholesterol particles by activation of lipase and lecithin-acyl cholesterol transferase (Mooradian et al., 2008). The mechanism responsible for the development of hypertriglyceridemia in insulin deficient STZ – diabetic rats may be due to a number of metabolic abnormalities that occur sequentially. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, resulting in increased secretion of VLDL–triglyceride from liver (Balasse et al., 1972). In diabetic rats, there is a decrease in lipase activity (Nikkila et al., 1977), resulting in impaired clearance of VLDL and chylomicrons from plasma (Bagdade et al., 1968).

The ethanolic extract of A.indica treated diabetic rats showed a reduction in LDL and VLDL cholesterol levels and an increase in HDL cholesterol levels. The lowering effect on serum lipids in the diabetic animals might be associated with a good glucose control by the plant extract. This might be due to the reduced hepatic triglyceride
synthesis and or lipolysis that might be due to the increase in serum insulin levels in the plant extract treated rats. The HDL increased significantly in the plant extract treated rats indicating a reversed atherogenic risk (Medvedeva et al., 2002; Sivaraj et al., 2009). The changes in lipid profile to normal level, could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics (Cho et al., 2002). Similar reports were observed in “Glyoherb” – a polyherbal formulation in streptozotocin – induced diabetic rats (Thakkar and Patel, 2010).

5.6. In vivo antioxidant potential and free radical scavenging activity of ethanolic extract of Acalypha indica

Free radicals and associated oxidative stress induced by STZ, elicited pathological changes in Diabetes mellitus. In recent years, several antioxidants of plant origin have been identified and used as protective agents against oxidative damage caused by free radicals. When there is an imbalance between free radical production and antioxidant defence, oxidative stress occurs resulting in deregulation of cellular functions (Bandyopadhyay et al., 1999; Resmi et al., 2006; Arulselvan and Subramanian, 2007; Ekor et al., 2010).

Many synthetic drugs protect against oxidative damage, but they have adverse side effects. An alternative solution to this problem is to consume natural antioxidants from food supplements and traditional medicines (Yazdanparast and Ardestani, 2007). Many natural antioxidants have been isolated from different plant materials (Packer and Ong, 1997; Jovanovic and Simik, 2000; Cheung et al., 2003; Parthasarathy et al., 2009). Antioxidants are compounds that prevent the oxidation of essential bio molecules by inhibiting the propagation of the oxidizing chain reaction (Kuo et al., 2005). The antioxidants are known to mediate their effect by directly reacting with ROS, quenching them and or chelating the metal ions (Robak and Marcinkiewicz, 1995; Lin et al., 2005).

5.6.1. Lipid Peroxidation (LPO)

Lipid Peroxidation is a characteristic of Diabetes mellitus. The increase of free radicals in diabetic condition may be due to the increased lipid peroxidation and damage of antioxidant defence system (Wolff, 1993). During diabetes, hypoinsulinemia increases the activity of fatty acyl coenzyme-A oxidase, which initiates β-oxidation of fatty acids,
resulting in lipid peroxidation. Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity and changes the activity of membrane-bound enzymes and glucose auto-oxidation can lead to the formation of free radicals which in turn can induce lipid peroxidation (Baynes, 1991).

The lipid peroxidation (LPO) levels were increased in STZ-induced diabetic rats (Table 27 & Fig.17). The increase in lipid peroxidation might be due to the increased generation of different radical species (Quinlan and Gutteridge, 1988). In diabetes, the increase in oxygen free radicals could be primarily due to an increase in blood glucose level, which upon auto-oxidation generate free radicals (Szkudelski, 2001). After supplementation with ethanolic extract of *A. indica*, LPO were found to be reduced in diabetic rats. In the present study, the ethanolic extract of *A. indica* may scavenge or inhibit the free radical formation and effectively prevent the damage of liver and kidney by reducing the causation of diabetes. These results are in agreement with the previous finding of Mansouriet al. (2011) in Grape seed proanthocyanidin extract.

5.6.2. Enzymatic antioxidants

Antioxidants are of two types – enzymatic and non-enzymatic antioxidants. Superoxide dismutase, catalase, peroxidase, glutathione synthetase, glutathione peroxidase and glucose-6-phosphate dehydrogenase are examples of enzymatic antioxidants. Superoxide dismutase and catalase are the primary enzymes as they are involved in the direct elimination of reactive oxygen species (Halliwell and Gutteridge, 1985; Gulcin et al., 2002 a, b). Earlier reports have demonstrated that oxidative processes results in the loss of key antioxidant enzymes. The damage brought about by oxidative stress is expected to be exacerbated, if the antioxidant enzymes themselves are damaged and inactivated by glycation – induced oxidative stress which ultimately resulting in the perturbation of cellular redox status (Shin et al., 2006).

Super Oxide Dismutase (SOD) is an important defence enzyme, which catalyzes the dismutation of superoxide radicals to oxygen and hydrogen peroxide (Mc Cord et al., 1976; Rathod et al., 2009). Catalase (CAT) is a hemoprotein, which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals (Chance et al., 1952; Jin et al., 2008). Glutathione synthetase, the most important bio
molecule protecting against chemical induced toxicity, participates in the elimination of reactive intermediates by reduction of hydroperoxide in the presence of Glutathione peroxidase (Meister, 1984; Nicotera and Orrenius, 1986; Ozdemir et al., 2005; Kumawat et al., 2009). Glutathione Peroxidase (GPx) a selenium containing enzyme present in significant concentration, detoxifies hydrogen peroxide to water through the oxidation of reduced glutathione (Bruce et al., 1982; Gonenc et al., 2006; Lee et al., 2007).

The activities of SOD, CAT, GPx, GR and GST in liver and kidney of STZ-induced diabetic rats were found to be significantly reduced (Table 28 & Fig.18 a & b and Table 29 & Figs.19 a, b & c respectively). The reduced activity of SOD and CAT of liver and kidney observed during diabetes may result in deleterious effects as a result of the accumulation of superoxide anion radicals and hydrogen peroxide (Searle and Wilson, 1980). The decreased level of glutathione synthetase observed in diabetic animals represents increased utilization resulting from oxidative stress (Anuradha and Selvam, 1993; Rahimi et al., 2005). Depression of glutathione peroxidase activity, observed in diabetic liver and kidney tissues had been shown to produce important adaptive response to increase peroxidative stress (Kinalski et al., 2000). A significant increase of SOD, CAT, GPx, GR and GST activity after the oral supplementation of ethanolic extract of A. indica, indicated that the extract may reduce oxygen free radicals and improve the activities of antioxidant enzymes (Tripathi and Chandra, 2010). The administration of extract improves impairments of SOD, GSH, GPx and CAT, suggesting that the extract prevents oxidative stress and acts as suppressor against liver cell damage and inhibits the progression of liver dysfunction induced by hyperglycemia (Sowmya and Rajyalakshmi, 1999; Lee et al., 2007).

Hepatic and kidney G6PDH activity was decreased in STZ-induced diabetic rats in the present study is in accordance with the earlier reports (Bugdayci et al., 2006; Karuna et al., 2010). The reduction of G6PDH in turn decreased the activity of NADPH, which is needed for the activation of the enzyme, aldolase reductase. As a result, glucose does not enter in to the pentose phosphate pathway in diabetic rats. The activity of G6PDH and NADPH/NADP ratio vary inversely in relation to blood glucose concentration. Studies of Diaz-Flores et al. (2006) inferred that reduction of hepatic G6PDH activity is depends on severity of hyperglycemia. As the G6PDH activity was
reduced in diabetes, the HMP shunt is unable to meet the requirement of NADPH for the enzymes that maintain Hb and GSH in reduced states. Further, the decline in the activity of G6DPH causes accumulation of glucose-6-phosphate, a potent glycosylating agent that may lead to GSH depletion and activation of glycation and final step of gluconeogenesis.

Oral administration of *A. indica* extract significantly increased the activity of G6PDH in diabetic rats which may be due to the supply of hydrogen that binds with NADP⁺ to produce NADPH, that in turn enhances lipogenesis ie, synthesis of fat from carbohydrates (Abdel-Rahim *et al.*, 1992) and finally reduced the plasma glucose levels. The findings were in similar line with the study of Ramesh and Pugalendi (2006), which revealed that Umbelliferone, a phenolic compound produced the same effect in STZ-induced diabetic rats.

The results clearly showed that ethanolic extract of *A.indica* possesses free radical scavenging activity, which could exert a beneficial action against pathological alterations caused by the presence of O₂⁻ and OH⁺. Similar findings were also observed in *Basella rubraa* in STZ–induced diabetic rats (Nirmala *et al.*, 2011a).

### 5.6.3 Non-enzymatic antioxidants

Non-enzymatic antioxidants can be divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants like Lipoic acid, Glutathione, L-arginine, Coenzyme Q₁₀, Melatonin, Uric acid, Bilirubin, Metal chelating proteins, Transferrin *etc* are the endogenous antioxidants produced by metabolism in the body (Droge, 2002; Willcox *et al.*, 2004). The nutrient antioxidants are the exogenous antioxidants which cannot be produced by the body, but provided through diet or supplements viz., trace metals (selenium, manganese and zinc), flavonoids, Omega-3 and Omega-6 fatty acids (Pham-Huy La *et al.*, 2008), vitamin E, vitamin C and vitamin A (Tiwari, 2001).

Non-enzymatic antioxidant defence system includes Ascorbic acid (Vitamin-C), α-tocopherol (Vitamin-E), β-carotene (Vitamin-A) and Glutathione (GSH). There is a balance between both activities and intracellular level of these antioxidants that are essential for the survival of an organism and their health (Raygani *et al.*, 2007). Glutathione or Reduced Glutathione (GSH) is a tripeptide, non-enzymatic biological antioxidant present in the liver which is important for maintaining the structural and
functional integrity of different organs (Ganie et al., 2010). GSH plays a common role in cellular resistance to oxidative damage as a free radical scavenger, as a protein-bound glutathione and by generation of ascorbic acid or tocopherol in liver (Mark et al., 1996).

Vitamin-C or ascorbic acid is an important water-soluble antioxidant reported to neutralize the oxidative stress (El-Gendy et al., 2010). Vitamin-C not only reacts with hydrogen peroxide, but also with $O_2^*$, $OH^*$ and hydroperoxides. It has an additional role in protecting or regenerating oxidizing carotenoids or tocopherols (Shao et al., 2008). Vitamin-E is a lipid-soluble antioxidant found in all plant parts and is a potential scavenger of ROS and free radicals (Ahmed, 2009) and it is the most effective chain-breaking antioxidant within the cell membrane and protects the membrane fatty acids from lipid peroxidation (Singh et al., 2004). Carotenoids (Vitamin-A) are efficient scavengers of free radicals (Di Mascio et al., 1989; Ho et al., 2007; Glauert et al., 2010) and have been shown to protect low density lipoproteins (LDL) against oxidation in vitro.

Reduction in the level of GSH, vitamin-C, vitamin-A and vitamin-E in liver and kidney tissues have been noticed in the present study (Table 30 & Figs. 20 a & b and Table 31 & Figs. 21 a & b). GSH level seems to be reduced in liver of diabetic rats which may be due to its increased utilization by the hepatic cells in order to counteract the increased formation of lipid peroxides (Gregus et al., 1996). However administration of plant extract restored the reduced level of GSH nearing normal level. The decreased level of vitamin-C in diabetic rats may be due to increased utilization against ROS and / or decrease in the non-protein thiols like GSH, as it is required for the recycling of vitamin-C (Jin et al., 2000). But treatment with ethanolic extract of A. indica in diabetic rats increased the level of vitamin-C in liver and kidney of diabetic rats, which may be expected to enhance the GSH level or stimulation of the system to recycle the dehydro ascorbic acid back to ascorbic acid. Decreased level of vitamin-A in liver and kidney may be due to more oxygen radicals in tissues, but the treatment with ethanolic extract of A. indica brought back the reduced level to normal level. This might be due to regeneration of vitamin-A from the radicals. There is a sharp decline in hepatic $\alpha$-tocopherol (Vitamin-E) in STZ-induced diabetic rats. This result suggested that the demand for the antioxidant vitamin-E is increased due to the activation of free radical related metabolism in diabetes.
(Higuchi, 1982; Kim, 2005). However treatment with ethanolic extract of *A. indica* restored the values nearing to control rats (Group I). Similar results were observed in supplementation of Rutin in streptozotocin – induced diabetic rats (Kamalakkannan and Prince, 2006).

The findings of the present investigation proved that ethanolic extract of *A. indica* possesses significant antioxidant and free radical scavenging properties. This may be helpful for developing new drugs from this plant for the management of diabetes and associated complications.

### 5.7. Histopathological Investigation

Diabetes mellitus is a syndrome resulting from a variable interaction and environmental factors and is characterized by depleted insulin secretion, hyperglycemia and altered metabolism of lipids, carbohydrates and proteins, in addition to damaged beta cells of pancreas and increased risk of complications of vascular diseases (Khan, 1991; Davis and Granner, 1996; Kamalakannan et al., 2003). A multitude of herbs and other plant materials have been described for the treatment of diabetes throughout the world (Kesari et al., 2005; 2006; Jayasri et al., 2008; Iweala and Oludara, 2011).

Histologically, liver section (Plate 3) of STZ-induced diabetic rats showed marked alteration in liver as a result of absence of insulin. The tissues exhibited mild sinusoidal dilation and congestion. It confirms that the degeneration of hepatocytes and necrotic cells are possibly associated with the generation of free radicals in the liver of diabetic rats (Packer et al., 2000) in addition to that apoptosis. (Sun et al., 2003; Vitaglione et al., 2004). This damage was partially reversed by the plant extract treatment in the present study, which correlates with the result observed by Ghosh and Surawanshi (2001) in *Vinca rosea* extract.

The histopathology report of kidney sections (Plate 8) of STZ-induced diabetic rats showed congestion of vessels (medulla), tubules showing fluid and no glomeruli while *A. indica* extract treated rats showed excellent recovery of renal functions which may be attributed to the regenerative capability of renal tubules by the selected plant extract. Similar result was shown by *Trigonella foenum graecum* treatment in alloxan- induced
diabetic rats (Thakran et al., 2004). The role of \textit{A. indica} in reversing the diabetic state at the cellular level besides the metabolic normalization further proves its potential as an antidiabetic agent.

The histopathological study of endocrine region of pancreas of diabetic rats (Plate 13) showed atrophy of islets. But after the treatment with selected plant extract (Plate 14 & 15) the pancreas revealed restoration of beta cells which corroborate the increased serum insulin levels. The results are in agreement with earlier reports of Ghosh and Surawanshi (2001) in \textit{Vinca rosea}, Gholamani et al. (2005) in fenugreek, onion and garlic and Dada et al. (2013) in \textit{Byrsocarpus coccineus} on alloxan – induced diabetes rats. The regenerative effect of the pancreatic cells by \textit{A. indica} may enlighten the positive effects of these agents on the production of insulin. This is in line with an earlier report of Hsu et al. (2009) in the water extract of \textit{Bidens pilosa} in STZ-induced diabetic rats.

Hayashida et al. (1983) observed reduction in population of beta cells as low as 50\% during diabetes but the supplementation of combined plant extracts revealed restoration of size of the islets along with beta cell repair.

The histopathological study of liver, kidney and pancreas showed that the ethanolic extract of \textit{A. indica} was found to improve the function of liver, kidney and pancreas effectively and reduced the lesions associated with diabetic state in STZ – induced diabetic rats. Furthermore, the effect of oral supplementation of 500 mg kg\(^{-1}\) body weight showed more efficacy than 250 mg kg\(^{-1}\) body weight treatment.

The results obtained from the present study revealed that the ethanolic extract of \textit{Acalypha indica} possesses antihyperglycemic properties due to \(\beta\)-cell restoration against STZ-induced damage. Reduction of marker enzymes of liver and kidney showing the organ protective mechanism of \textit{A. indica} in diabetic condition and strengthening of the antioxidant potential in diabetes mellitus. Hence the present study plant \textit{A. indica} merits its role as an adjuvant in the management of diabetes mellitus and its associated complications.