

An executive summary of the final report of work done on the Minor Research Project of **MR HARIPRASAD SHETTY** entitled **“IMMUNE MODULATION OF BRAIN FUNCTION IN CANCER MODEL: A STUDY AT THE PHYSIOLOGICAL, NEURAL AND BEHAVIOURAL LEVELS”**, sanctioned by UGC, vide sanction letter No. **MRP(S)-0114/12-13/KAMA002/UGC-SWRO Dated 29.03.2013**

The Wistar rats were randomly divided into two groups (n=6 each). DEN (; Diethylnitrosamine, Sigma, 200mg/ml) was dissolved in physiological saline and administered intraperitoneally. The control group received only saline. And animals have been kept for 10 week to develop hepatic cancer. On the test day, the Normal and DEN induced Wistar rats were randomly divided into two groups (n=6 each). Lipopolysaccharide (*Escherichia coli* 0111:B4; Sigma, 1mg/ml) was dissolved in physiological saline and administered intraperitoneally. The control group received only saline. At intervals of 2 hours each post injection, the animals were exposed to different behavioural testing paradigms .The recorded behavior through ethovision of activity test (baseline activity), activity box (Novel, non-anxiogenic environment) and EPM (anxiogenic environment) were monitored and analyzed. Antioxidants and Brain-associated enzymatic markers -assay have been carried out. The *Go-Ark* is a product of cow urine prepared by condensing the vapors of fresh cow urine using a glass distillation apparatus. Various qualitative and quantitative biochemical analysis of *Go-Ark* was done. Soil was treated with *Go-Ark* and soil analysis was done using Soil analysis kit. Anti-bacterial activity was tested by using increasing concentrations by the disc diffusion technique against cultures of *E.coli*, *B.subtilis* and *S.aureus*. 05 rats were administered *Go-Ark* at a dose of 1ml/kg body weight and 05 were administered vehicle by intubation between 8:30 to 11:00 am for 10 weeks. 16 male wistar rats (~150g) were procured and were injected with DEN carcinogen (200mg/Kg body weight) I.P. and 08 animals were intubated with the *Go-Ark* (1ml/kg body weight) for 10 weeks and 08 were intubated

with vehicle by intubation between 8:30 to 11:00 am for 10 weeks. The tail suspension test was carried out. For the formalin test rats were injected with 100µl of 5% formalin on the left paw and saline on the right paw, on the dorsal side. After 4 hrs of formalin injection, the paw edema diameter was measured using a Vernier's callipers. A very next day rats were sacrificed and liver was extracted from all the animals and collected livers have been treated with liquid nitrogen and stored in -4⁰ C for various liver enzyme assay. The behavioral changes, collectively termed "sickness behavior," include reduced exploration, increased anxiety, cognitive dysfunction, and social withdrawal in rodents. But DEN animals have not showed any Anxiolytic behavior. DEN with saline animals showed more risk assessment behavior in open and closed arm. A peripheral inflammation can have an effect on the brain where certain enzymes like AChE exhibit their functions. At 6 hrs post Normal- LPS injection, there is a significant increase in AChE levels in the forebrain and decrease in the brainstem. At 6 hrs post DEN-LPS injection, there is no significant differences in AChE levels in the forebrain and in the brainstem. At 6 hrs post for Normal and DEN, LPS injection, there is an increase in malondialdehyde levels as indication of lipid peroxidation in the brain stem. The forebrain shows no significant difference. Prolonged generation of ROS can eventually lead to lipid peroxidation, wherein the phospholipid bilayer gets increasingly porous, culminating in necrosis. LPS induced alterations in the brain and dependent on the dose of LPS, different responses are observed in different brain regions. MDA levels were elevated in different brain regions suggesting a probable mechanism to overcome oxidative stress. At 6 hrs post LPS injection for normal and DEN animals, there is no difference in reduced Glutathione levels in the forebrain and brainstem. At 6 hrs post LPS injection for normal and DEN animals, there is no significant difference in nNOS levels in the forebrain and the brainstem. Results from the biochemical tests indicated the absence of carbohydrates, protein and ammonia, while Urea, creatinine, sulphate and phosphate were detected. For all the data, *Go-Ark* demonstrated significant

antimicrobial activity against Gram negative *E.coli* in a dose-specific manner. No response was observed against Gram positive *S.aureus* and *B.subtilis* across the various doses tested. The *Go- Ark* treated animals exhibited a significantly faster response in the tail suspension. In the present investigation, it was observed that the animals treated with DEN showed significant hepatic damage which is represented by the elevated levels of liver markers SGOT and SGPT. The rise in the SGPT in *Go-ark* treated animals shows less increase in the levels of SGPT. These results proved to be an evidence for increasing link between peripheral infections and central neural functioning. During immune challenge and cancer the infection may lead to increased susceptibility for brain damage and associated disorders such as depression. The major outcome of this project is in our understanding of the link between nerve and immune-related events which in turn will help in the evolution of therapeutics. *Go-Ark* possesses potential antibacterial and antifungal activity and deserves can further studies on the development of new drugs. Even immune modulatory experimental findings suggest that *Go-Ark* would be a promising anti-inflammatory agent for inflammation treatment.

Date:

(Name & Signature of Principal Investigator)

Signature of the Principal