ABSTRACT

Leptospirosis is a zoonosis of ubiquitous distribution, caused by infection of pathogenic *Leptospira* species. The spectrum of human disease caused by leptospires is extremely wide, ranging from subclinical infection to severe syndrome of multiorgan infection with high mortality. Since 1980’s the disease has been reported from various states during monsoon months. The disease is endemic in Karnataka, Kerala, Tamil Nadu, Gujarat, Andaman and Maharashtra. Leptospirosis has been under reported and under diagnosed from India due to lack of awareness of the disease and a lack of appropriate laboratory diagnostic facility in most parts of the country. The infection is commonly transmitted to humans by allowing fresh water that has been contaminated by animal urine to come in contact with unhealed breaks in the skin, eyes or with the mucous membranes.

Collection of samples (*i.e.* biological materials like serum, plasma, urine and others) was carried out from different places of Karnataka state, India, and isolates were also obtained from serum institutes and other reference laboratories. Bacterial samples were subcultured in a semisolid medium containing BSA and either Tween 80 or Tween 40 or EMJH. The time required for detection of a positive culture varies with the leptospiral species and the number of organisms present in the sample. The cultures were examined under a dark field microscope every week. The presence of leptospires was confirmed by conducting ADMAS *Leptospira* staining technique. Through this observation 110 samples were found positive for *Leptospira*.

We have developed PCR assay targeting partial *LipL21, LipL32, LipL41* and *rpoB* gene of pathogenic leptospires using in-house designed primers. PCR-based protocols offer several advantages over standard culture techniques. In the present work an attempt has been made to develop sensitive and specific confirmative diagnostic technique for *Leptospira*. Also the usefulness of the partial *LipL21, LipL32, LipL41* and *rpoB* gene for the diagnosis of leptospirosis over biochemical tests has been assessed. The SSCP analysis is designed for the detection of minor sequence changes in DNA amplified by PCR of single base substitution because of its simplicity and rapidity, ease and generally high sensitivity. This study demonstrates that the SSCP with our own designed primer readily distinguish eight species in the genus *Leptospira*. The present study showed that RNA polymerase β-subunit encoding gene (*rpoB*) sequence analysis can be a powerful tool in the identification of *Leptospira* species.
From the phylogenetic tree, it can be seen that this cluster shows higher divergence among them compared to Indian cluster. Using a partial rpoB gene sequence, it was possible to distinguish the *Leptospira* isolates. This demonstrates the usefulness of *rpoB* sequence in the identification of *Leptospira* and distinguishing from other species.

Since there are scanty reports on spectral characterization of the novel compounds from *Phyllanthus amarus* (L.) and *Eclipta alba* (L.) against *Leptospira*, the studies were taken up to find out the antioxidant activity by ABTS and DPPH assays. Exposure of the DNA of *Leptospira* to the methanol extract of *E. alba* and *P. amarus* at the concentration of 1 µg/ml resulted in DNA cleavage.

Silica gel chromatography technique was used to collect biologically active fractions. Silica gel column chromatography of crude methanolic extract yielded 48 fractions. Among these F1 to F35 showed no activity for DPPH and ABTS scavenging activity, but further purification of the fractions from F36 to F48 yielded three fractions (F37, F42 and F46) which exhibited high scavenging activity. BC₁, BC₂ and BC₃ represent fractions as F37, F42 and F46 in this study. Based on the performance of the fractions in tube dilution technique (TDT) and micro dilution technique (MDT), F46 has recorded maximum inhibition of *Leptospira*. Hence, only F46 was used for *in vivo* studies. In *in vivo* studies, inoculated mice kidney exhibited shrunken glomeruli on histopathological examination of kidney, while glomeruli were nearly normal in the F46 treated with 0.075 and 0.1 mg/ml. Histopathological assessment of liver shows prominent changes including centrilobular necrosis, bile duct proliferation, and disorganization of normal radiating pattern of cell plates around central vein in mice induced with *Leptospira*. Induced mice treated with 0.075 and 0.1 mg/ml concentrations of F46 showed a significant antileptospiral property. These promising fractions of *P. amarus* were further subjected for spectral characterization by IR, ¹H-NMR, ¹³C-NMR and MS techniques. Based on the spectral data, the structure of the compounds BC₁, BC₂ and BC₃ were deduced using ChemDraw drawing tool, PerkinElmer, USA.

A series of pseudo-peptides against *Leptospira* were established and characterized by spectroscopic (¹H-NMR, ¹³C-NMR and MS) studies. Preliminary pharmacological assays for leptospirosis were studied by TDT and MDT. In particular, all the analyses led to the conclusion that the synthesized compound inhibiting the *Leptospira* significantly. Out of three compounds the compound 4c at 50 and 75µg/ml showed significant inhibitory activity on all species of *Leptospira*. 