

SUMMARY AND CONCLUSION

The results obtained from the various studies conducted with the flavonoid fraction isolated from leaves of *A. echinoides* are summarized below.

- The aqueous suspension of powder of leaves of *A. echinoides* (CASAE) at a dose of 250 mg/kg bw has produced significant antihyperglycemic activity at 6th hour of the treatment in STZ induced diabetic rats.
- The flavonoid content present in the AETOF fraction obtained from leaves was quantitatively found to be 174 ± 9.4 μg quercetin equivalents (QE) /mg.
- When exposed to iodine vapour, the thin layer chromatogram of the AETOF fraction revealed the presence of multiple compounds in the AETOF fraction. Further, when subjected to LC-ESI-MS/MS analysis, revealed the presence of 32 compounds in this fraction.
- There was significant reduction (68.4%) in blood glucose levels of diabetic rats after oral administration of 50 mg AETOF/kg bw and hypoglycaemia was observed at a dose of 70 mg/kg bw. But there was no hypoglycaemia in normal rats treated with 70 mg AETOF /kg bw, which could be due to the effective functioning of counter regulatory hormones to insulin.
- Oral Glucose Tolerance Test (OGTT) in diabetic rats revealed that there was a consistent decrease in the blood glucose levels with a maximum fall of 51.5 % after 3 hours of treatment with the AETOF fraction. The antihyperglycemic effect of AETOF fraction was much higher than that of glibenclamide. There was no change in blood glucose levels in normal rats treated with AETOF fraction.
- In the long term study, maximum (71.7%) decrease in FBG levels of diabetic rats were observed at a dose of 50 mg of AETOF/kg bw.
- Treatment with AETOF fraction significantly elevated the serum insulin levels in STZ induced diabetic rats when compared with the controls. Similar effect was shown in glibenclamide treated diabetic rats also.

- After 40 days of AETOF administration, the body weights of diabetic rats were increased in comparison with diabetic controls. The body weight gain could be due to improved insulin secretion and glycemic control in the treated diabetic rats.
- Increased glycated haemoglobin (HbA_{1c}) levels in diabetic animals was brought down to near normal after treatment with AETOF fraction and the haemoglobin level which was lowered in diabetic animals was improved to normal value after the treatment.
- The plasma protein and glycogen levels in liver and kidney were decreased in diabetic control rats but the 40 days treatment with AETOF fraction, the levels of plasma protein and glycogen in tissues were improved to near normal. Similar effects were observed in the diabetic animals treated with glibenclamide.
- Decreased activities of glycolytic enzymes, hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH) were observed in diabetic rats but after administration of AETOF fraction for 40 days, the activities of these enzymes were increased to near normal.
- The activities of gluconeogenic enzymes, Glucose-6-phosphatase (GP) and Fructose 1,6bisphosphatase (FB) were elevated in diabetic rats but after treatment with AETOF fraction they were brought down significantly.
- The serum lipids, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol were increased while the good cholesterol i.e high density lipoprotein (HDL) was decreased in diabetic control rats compared to those in normals. After the AETOF administration for long term these lipid levels were improved to near normal.
- We observed a significant increase in lipid peroxide levels (TBARS) in plasma and tissues (liver and kidney) of diabetic rats compared to normal rats. Administration of AETOF fraction or glibenclamide potentially decreased the levels of TBARS in plasma and tissues of diabetic rats.

- In our study, the activities of hepatic and renal SOD, GPx and GST were decreased in diabetic rats compared to normal rats but long-term treatment with AETOF fraction had increased the activities of these enzymatic antioxidants. CAT was significantly increased in liver and kidney of diabetic untreated rats but this reverted back to normal after glibenclamide/AETOF fraction administration.
- The long term treatment of diabetic rats with AETOF fraction also increased the activities of enzymatic (SOD, GPx and GST) antioxidants and concentration of non enzymatic (GSH, Vitamin C and Vitamin E) antioxidants in plasma where as in tissues Vitamin C and GSH levels were increased and Vitamin E levels were decreased reflecting the antioxidant efficacy of the AETOF fraction.
- The activities of serum ALT, AST and ALP were increased in diabetic rats compared to the normal rats. Treatment with AETOF fraction for 40 days lowered the serum hepatic function markers in diabetic rats. Glibenclamide treated diabetic rats also have shown decreased levels of ALT, AST and ALP after long term treatment
- The renal function markers i.e serum Urea and Creatinine were increased in the diabetic control group but on treatment with AETOF fraction significantly decreased these levels to near normal. The levels were also decreased on treatment with standard drug glibenclamide.
- In the molecular dynamics study, docking of 32 phytoconstituents of AETOF fraction with ATP I and ATP II domains of Sur1 receptor, on β -cells revealed that three flavonoids viz. Skullcap flavone I, Echioidin and Echioidinin have shown highest binding affinity with ATP II binding pocket in comparison with standard drug Glibenclamide and its natural substrate Adenosine triphosphate.

The following histological changes were observed after long term treatment of diabetic rats with AETOF fraction:

- a. In diabetic untreated rat pancreas there was insulinitis with lymphocytic infiltrations along with atrophy and destruction of β -cells. Regenerative changes in the tissue architecture of pancreas were observed in the diabetic rats treated with AETOF fraction.
- b. Diabetic untreated rats showed degenerative liver with severe congestion of central vein, hemorrhages in the sinusoidal spaces and granular appearance of the hepatocytes (degenerative change) with cloudy swelling (hazy nucleus). Treatment with AETOF fraction in diabetic rats showed normal liver architecture with slight congestions in central vein, normal sinusoidal spaces and normal hepatocytes.
- c. The kidney of diabetic untreated rats showed atrophy of the glomeruli, necrotic tubular epithelial cells and dark pyknotic nuclei. The kidney of diabetic rats treated with the AETOF fraction showed normal glomeruli, normal intertubular vessels and tubular epithelial cells indicating regenerative changes.

From all these observations, it is concluded that the AETOF fraction of leaves of *A. echioides* possess significant anti hyperglycaemic, anti hyperlipidemic and antioxidant activities. The anti-hyperglycemic activity of the AETOF fraction could be due to its flavonoids derivatives as evidenced by LC-ESI-MS/MS analysis. These flavonoids may be acting in a synergetic manner by improving Insulin secretion from the remnant and regenerated β -cells and also by peripheral glucose utilization in tissues by normalizing the activities of carbohydrate metabolising enzymes. Lipid metabolism and antioxidant defence system were also improved to normal levels in the diabetic rats treated with the AETOF fraction. Hence, AETOF fraction of the *A. echioides* useful as a source of the natural antihyperglycemic, antihyperlipidemic agent and antioxidant supplement during persistent hyperglycemic condition.