7. SUMMARY AND CONCLUSION

1. Leptospirosis the re-emerging, life threatening zoonanthroponosis is not well studied among the pediatric cases of Salem. The MAT sero-study among the age groups (1-5, 6-10 & 11-15 years) tested (Figure: 1a & 1b) have shown the disease incidence very clearly to this region (Table: 1). The second age group of the present study (6-10 years) was found to be the worst affected with 25% and a clear male preponderance was noted (Table: 2 and Figure: 4 & 5). *L. autumnalis* was the predominant serogroup causing the infection to this region. However, a clear shift from *L. autumnalis* predominance to *L. icterohaemorrhagiae* predominance was observed during the shift in season from winter to summer (Table: 3-6).

2. Though WHO is recommending MAT as the standard sero diagnostic tool it is not suitable for routine diagnostic purposes. Therefore an in-house CIE (Figure: 6) was standardized which has got merits such as non-hazardous, non-cumbersome, easy, economic, rapid and not demanding high skilled labor. A few cases (Four samples GH 21, 84, 151 & 168) were MAT negative at acute phase became positive during convalescent phase. However, in both acute phase and convalescent phase the CIE was consistently giving positive results (Table: 7 & 8). Wherever discrepant results were found PCR was taken as the gold standard of diagnosis for this comparative study. MAT sensitivity (95.90%) was found to be almost equal to CIE (96.80%). The specificity (89.83%) and PPV (95.23%) for MAT and CIE (89.28% & 95.08%) were found to be more or less the same (Table: 9). NPV (91.37%) and ET (93.92%) were found higher than CIE the respective values were 92.59% & 94.47%. The *p* value for MAT (0.6796%) was also found to be almost equal to CIE (0.7017%) (Table: 10).
3. Multidrug resistance, antibiotic sensitivity and allergic complications including many side effects have made Indian medicine especially from herbal sources as a very important infectious diseases management tool. Though *P. amarus* and *E. alba* have also proven to be important among medicinal plants with anti-microbial, anti-oxidant, hepatorenal protective activities studies pertaining to anti-leptospiral concepts are obscure. Hence the present study was executed and it revealed the presence of tannins, alkaloids, saponins, flavonoids, terpenes and anthraquinones in the leaves of these plant extracts. Thus the study has given hope of possible Indian medicinal treatment for the human leptospirosis with these Indian medicinal plants (Table: 11). By observing the drug dose concentration the extracts of *P. amarus* was found to be having a better active principle (160 μg/ml) than the extracts of *E. alba* (320 μg/ml) (Figure: 7 & 8).

4. Several virulent factors are important for the successful establishment of leptospirosis syndrome. Among them sphingomyelinase of *Leptospira* protein is very important in the hemorrhagic effect which helps in the bacterial entry into the animal cells. To understand the extracts active principle on the virulent factor at molecular level, a protein and a DNA studies were carried out. By doing SDS-PAGE protein analysis low molecular weight polypeptides 22, 29, 32, 38, 45 kDa and high molecular weight polypeptide bands were observed respectively. After the extracts exposure these protein profile’s damages were analyzed. By observing proteins damage at 29, 32, 38, 65, 83, 94, 101 and 105 kDa regions it is evident that sphingomyelinase, OMP and inner membrane proteins of *Leptospira* were damaged due to these extracts (Figure: 9). Complete and partial lysis of protein bands such as 29, 32, 38, 65, 83, 94, 101 and 105 kDa were observed on *P. amarus* and *E. alba* extracts (PEA and EAE) exposed cultures respectively.
5. Eventually after observing the leptospiral proteins damages observed in the medicinal plant extracts exposed *Leptospira*, a molecular (DNA) study was conducted to decipher the respective nucleic acid damage if any as the reason for these protein damages. Interestingly the extract treated leptospiral DNA template did not get amplified for the *sphH* gene in spite of five repeated attempts. Successful demonstration of the specific DNA band at approximately 1.6 kbp on 1.5% agarose gel electrophoresis stained with Ethidium bromide in the positive control (*Leptospira* culture) is the clear indication of reliability on the standardized in-house PCR assay (Figure: 10 & 14).

6. After understanding the leptospiral protein damage by SDS-PAGE and DNA damage by PCR before and after the herbal extracts exposure. In furtherance to understand the active molecules present in the best herbal extract which are having the active principle against the pathogen, a Gas Chromatography and Mass Spectrometry (GC-MS) study was conducted on the methanolic leaf extracts of *P. amarus*. Seventeen important biochemical moieties (Table: 12) were identified in the *P. amarus* extracts, perhaps these moieties active principles individually or synergistically acted upon the pathogen in conferring the anti-leptospiral activity. Thus, this study has given an excellent hope for the development of an anti-leptospiral candidate drug from this plant leaf extracts (Figure: 11-13). This study has given hope not only on their therapeutic application but also on prophylactic potency for preventing leptospirosis in controlling its sporadic and epidemic episodes in India.
Thus the present study has not only confirmed the re-emergence of leptospirosis among Salem population but also given an alternate tool for the MAT which is non-hazardous and can be used for routine lab diagnosis for countries like India. *P. amarus* extracts efficacy on the leptospiral protein and DNA damage with one or more of its synergistic active molecules has thrown light on the possibility of effective leptospirosis management by Indian medicine. However, based on this present investigation it was recommended that further studies are needed on the isolation and purification of the bioactive compounds having anti-leptospiral potentiality from not only *P. amarus* and *E. alba* but also in other hepato-renal protective Indian medicinal plants for the betterment of leptospiral management.