CHAPTER 5. DISCUSSION

DM is a chronic disorder of carbohydrate, fat and lipid metabolism associated with long term microvascular and macrovascular complications. Complications like diabetic nephropathy, neuropathy, retinopathy and cardiomyopathy result from substantial disturbances in metabolism and cellular homeostatic mechanisms occurring due to lack of insulin or insulin resistance.

One of the common complication of DM, diabetic neuropathy has been found most difficult to prevent and manage as it starts very early in the course of DM and the damage is irreversible in nature (Boulton et al., 2005). UK prospective Diabetes Study (UKPDS) and Diabetes Control of Complications Trial (DCCT) has established that, Diabetic nephropathy is the leading cause of end stage renal disease. However, aggressive treatment of microalbuminuria may prevent progression to frank macroalbuminuria (Bakris et al., 2007). It was also proposed that protection from macrovascular complications requires a multifactorial approach with tight management of blood glucose, blood pressure and lipid levels (Vithian and Hurel, 2010). Several other studies have shown the importance of reducing oxidative stress (Giacco and Brownlee, 2010), insulin resistance (Groop, Forsblom and Thomas, 2005) and inflammation (Kaul, 2013) as important targets for prevention of complications of DM. This makes it apparent that a drug should encompass a blend of effects to be effective in preventing development of diabetic complications.

Herbal drugs have more than one constituents with varied effects and mechanisms making them suitable for management of multifactorial diseases like DM. Several herbal drugs are under study for their potential use in such multifactorial diseases. Our present study was aimed at the evaluation of AA in diabetes and its complications as it is traditionally used in treatment of DM and has been reported to have several beneficial effects like antidiabetic, anti-oxidant, anti-inflammatory and neuroprotective activity.
In the present study, we explored the pharmacological effects of methanolic extracts of AA on STZ and Fructose+ STZ induced DM and diabetic neuropathy, nephropathy and cardiomyopathy.

Insulin resistance is the key feature that differentiates T1DM from T2DM. We assessed the insulin sensitivity in both animal models used to confirm the type of pathology they develop.

Single high dose STZ i.p. injection in rats is known to develop a disease similar to T1DM by damaging beta cells of pancreas. It is a glucose and N-acetyl Glucosamine (GlcNAc) analogue, which enters the beta cells of the pancreas via GLUT 2 transporter. It damages beta cells via three mechanisms:

i) DNA methylation and fragmentation  

ii) NO production  

iii) Free radical generation.  

iv) NO production and free radical generation increase oxidative stress and cause cell death (Eleazu et al., 2013)

T1DM is characterized by pancreatic beta cell death and insulin deficiency with normal insulin sensitivity. The mechanism of cell death in T1DM is autoimmune or infectious in nature. However, STZ induced DM replicates the pathology of T1DM most closely. Thus, it is believed to induce T1DM in animals. On the other hand, several studies have demonstrated that fructose administration causes development of insulin resistance (Zavaroni et al., 1980; Martinez, Rizza and Romero, 1994; Thorburn et al., 1989) and hyperinsulinaemia. Fructose enters the cell via GLUT 5. Expression of these transporters is independent of insulin. Thus, in contrast to glucose intake, fructose intake in body does not evoke release or insulin. Fructose serves as an unregulated source of glycerol-3-phosphate and acetyl-CoA for hepatic lipogenesis, thus it is highly lipogenic. These effects of fructose combined with beta cell damaging effect of STZ are believed to induce a syndrome similar to T2DM.

This is confirmed with our observations in an insulin tolerance test done on both types of induced DM animals. The insulin sensitivity index (K_{ITT}) of STZ injected animals was > 1.5%/min indicating normal insulin sensitivity and a model of T1DM. Animals with fructose + STZ induced DM, demonstrated K_{ITT} < 1.5%/min i.e. reduced insulin sensitivity.
Discussion

or had insulin resistance. Thus, we considered this model for assessment of effects of AA in T2DM. Animals used in present study also developed same pathologies as confirmed by lower insulin sensitivity and HOMA IR values.

All STZ injected animals demonstrated high plasma glucose levels at 0 day, while both AA extracts could reduce this hyperglycemia significantly which was found to be similar to standard treatment with insulin. Studies by Manosroi et al and Hemamalini et al have also shown hypoglycemic activity of AA aqueous and Methanolic extracts, respectively in alloxan induced DM in rodents (Manosroi et al., 2011; Hemamalini and Vijusha, 2012). The diabetic animals in the present study developed classical signs of diabetes such as loss of body weight, hyperphagia and polydipsia. AA treatment significantly prevented these signs, indicating a better control of the disease.

Oxidative stress is generated in state of hyperglycemia via different pathways like the hexosamine pathway, mitochondrial electron transfer system and by the Amadori compounds. This oxidative stress further reduces insulin secretion and increases insulin resistance. It also promotes disease progression and target organ damage (Kawahito, Kitahata and Oshita, 2009). Lipid peroxidation is an important mechanism that results in cell membrane damage and induction of apoptosis or necrosis. Malondialdehyde (MDA) is one of the end product and marker of lipid peroxidation (Ayala, Muñoz and Argüelles, 2014). In line with this fact, our diabetic control animals also developed severe state of oxidative stress as indicated by an increase in serum MDA levels. However, treatment with AA extract could efficiently suppress the lipid peroxidation in diabetic animals. Glutathione is a tripeptide present in all cells of the body as a constitutive defense mechanism against oxidative damage and decrease in GSH levels is indicative of oxidative stress in the tissue. In the present study also diabetic control animals have demonstrated significantly lower reserves of reduced glutathione, while that in AA treated rats was higher than DC rats. Catalase is the enzyme which breaks down the highly damaging hydrogen peroxide into water and oxygen. Decreased catalase levels may result in increased damage to various proteins, lipids and DNA (Tiwari et al., 2013; Asmat, Abad and Ismail, 2016). In the present study, catalase levels of untreated diabetic rats were found to be significantly lower than the normal control animals. This indicates a disturbed antioxidant system of the animals. Treatment with AA extracts could prevent such depletion of catalase in a dose dependent manner. It would be interesting to note that, AA
bark extract has been reported previously to possess potent anti-oxidant activity probably attributable to its high phenolic content. Several constituents present in AA, like, gallic acid, quercetin, pterostilbene, flavano-ellagitannins etc. are proven antioxidants. Moreover, AA has also demonstrated potent antioxidant activity in in vitro assays (Moses, Manosroi and Manosroi, 2009; Manosroi et al., 2011). Such strong antioxidant activity is a desirable feature for an anti diabetic agent as oxidative stress is a common pathway leading to cell death after different cell damaging stimuli.

Diabetic neuropathy (DN) is the most common type of diabetic complication occurring in 60-70 % of DM patients in different forms. It results from endoneurial capillary dysfunction, nerve injury resulting in demyelination, apoptosis or necrosis of peripheral nerves, including cranial nerves, spinal nerves and their branches. STZ induced DM results in peripheral neuropathy with early signs such as allodynia and hyperalgesia, while later symptoms of DN include slow nerve conduction velocity, hypoalgesia, motor incoordination, degeneration and demyelination of nerves and loss of epidermal nerve fibers.

Hyperalgesia to thermal nociceptive stimulus occurs between the duration of 2-6 weeks after induction of diabetes (Somani and Shaikh, 2010). Persistent hyperglycemia leads to dysfunction of various local cytokines, leading to enhanced inflammatory effects of these cytokines. Such sub-clinical inflammation and oxidative stress at peripheral nerves lead to increased nociceptive response to various stimuli. The same phenomenon was observed with our STZ induced diabetic rats when they were tested for thermal nociception. This indicates that those animals had ongoing neurological damage associated with DN. However, animals treated with AA extracts resulted in preserved nociception in a dose dependent manner. Insulin treatment also showed a better nociceptive function, albeit less significantly than AA response. More significant effect of AA than Insulin on neuropathic parameters indicates accessory beneficial effects exerted by AA extract on the nervous system. Protection from oxidative damage, inflammatory mediators, apoptotic signals, polyol mediated damage may be some of the beneficial effects produced by AA extract responsible for its protection against diabetic neuropathy.

In addition to this, chemical allodynia to hind paw injection of 0.2% formalin worsens in STZ diabetic rats between the duration of 4 to 8 weeks (Freshwater and Calcutt, 2005; Joshi and Honore, 2006). This was also observed in our study in which diabetic control rats
showed significantly elevated number of hind limb flinches i.e. paw scrapping and paw licking response as compared to normal non diabetic animals. Treatment with AA leaf as well as bark extract could prevent such allodynia as indicated by their significantly lower number of limb flinches. Several antioxidant agents have shown to be effective in preventing neuronal damage and loss in sensory function (Ametov et al., 2003). Oxidative stress causes microvascular impairment leading to decrease in nerve blood flow, resulting in endoneurial hypoxia, and stimulation of inflammatory processes in the neurons. The strong anti-oxidant effect of AA as discussed earlier may be responsible for its protective effect against DN. Moreover, AA has also shown to possess neuroprotective, NO scavenging and COX 2 inhibitory activity in a rat model of transient focal cerebral ischemia (ArunaDevi et al., 2010). Anolignan B found in AA has also shown to possess anti-inflammatory and COX inhibitory activity (Eldeen et al., 2006). This suggests that these actions in addition to hypoglycemic effect may be responsible for its ability to prevent hyperalgesia and allodynia in peripheral neurons. Advanced Glycemic End product formation also plays an important role in the development of neuropathy. Vescalagin -an ellagitannin dimer present in AA has been shown to inhibit AGE formation in metabolic disorder in rats (Chang, Shen and Wu, 2013). Quercetin present in AA is a highly potent antioxidant and has shown neuroprotective action (Costa et al., 2016).

Gastroparesis and constipation are common symptoms and consequences of autonomic neuropathy. Abnormalities of cholinergic neurons controlling gut motility results in reduced gut motility as indicated by less distance travelled by the orally administered charcoal meal in this test. In the present study, the untreated diabetic rats exhibited significantly lower charcoal meal transit as compared to normal rats, indicating presence of autonomic neuropathy. However, rats treated with AA extracts as well as insulin demonstrated significantly higher transit of charcoal meal, indicating a preserved neuronal function of enteric nervous system by this treatment.

Hyperglycemia in untreated diabetic rats in present study resulted in reduced nerve conduction velocity. Insulin and all AA treated groups exhibited a significant improvement of NCV as compared to diabetic control rats. However, animals treated with 300 mg/kg of leaf extract had the most significant improvement which was concomitant with its highest antihyperglycemic action. Hyperglycemia results in entry of excessive glucose in Schwann cells of nerves. Such accumulated glucose activates aldose reductase pathway leading to formation and accumulation of sorbitol and fructose. This also results in depletion of myo-
inositol thus compromising glutathione cycle and activity of sodium potassium ATPase pump. Na⁺/K⁺ ATPase plays a vital role in restoring the balance of sodium and potassium on either sides of the neuronal membrane. These disturbances hamper the impulse conduction via neuron, changes being more prominent in long nerves like sciatic nerve. Castalagin -an ellagitannin present in AA has demonstrated AGE formation and aldose reductase inhibitory activity (Zhang et al., 2014).

Persistent hyperglycemia and other pathological factors like ROS, glycation of proteins, lipids and vital molecules of the cell, inflammatory cytokines etc. cause substantial damage to kidney. Such changes in diabetic kidney result in loss of nephrons and therefore, decreased formation of urine. In addition to this, glomerular basement membrane thickening, glomerulosclerosis and increase in mesangial matrix deposition also results in loss of renal function. In our study, the urine volume was higher in diabetic animals owing to osmotic effect of hyperglycemia, when measured at 2 weeks after development of DM. However, the urinary volume at 8 weeks was significantly lower as compared to normal animals, indicating renal damage and loss of excretory function. Treatment with Insulin and AA extracts could restore the abnormalities in urine volume at both stages, the most significant effect being that of 300mg/kg dose of leaf extract of AA.

Proteinuria is considered to be the gold standard for screening and staging of diabetic renal damage (De Zeeuw et al., 2006). Not only glucose toxicity but also the low grade inflammation of renal tissue lead to renal tubular damage as well as alteration of glomerular filtration barrier, loss of glomerular charge selectivity (Nakamura and Myers, 1988; Pietravalle et al., 1991; Morano et al., 1993) and loss of glomerular size selectivity (Deckert et al., 1993; Torffvit and Rippe, 1999; Gall et al., 1994). Diabetic nephropathy progresses from microalbuminuria to macroalbuminuria, and finally to End Stage Renal Disease (ESRD). Microalbuminuria is also a strong predictor of cardiovascular disease in patients with diabetes (Ruggenenti and Remuzzi, 2006; Dinneen, 1997). Diabetic control animals in our study developed prominent proteinuria. However, AA treatment could prevent it at both the dose levels. This indicates a protective effect of AA against renal glomerular and tubular damage.

Loss of excretory function of kidney results in accumulation of waste products in the blood. Serum creatinine and Blood Urea Nitrogen are sensitive markers of renal function. Diabetes in untreated animals in the present study, resulted in significant increase in serum
creatinine and BUN levels indicating a worsening of kidney function, in concordance with the reduced urine volume at 8 weeks. Treatment with both extracts of AA could reduce both markers, demonstrating the protective effect of AA on diabetic nephropathy.

Tubulointerstitial fibrosis and glomerulosclerosis are prominent signs of nephropathy in diabetic patient. These changes result from deposition of myofibroblasts and extracellular matrix in the diabetic kidney. Such fibrous tissue deposition occurs due to stimulation of various growth factors such as PDGF, TGF-β and IL-1 owing to ongoing inflammation. Such fibrosis and inflammation results in increased relative kidney weight in animal with diabetic renal disease, which was also observed in our diabetic control animals. AA treatment could reduce the kidney hypertrophy and inflammation as indicated by lower kidney weight ratios. Quercetin present in AA has shown protective effect on diabetic nephropathy (Gomes et al., 2014).

Fructose and STZ injected model in our study represented Type 2 DM as confirmed by Insulin tolerance test discussed earlier. Diabetes in these animals resulted in increased food intake more prominently in early phase of diabetic state. However, the increase in food intake does not proportionately increase the body weight of animal. This may be due to lack of anabolic action of insulin on protein metabolism, which also results in muscle wasting and increase in adipose tissue mass. However, due to loss of muscle mass the body weight of animals decreases. In our study also there was a continuous loss of body weight over a period of 12 weeks in diabetic control group of animals. However, AA treatment could prevent such weight loss in treated animals and a normal pattern of increase in body weight was observed, which indicated overall better health of these animals. Diabetic animals also demonstrated an increase in water intake due to hyperglycemic state. The animals treated with AA presented with less polydipsia, which was proportionate to their plasma glucose levels. Induction of T2DM in animals resulted in very high increase in plasma glucose levels. AA treatment could reduce the plasma glucose levels in a dose dependent manner. Glycated haemoglobin levels were accordingly high in untreated diabetic animals indicating persistently elevated levels of plasma glucose in these animals. Higher doses of AA could bring down the HbA1C level to a significantly lower value. This supports the anti-hyperglycemic action of AA extracts in T2DM. However, as the average life span of RBC is 120 days and animals were tested on 77th day of induction of DM, haemoglobin glycated during this day of DM induction can not be fully reversed until
nearly 120 days. Thus, even though lower doses of AA could significantly reduce plasma glucose levels, they were not able to reduce the HbA1C value significantly.

In the condition of insulin resistance and elevated lipid levels, there is an increased lipolysis leading to release of free fatty acids from adipose tissue (Volkova and Deedwania, 2007). Moreover, there is an increased use of fatty acid as energy substrate in diabetic hearts. However, fatty acid uptake of heart exceeds this utilization and results in accumulation of lipids in the heart leading to lipotoxicity and apoptosis of cardiomyocytes (Zhou et al., 2000). Dyslipidaemia has been the major predictor of cardiovascualr adverse events in diabetic patients. Metabolic syndrome in diabetic rats lead to elevated total cholesterol, triglyceride, LDL levels and decrease in HDL levels. AA treatment was able to reduce the lipid levels and increase HDL levels, indicating a beneficial effect on cardiovascular risk of diabetic animals.

Treatment with AA demonstrated a highly significant reduction in oxidative stress in the diabetic animals, in contrast to glibenclamide treatment which produced only nonsignificant changes in oxidative stress parameters. AA extracts were found to be rich in phenolic compounds, which are known to be strong antioxidants. Increased production of ROS and reduced cellular antioxidant mechanisms contribute to myocardial damage, apoptosis and then remodeling. Moreover, it also leads to disturbances of myocardial energetic leading to loss of efficiency of myocardium. Thus, oxidative stress is a major factor contributing in development of diabetic cardiomyopathy. Thus, its anti-oxidant mechanism may be one of the factor responsible for its protective effect against cardiomyopathy in DM.

DC rats had higher insulin levels as compared to normal rats. However, higher levels of insulin in DC rats were insufficient when seen in proportion to the plasma glucose level in these animals. This may be due to exhausted beta cell function resulting from insulin resistance and glucose toxicity. AA treatment reduced the insulin levels. Although, no significant difference was found in insulin levels between control and treated groups, it reveals a beneficial effect on sensitivity of available insulin and functioning of beta cells when seen in conjugation with glucose levels. Studies by Manosroi et al., 2011, Hemamalini et al., 2012 and Zaruwa et al, 2015 also demonstrated anti diabetic effect of methanolic or aqueous extracts of AA in alloxan induced DM. However, our study is the first to demonstrate the effectiveness of AA in animal model of T2DM. We determined the
effect of AA treatment on insulin resistance (IR) and beta cell function using HOMA method. AA treatment could significantly reduce the IR in diabetic rats supporting its effectiveness in T2DM. Other plants rich in flavonoids like *Prunus dulcis* (Mill.) D.A. Webb (Sendrayaperumal, Pillai and Subramanian, 2014), *Eriodictyon californicum* (Hook. & Arn.) Torr (Zhang et al., 2012), *Cochlospermum vitifolium* (Willd.), oranges etc (Mulvihill et al., 2009) have also demonstrated IR lowering effect, suggesting the contribution of rich phenolic content of AA to be responsible for its antidiabetic effect. Quercetin, a flavonoid present in AA demonstrated a flavonoid effect and improved insulin sensitivity (Yan et al., 2015).

To further assess its efficacy on insulin resistance, we assessed PTP1B inhibitory activity of AA extracts *in vitro*. Both AA extract demonstrated a significant inhibition of PTP1B enzyme, which is the negative regulator of insulin sensitivity. Thus, reduction of IR mediated via PTP1B inhibition may be one of the mechanisms of antidiabetic action of AA. Apart from IR, AA extract also significantly increased the beta cell function of diabetic rats as demonstrated by HOMA β values. This indicates that it has either preserved the beta cell viability or has insulin secretogogue action or both. It is a known fact that long term hyperglycemia exerts glucose toxicity on pancreatic beta cell leading to cell death, the mechanism responsible for secondary failure of several oral hypoglycemic therapies in T2DM patients. Vescalagin present in AA has been shown to prevent β cell death by inhibiting cytokine and TNF α release (Chang, Shen and Wu, 2013). The effective blood glucose control and strong antioxidant property of AA may be pivotal in prevention of such glucose toxicity and beta cell death in treated animals. However, it is possible that it may also have insulin secretogogue activity as several phenolic compounds present in AA, like, pterostilbene (Manickam et al., 1997), gallic acid (Sameermahmood et al., 2009), quercetin (Youl et al., 2010), have previously demonstrated the insulin secretogogue action.

T2DM induced by fructose and STZ resulted in classical metabolic disorder developing not only hyperglycemia but also severe oxidative stress in the animals as was evident from the reduced glutathione and catalase levels and increased MDA levels in the diabetic rats. It is apparent that AA extracts owing to their phytoconstituents could alleviate this oxidative stress. AA treatment increased the levels of catalase and glutathione, while reduced the levels of MDA indicating reduced lipid peroxidation. However, as discussed earlier, potent antioxidant activity of AA has been previously demonstrated in *in vitro* and *in vivo* studies.
(Moses et al, 2009, Manosroi et al., 2011). But its antioxidant activity in T2DM presents a potential benefit over the conventional treatments (like Glibenclamide, which has shown little protection against oxidative stress in present model), which do not interfere with disease progression process, resulting in loss of pancreatic beta cell viability and target organ damage on long term.

Insulin resistance is a cause of dyslipidemia and elevated TG, FFA levels also promote insulin resistance. Both insulin resistance and dyslipidemia are major underlying abnormalities leading to cardiovascular disease. Thus it is clear that control of dyslipidemia is also a modality for prevention of cardiovascular disease (Ginsberg, 2000). Treatment with AA extracts could significantly improve the lipid levels of the animals. It could reduce levels of TG, Total cholesterol, and LDL, and increase levels of HDL in a significant manner at 2 and 12 weeks. Hypertriglyceridemia is a characteristic feature of disease induced by fructose. Our animals also developed severe elevations in TG levels. TG accumulation, so-called lipotoxicity is detrimental for pancreatic β-cells where it reduces glucose dependent insulin release and promotes apoptosis of these cells (Yang and Li, 2012). Pterostilbene a lignin present in AA has been found to possess hypolipidaemic activity via activation of peroxisome proliferator-activated receptor alpha (PPAR α) receptor (Rimando et al., 2005). One of the ellagitannin, Vescalagin found in the AA is known to possess hypotriglyceridemic action (Shen and Chang, 2013). Flavonoid Quercetin present in AA is also known for its hypolipidemic action (Yan et al., 2015).

Serum Lactate dehydrogenase and CK-MB are sensitive biomarkers of cardiac damage (Fontes, Goncalves, and Ribeiro, 1999). LDH is released as a result of myocardial tissue necrosis, and correlates with the extent of myocardial damage. Both LDH and CK-MB are found to be increased in diabetic cardiomyopathy (Hall, 1991). In present study also there was significant increase in both enzymes at 12th week of DM induction, thus indicating profound myocardial damage, however, their levels in AA treated groups were significantly lower indicating a protective effect on heart.

Diabetes in present animals also resulted in increase in mean blood pressure and decrease in heart rate, indicating the condition of cardiomyopathy in animals. However, there was no significant difference in force of contraction observed between the groups. Mean blood pressure was significantly reduced in animals treated with higher doses of AA. Elevated blood pressure is a prominent risk factor for coronary artery disease (McInnes, 1995) and
also for cardiomyopathy (Boudina and Abel, 2010). It indicates increased burden on heart, which may result in cardiac hypertrophy and/or ischaemic heart disease (Hayat et al., 2004). Thus, ability of AA to reduce mean blood pressure in diabetic rats indicates a beneficial effect on cardiovascular system of these rats. One of the complex tannin Castalagin present in AA has been shown to possess hypotensive effect in spontaneously hypertensive rats (Lin, Hsu and Cheng, 1993). Thus, it is possible that effect of AA on blood pressure may be due to its complex tannin content. AA also could prevent the bradycardia associated with diabetic cardiomyopathy as reflected by significantly higher heart rate in AA treated groups as compared to diabetic control animals, indicating a preserved cardiac function.

Experimentally induced diabetes has been shown to cause an increase in accumulation of protein and collagen formation. This causes structural and functional changes in the cardiac tissue. (Regan et al., 1981). Moreover, deposition of collagen in left ventricular interstitium causes myocardial fibrosis and stiffness resulting in cardiac dysfunction (Nagai et al., 1988; Weber, 1994). Several studies have indicated that fibrous tissue formation and accumulation of collagen increases the left ventricular mass in diabetic rats (Biernacka and Frangogiannis, 2011). Biopsy of diabetic human hearts has also supported the finding observed in rat hearts (Shimizu et al., 1993). Cardiac and left ventricular hypertrophy has shown to be represented by heart weight to body weight ratio and left ventricular weight to heart weight ratio respectively (Balakumar and Singh, 2006). Treatment with AA extracts could attenuate both cardiac and left ventricular hypertrophy significantly, indicating a preventive effect on cardiac remodeling. Quercetin, one of the flavonoid present in AA could prevent cardiac fibrosis in a study by inhibiting overexpression of transforming growth factor β1 (TGF-β1), connective tissue growth factor (CTGF), and excessive deposition of extracellular matrix (ECM) (Li et al., 2013). Thus, it is possible that effect of AA may be at least in part be attributable to its quercetin or other flavonoid content.

Consistent with this supposition, the phytochemical analysis of AA extract shows presence of tannins, Flavonoids, alkaloids, saponins and proteins or amino acids. It is a well known fact that Combretaceous plants are rich in tannin and flavonoid content. AA extracts demonstrated a high tannin and flavonoid content; therefore it was quantified using standard methods. Both leaf and bark extracts demonstrated good amount of tannins and flavonoids. However, leaf extract had very high amount of tannins, while bark extract
showed presence of high amount of flavonoids. This was further supported by result of HPTLC fingerprinting analysis. Gallic acid and quercetin were used as markers. One of the peak of leaf extract corresponded with gallic acid peak, while one peak of bark chromatograph matched with quercetin peak. This, indicated that leaf had gallic acid in addition to other constituents, while quercetin was one of the constituent of bark extract. There were several peaks which were more prominent and common in both extracts. These may be other constituents potentially responsible for potent pharmacological actions observed in the present study. Several studies have revealed presence of unique complex tannins (flavano-ellagitannins) like, acutissimin A and C, eugenigradin etc. in AA in addition to simple tannins like gallic acid, ellagic acid etc. Ellagitannin dimers like castamollinin, anogeissusin A and B, anogeissinin, castalagin, castalin, vescalagin carboxylic acid, grandinin are also found to be present in AA. Apart from this various lignans like pterostilbene, anolignan A, B and C, secoisolariciresinol and leiocarpan are also present in AA. Several compounds out of this have demonstrated varied pharmacological actions. This justifies the beneficial effects of AA extract on T1DM, T2DM, neuropathy, nephropathy and cardiomyopathy. Anti-oxidant effect of tannin rich plants is most potent among all pharmacological activities. Our results of in vitro anti-oxidant tests done with AA extracts confirm this notion. Both leaf and bark extracts demonstrated potent DPPH radical scavenging activity. DPPH is a colored and relatively stable free radical used in this assay. DPPH free radical reacts with reducing agent and loses its color. Thus, discoloration of reaction mixture is proportionate to the reducing power of the analyte. AA extracts showed potent activities with EC50 values comparable to that of Vitamin C. Our results are in agreement with results of study done by Manosroi et al in which they found AA aqueous extracts to have potent DPPH radical scavenging action (Manosroi et al., 2011).

Reducing power assay involves the reduction of Fe$^{3+}$/ferricyanide complex to the ferrous form of Perl’s Prussian blue. The Perl’s Prussian blue formed has absorbance peak at 700nm. Both extracts of AA demonstrated a higher formation of Perl’s Prussian blue as indicated by their higher absorbance values. This implies that they are having potent reducing abilities and thereby can act as potent antioxidant defense for the tissue.

TBA assay represents the ability of analyte to prevent the lipid peroxidation of fatty acid present in the reaction mixture. Lipid peroxidation is an important mechanism of damage to lipid components of cell, like, cellular and subcellular membranes. Damage of lipid
bilayer of cell membrane is an important mechanism of cell damage which can lead to apoptosis or necrosis. Thus, ability to prevent lipid peroxidation may be a desirable property of any drug acting on chronic diseases. It is also an important pathway causing macrovascular and microvascular damage in long standing diabetes (Arora, Vig, and Arora, 2013). Both extracts of AA exhibited an ability to prevent lipid peroxidation comparable to that by vitamin C.