CHAPTER: 5

DISCUSSION

The two plants viz., *Kandelia rheedei, Euphoria lopogona* selected for the present investigation are medicinally important. Roots of *Kandelia rheedei* have been used in traditional medicine to treat headache, antisyptic, antispasmodic and diaphoretic. The roots have been employed as emmenogogue and as an abortifacient. Infusion of the flowers is drunk as tranquilizer and tonic [52]. Leaves of *Euphoria lopogona* have been used in folk medicine for the treatment of rheum, dysentery, pruritus, malaria and burn. It has considerable reputation as a potent adjunct in the treatment of various ailments such as jaundice, inflammation and fever [53]. Various extracts of roots of *Kandelia rheedei* and leaves of *Euphoria lopogona* have been screened for chemicals and different pharmacological assays. Ethyl acetate extract of root of *Kandelia rheedei* was found to contain a number of flavanoidal compounds [60, 61]. It was also reported that ethyl acetate extract of leaves and roots posses’ protective property against many carcinogens and cytotoxic activity against different human cancer cell lines respectively [62, 63]. Thus, it can be seen that though the extracts of KR and EL found to contain different classes of chemicals of pharmacological importance, have been screened for very few biological/pharmacological activities. In the present investigation, methanolic extract and its fractions of roots of KR and leaves of EL were screened for hypoglycemic and antidiabetic activities in rats. Further, the effective fractions were subjected to glucose uptake assay, insulin secretogogue assay in cell lines and glucose uptake assay using isolated rat hemi-diaphragm. The physical status and percentage yield of methanolic extract and its fractions of KR and EL were recorded for future reference. In acute toxicity study, methanolic extract of roots of KR (MKR) and leaves of EL (MEL) were found to be non toxic up to the dose 2 g/kg b.w. Hence, the work on these extracts with four doses (100, 200, 400 and 800 mg/kg b.w.) was continued. It is evident from results that MKR and MEL exhibited significant hypoglycemic and antihyperglycemic activities in normal and streptozotocin induced diabetic rats respectively. The hypoglycemic effect of MKR and MEL was dose dependent. MKR and MEL significantly decreased the body glucose levels with 400 and 800 mg/kg
b.w. doses after 4 h and lasted the effect up to the end of 8 h and the effect was highly significant after 6 h. MKR and MEL with these doses differ from glibenclamide (10 mg/kg b.w.), the reference drug in their onset of action, but they produced prolonged significant hypoglycemia. On the contrary, MKR at 400 and 800 mg/kg b.w. doses produced a significant and similar hypoglycemic effect to that of glibenclamide, i.e., after 2 h, which lasted for 6 h. However, the hypoglycemic potential of these extracts was comparable to that of glibenclamide (10 mg/kg b.w.), the reference hypoglycemic drug of sulphonyl urea type [54].

In streptozotocin induced type 2 diabetic rats MKR and MEL lowered the blood glucose level in a dose dependant manner, indicating their antihyperglycemic effect. MKR at 400 and 800 mg/kg b.w. exhibited significant (p<0.05) onset of action after 2 h and continued the effect up to the end of 6 h, which was comparable to that of glibenclamide (10 mg/kg b.w.). MEL at the same doses showed the effect in a different way i.e., late onset of action (after 4 h) and prolonged duration of action (8 h). As the treatment of one day old rats with streptozotocin produced a relatively moderate increase and decrease in fasting blood glucose and insulin levels, respectively (i.e., a rat model of type 2 diabetes)[59]. The antihyperglycemic effect of MKR and MEL obtained in this study in type 2 diabetic rats could be explained in terms of potentiating glucose-induced insulin secretion. Pretreatment of non-diabetic rats with MKR and MEL at 400 mg/kg b.w. dose produced an improvement of oral glucose tolerance after 30 min onwards. The blood glucose levels reached nearly to normal or less than normal in both the extracts treated and glibenclamide (10 mg/kg b.w.) treated groups after 120 min, in a similar way. The improved glucose tolerance exhibited by MKR and MEL in this study supports the possible way of antihyperglycemic effect of these extracts in streptozotocin induced type 2 diabetic rats by potentiating glucose induced insulin release. However, it can be confirmed only after analyzing the other biochemical parameters related to type 2 diabetes. Since the hypoglycemic and antihyperglycemic potential of the methanolic extracts, viz., MKR and MEL were comparable to that of glibenclamide, the reference drug, these extracts were fractionated with toluene, ethyl acetate, butanone and n-butyl alcohol in succession separately and screened then for antidiabetic activity to isolate the active
fraction. This process not only helps in identifying and isolating the active fraction but also gives information about the nature of chemicals with respect to their polarity.

The respective fractions of MKR and MEL at 100 and 200 mg/kg b.w. in streptozotocin induced type 2 diabetic studies in rats revealed that TFKR and TFEL at 100 mg/kg b.w. can not reduce blood glucose level significantly at any time point while at 200 mg/kg b.w. can show significant reduction in blood glucose reduction after 6, 4 and 8 h respectively. ii. EAFKR and EAFEL at both the test doses exhibit significant antihyperglycemic activity with differences in the onset of action (after 4, 4 and 2 h respectively) and prolonged duration of action (8, 6 and 6 h respectively). The antihyperglycemic effect of these extracts are comparable that of glibenclamide, the reference drug. iii. BNFKR and BNFEL and BLFKR and BLFEL possess antihyperglycemic activity. The effect is relatively less intense than their corresponding EAFKR and EAFEL and the reference drug. In this study, ethyl acetate fraction of MKR and MEL viz., EAFKR and EAFEL were found to be effective active fractions, even at the lower test dose 100 mg/kg b.w. The activity may be attributed to the polar constituents present in them as ethyl acetate is a polar solvent. EAFKR and EAFEL at 100 mg/ kg b.w. showed improved glucose tolerance in streptozotocin induced diabetic rats. These extracts exhibited same intense of activity with a difference in the significant onset of action, which was late for EAFEL. The glucose tolerance effect of these extracts was comparable to that of glibenclamide, although the later exhibits early onset of action. The findings of this study support the antidiabetic activity of EAFKR and EAFEL. In the subacute study, administration of EAFKR and EAFEL at 100 mg/kg b.w. dose, glibenclamide (10 mg/kg b.w.) and metformin (250 mg/kg b.w.) once a day for 28 days to streptozotocin induced type 2 diabetic rats brought about the following changes on different biochemical parameters.

i. A significant improvement in body weight after 28 days, indicating their beneficial effect in preventing loss of body weight in diabetic rats [45]. As the effect was quite similar with that of standard drugs glibenclamide and metformin, it can be said that these extracts do not have any effect on degradation of depot fat and they can maintain the body weight. The ability of the extracts to prevent body weight loss
seems to be due to their ability to reduce hyperglycemia. In both the extract and reference drugs treated groups, there was a gradual reduction in blood glucose level after 7 days onwards to the end of completion of the study (28 days).

ii. The highly significant (p< 0.01) antihyperglycemic effect observed for EAFKR and EAFEL after 14 days onwards to the end of the study was comparable to that of both the reference drugs. Further the effect of EAFKR was similar to that of glibenclamide at any time point of the study. Incase of EAFEL, the highly significant (p<0.01) effect was observed after 21 days to up to the end of 28 days. The maximum reduction in blood glucose level after 28 days in both the extract and reference drugs treated groups almost same. This phenomenon clearly indicates that the extracts can potentially control the hyperglycemic state of type 2 diabetes [45].

iii. EAFKR and EAFEL lowered serum triglyceride level significantly (p<0.05) after 21 days to the end of study i.e, 28 days (p<0.01). They also showed significant (p<0.05) decrease in cholesterol level after 28 days. The antihypertriglyceridemic and antihypercholesterol- esterolemic effect of the extract were comparable to that of both the reference drugs. Clinically, it has been observed that there is presence of altered fat metabolism in type 1 and type 2 diabetes leading to altered serum cholesterol and triglyceride levels [73]. Hypercholesterelomia and hypertrygliceredemia have been reported to occur in streptozotocin diabetic rats. In insulin deficient subjects, it fails to activate the enzyme lipoprotein lipase and causes hypertrigly- ceredemia. Hence, it is possible that the mechanism of reduction of serum lipid levels with EAFKR and EAFEL may be through insulin release or by enhancing insulin sensitivity in the tissues.

iv. EAFKR, EAFEL and glibenclamide increased serum insulin level significantly (p<0.01) after 28 days of the study while EAFKR and metformin produced no significant change in insulin level. The effect of EAFKR and EAFEL was similar as that of the reference drug glibenclamide, indicating that they might have insulin secretogogue activity, which in turn controls the hyperglycemic state of type 2 diabetes. The ability of glibenclamide, a sulphonyl urea agent to block the beta- cell KATP channels directly explains its stimulatory effect on insulin secretion [59]. In this study, like metformin, EAFKR did not influence serum insulin level.
v. SGOT and SGPT levels decreased significantly (p<0.05) in EAFKR and EAFEL and the reference drugs treated groups after 28 days of the study. Liver and kidney GOT and GPT activities are elevated in streptozotocin induced diabetic rats [56]. Increased gluconeogenesis and ketogenesis are observed in diabetes which may be due to high level in the activities of these transaminases [57]. Hence, the diminution of SGOT and SGPT level after supplementation of EAFKR and EAFEL further strengthen the antidiabetogenic effect of the extracts.

vi. In both the extract and the reference drugs treated groups, the serum protein level was increased significantly (p<0.05) after 28 days. In type 2 diabetes, changes in protein metabolism takes place, leading to reduction in serum total proteins which is due to deficiency of insulin. Insulin stimulates uptake of amino acids into muscle and increases protein synthesis [54]. Therefore the increased serum protein level by the extracts explains their antidiabetogenic effect, which may be due to increased serum insulin level as mentioned before. In DPPH radical scavenging assay EAFKR and EAFEL exhibited free radical scavenging ability or antioxidant activity in a concentration dependant manner. The order of antioxidant activity of these extracts is as follows: EAFKR> EAFEL. The antioxidant property of these extracts could be use full in the treatment of free radical pathologies such as cancer, arthritis, diabetes etc. Diabetes mellitus of long duration is associated with several complications such as nephropathy, atherosclerosis etc, which have long been assumed to be related to chronically elevated glucose level and subsequent oxidative stress. Mechanisms that contribute to increased oxidative stress in diabetes include non-enzymatic glycosylation, auto oxidative glycosylation and metabolic stress. Oxidative stress in diabetes may partially be reduced by antioxidants [58]. In subacute study in streptozotocin induced diabetic rats, EAFKR and EAFEL exhibited significant antioxidant activity which was evident from the decrease in the level of lipid peroxidative marker, malonoldehyde (MDA) in serum after 28 days. Lipid peroxidation, a general mechanism of tissue damage by free radicals is known to be responsible for cell damage and may be induce many pathological events. The results suggest that EAFKR and EAFEL may protect pancreatic β cells from degeneration and diminish lipidperoxidation of cells [59]. Further these extracts could exert a beneficial action against pathological alteration caused by the presence of free
radicals in streptozotocin diabetes [65, 66]. Differentiation of preadipocytes, 3T3-L1, to adipocytes is a complex process involving various pathways and signals. In this process, insulin and PPAR play critical roles in promoting upregulation of adipogenesis.

Pioglitazone, the standard drug, has shown dose dependant adipogenesis inducing activity up to 4 p.m and then further increase in concentration decreased the adipogenesis inducing activity. Oil red '0'& Eosin staining as well as glucose uptake study have proven adipogenesis inducing activity of the pioglitazone, which is higher at 4 g. Both basal and stimulated glucose uptake were significantly elevated by the pioglitazone. Insulin concentration that promotes high glucose uptake was optimized by subjecting various concentrations of insulin to adipocytes and glucose uptake was read by scintillating counter. Glucose uptake was reached to its maximum at 1 p.m and further increase in the concentration severely impaired the glucose uptake. Hence, insulin concentration 1 M was used for all the study to stimulate the glucose uptake. EAFKR and EAFEL elevated the glucose uptake in the basal and stimulated, insulin treated conditions in the 3T3-L1 cells. Initially glucose uptake activity of the extracts was evaluated at a fixed concentration (1001.1g/m1) to test the comparative potencies of the extracts, followed by dose dependant activity. EAFKR at 100 pg/m1 concentration exhibited very significant (p< 0.001) activity both in basal and insulin stimulated conditions. On the contrary and EAFEL have shown the activity only in insulin treated condition at 200 µg/ml (p< 0.05) and 50-200 µg/ml (p<0.05) respectively. The results suggest that EAFKR could enhance the basal level of glucose level even in absence of insulin, indicating its insulin like activity in enhancing the glucose uptake which is comparable to that of pioglitazone (41.11\4).

Glibenclamide was used as a standard for the insulin release studies. Glibenclamide stimulates voltage sensitive potassium channels resulting in the degranulation, followed by release of insulin from the vesicles [67]. Calcium ionophores are known to stimulate insulin release by allowing the calcium ions to influx. Glibenclamide at a concentration of 3 111\4 stimulated the RIN cells to produce high levels of insulin. EAFKR and EAFEL at different concentrations did not stimulate insulin release from the RIN cells, where as EAFKR could stimulate insulin release at 100-250 µg/ml. The
insulin secretogogue activity of EAFEL was comparable to that of glibenclamide (3.1 M/ml). The results indicate that EAFEL acts as antidiabetic by a mechanism similar to that of glibenclamide, a sulphonylurea drug used for management of diabetes. The uptake of glucose by isolated rat hemi-diaphragm is a commonly employed and reliable method in vitro study of peripheral uptake of glucose. EAFKR and EAFEL also enhance the uptake of glucose significantly (p<0.001 and p<0.01) in the peripheral tissues and were found to be more effective than insulin. It appears that these extracts have direct peripheral action. These extracts in presence of insulin also exhibit the effect and EAFEL was found to be more effective, indicating its synergetic effect.