7. SUMMARY AND CONCLUSION:

The present research work was aimed to access the safety and efficiency of aqueous extracts of EJ, MC and GA as antidiabetic agents. After detailed literature review and survey of available antidiabetic formulation in Indian, Chinese and Srilanka market it was decided to carry out research on aqueous extracts of EJ (Seeds), MC(Fruit) and GA (Bark). There are number herbal of preparations (more than 100) available in market which are meant for its use for the management of hyperglycemia. Some of them are available as juice, powdered form of plant part, extract and also the formulations are available in tablet and capsule forms. However, none of the products have been approved by health authority till date for use as antidiabetic medicine. The primary reason for this is that none of the companies or researchers have submitted the systematic data of their formulation to regulatory authority i.e right from collection, authentication, standardization, preclinical testing, formulation and clinical testing. Most of the manufactures are depending on the data available is ancient ayurvedic books but looking at the swift changes in environment and biodiversity the health authority are reluctant to approve the new formulation or herbs before reviewing details preclinical and clinical data. Literature review revealed that there are number of literature published on EJ and MC for their use as antidiabetic agent and most of them were based on results obtained on preclinical setting with limited data. There are very few reference available for aqueous extract of GA for its use to manage hyperglycemia. The MC contains peptide which is similar to insulin and due to same it shows insulinomimetic effect and also used for management of other diabetic complications. The proposed mechanism of action in literatures for EJ gives an idea that EJ acts with same mechanism as biguanides. So, in the present study safety and efficacy of EJ, MC and GA are assessed individually and with combinations in preclinical and clinical setup.

The selected plant material were initially assessed for morphological characteristics, loss on drying, moisture content, extractive values then evaluated for physicochemical and phytochemical properties. Preliminary phytochemical screening reveled that the aqueous extract of EJ contains alkaloids, tannins, saponins, steroids and aqueous extract of MC contains protein, amino acid, glycosides and saponins. The aqueous extract of GA also contains tannins and saponins. All the three test extracts were complied with microbiology limit test and quantities estimation of alkaloid resulted in 5 % of alkaloid in MC. Aqueous
extract of EJ contains 4 % saponins and GA contains 2.5 % saponins and 2.31 % of Phenolic compounds.

After standardization the extract were subjected to preclinical safety studies i.e acute toxicity study and repeated dose toxicity study. The results of acute toxicity study confirmed that all the combinations i.e A, B and C were non-toxic when administered to mice as a single oral dose at 300, 2000 and 5000 mg/kg. Oral administration of these extracts at given dose did not show mortality or symptoms of toxicity. According to Hilaly et al. (2004), Study of oral administration no-observed adverse effect (NOAE) dose was found to be 5000 mg/kg of body weight and same dose can be considered as maximum tolerated dose (MTD). There was no mortality for any of the tested doses at the end of the 14 days of observation. After confirming the safety in acute toxicity study for all three extracts the repeated dose toxicity study was performed for a period of 28 days in rats. No mortality or significant changes in general behaviour or other physiological activities were observed at any point in the present study. Neither clinical signs of any toxic nor adverse effect were noticed throughout the study. Also there were no significant changes in terms of body weight and food intake on administration of combined extract at doses 500, 1000, and 2000 mg/kg of body weight for 28 days.

After confirming the safety of the test extract the antidiabetic potential of the test extracts were evaluated in Alloxan induced diabetes rat model. The study was planned with broad objective for elimination of less effective combination and confirm the efficacy at selected dose in animals. The dose of 500 mg/kg body weight dose was chosen on the basis of market survey and literature review. In the present study the five different extract were tested i.e three combinations and two individuals. Glibenclamide was considered as an active standard. The results obtained were compared with diabetic control group and normal control group. The four week treatment with individual and combined extracts decreased blood glucose level by 100 to 200 mg/dl in various test groups. The group A (EJ+MC+GA) and C (EJ+MC) and reference drug F (Glibenclamide) shows almost equivalent response at day 14, 21 and 28 when compared with diabetic control group G. The results of the study revealed that combined aqueous extract’s i.e. A and C showed better hypoglycemic effect than the individual extract i.e D (EJ) and E (MC) when compared to diabetic control group G. Group C showed better hypoglycemic effect when compared with group A and B. The group A and
C showed significant decrease in plasma glucose on day 14, 21, 28 at the selected doses. The results of group A do not indicate any added advantage over the group C. In this study, it took a shorter period to attain a significant reduction in the blood glucose level when aqueous extract of MC combined with EJ showing its additive effect on hypoglycemic activity.

The next study was performed to assess the insulin potentiating effect of test extracts at dose of 5000 mg/kg body weight in alloxan induced hyperglycaemic rats. The results of this study indicated that the combined extract of EJ+MC+GA and EJ+MC significantly potentiate the hypoglycemic effect of insulin at 120, 180 and 240 minutes. Also the combination of three extract of i.e. EJ+MC+GA did not show any added advantage over combination of EJ+MC. So the combination of two extracts EJ+MC (1:1) were continued for further study instead of combination of three extracts i.e. EJ+MC+GA (1:1:1). The calculated KITT factor for group A, B and C was 0.0057, 0.0077 and 0.0077 respectively.

After confirming safety and efficacy of individual and combined extracts in preclinical models the clinical study was carried out in the patients with NIDDM and already receiving treatment with OHA. The study was carried at three sites with thirty-two randomized patients. There were more than 100 patients pre-screened across three centres and 53 patients consented and screened as per inclusion and exclusion criteria. Out of 53 screened subjects 32 subjects were eligible to randomize in the study and 21 subjects were screen failure due to non compliance of inclusion criteria. The majority of subjects were screen failed because of low FBS than 110 mg/dl at screening i.e controlled diabetes. Patient who were on insulin and newly detected diabetics were not screened for this study. Out of 32 randomized patients 30 patients were eligible for efficacy analysis. Out of 30 randomized patients 15 patients were randomized to test drug and 15 patients had received placebo. All the patients randomized in the study had received Metformin as per prescribed dose. The FBS and PPBS were assessed at each study visit. The mean FBS for Group A at baseline visit was 133± 6.5 mg/dl and at day 90 it was 95 ± 4.3 mg/dl. The same pattern was also observed for PPBS of Group A. A smooth and uniform fall in blood sugar was noticeable at the end of one month only in the patients randomized to test drug and this was maintained at 150 day follow-up visit. In the patients randomized to placebo the fall in blood glucose level was in line with group A till day 15 and after that group A showed better hypoglycaemic response in terms of FBS and PPBS till the end of study (p <0.01). There was also marked increase in blood glucose level at 150
day follow-up visit for Group- B patients compared to Group-A patients. There were 18 AEs recorded in the study and out of which there were 6 episodes of Nausea and 5 of vomiting. As per investigators opinion the adverse event nausea and vomiting were related to the test extract and there was no SAE recorded throughout the study period.

Another study was done on newly detected diabetic patients who have not received any treatment for diabetes. There were 130 subjects pre-screened for this study and 49 subjects were consented and screened for the study. The detailed screening of 45 patients could not be done due to consent refusal and 32 patients were detected with FBS more than 140 mg/dl so they were not screened for the study. Out of 49 screened patients 23 patients meeting all inclusion/exclusion criteria were randomized for the study. From 23 randomized patients three patients were early terminated i.e one due to SAE and two patients were lost to follow-up making patient compliance to 85 %. The 20 patients who had completed the study were considered for final evaluation. The FBS and PPBS level of all the randomized patients were assessed at each study visit i.e screening, day 15, 30, 60, 90 and also at safety follow-up visit on day 150. The statistical analysis by applying descriptive statistics. At the end of treatment period i.e 90 days the mean FBS level was decreased by 29.33 % (P < 0.001) and PPBS level was decreased by 26.09 % (P < 0.001) as compared to the mean baseline value. The reduction in blood glucose level were also observed in the subjects receiving placebo as the mean FBS level was decreased by 10.64 % (P > 0.05) and mean PPBS level was decreased by 13.26 % (P > 0.05) as compared to baseline reading. So the difference between percentage blood sugar reduction of test and control arm is more than 19 % and it reflected that better glycemic control is achieved in the patient receiving test extract. The change in diabetes related symptoms were monitored throughout the study. This significant improvement in NDDM related symptoms in patients randomized to test arm also resulted in better patient satisfaction and compliance for the treatment and study visits. There were no clinically significant changes in the haematological and biochemical parameters noted during the study. Also there were no clinically significant changes observed in vital parameters in the group A and B patients. There was one SAE recorded and 12 AEs (4 related to IP) recorded in the study.
Conclusions:

The results of present work conclude that:

1. The aqueous extracts EJ, MC and GA safe at the tested dose in animal
2. The oral administration of aqueous extract of EJ, MC and GA significantly reduces the blood glucose level in alloxan induced hyperglycemic rats.
3. The aqueous extracts EJ and MC potentiate the effect of insulin in Alloxan induced diabetic rats
4. Oral administration of aqueous extracts EJ and MC is safe at the tested dose in NIDDM patients
5. The aqueous extracts EJ and MC is an effective antihyperglycemic agent for NIDDM patients

Scope for Future Work:

- Commercial Clinical Studies to prove efficiency in larger patient population needs to be conducted
- Further studies on optimization of formulations need to be carried out