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The in vivo assessment of antiplasmodial activities of leaves and stem bark extracts of Mangifera indica (linn) and Cola nitida (linn)

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Background: Malaria is a serious parasitic disease from tropical regions caused by species of *Plasmodium* and transmitted by *Anopheles* mosquitoes. It is prevalent in countries in Africa, Southeast Asia and South America. High mortality rate is reported in these regions. The exponential increment of resistance of the most severe and commonest form of *Plasmodium* species, *Plasmodium falciparum* to chloroquine, a prominent anti malarial drug and first line drug over the past two decades has necessitated the investigation into tradinationally calmed anti malarial plants. Amongst the commonly used anti malarial plants in Nigeria are *Mangifera indica* and *Cola nitida*.

Methods & Materials: The air dried leaves and bark of *Mangifera indica* and *Cola nitida* were powdered and extracted using aqueous and ethanol as solvents. The solvents along with extracts were drained out and filtered. The semisolid extracts were obtained in vacuum using rotary evaporator. The malaria screened, twenty seven albino mice of both sex with body weight range of 18 to 25g were obtained from Obafemi Awolowo university, Ile Ife, Osun State, Nigeria and were allowed to acclimatize for one week. Each of the Swiss albino mice was intraperitonally administered with 0.2ml *Plasmodium berghei* parastized red blood cells and the parasitemia level were montored for five days.After the establishment of infection, the extracts, chloroquine and artesunate(used as positive control were administered orally through intra gastric route using the stomach tube to ensure the safe ingestion of the treatment doses to the tested groups daily for four subsequent days.

Results: The fourth day suppression results revealed a significant reduction in the parasiteamia level of the different treatment groups. A percentage suppression of 97.05% was recorded in the mice group treated with ethanolic *Cola nitida* leaves extract while 95.82% was recorded in the group treated with ethanolic *Mangifera indica* leaves extract.

Conclusion: The high suppressive values obtained justified the local usage of the plants as anti malarial plants and can be employed as a potential extracts for the development of active novel drugs against malaria parasites.

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Comparison of the incidence of dientamoeba fragilis in a cohort of paediatric children with allergic asthma and aontrols: Is it a pathogen or protector?

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Background: *Dientamoeba fragilis* is a neglected intestinal protist associated with clinical outcomes in humans. There is still an obscurity about its pathogenicity in humans, including whether it can modulate immune responses, through which mechanisms it initiates infection, or it is mostly a silent resident of human gut. There are many studies and case reports which all indicate *D. fragilis* as the causative agent of patients with diarhhea, abdominal pain, meteorism and urticaria. Yet, *D. fragilis* may have pathogenic subtypes and/or act as a pathogen under certain circumstances through interaction with the host's immune system. It was initially identified by conventional methods. After PCR became available, it was identified surprisingly common in regions where parasitosis is uncommon, such as Denmark and the Netherlands. Indeed, results of many controlled studies showed higher *D. fragilis* presence in healthy controls compared to study groups, which suggested that *D. fragilis* may be a natural resident of human gut, rather than being a pathogen. Combining these data with current debates about the influence of gut microbiota on human health, we aimed to compare the incidence of *D. fragilis* in a cohort of children with allergic asthma and healthy controls in this preliminary study.

Methods & Materials: A total of 50 children who were diagnosed as allergic asthma and 46 age-matched healthy controls were enrolled in the study. Personal information was collected from all participants initially. The mean age of asthmatic children was 5,67+ 2.1 and that of healthy controls was 5,43 + 3.2. Fresh stool samples were collected, their DNA content was isolated, and Real-Time PCR that targeted the ITS-1 region of *D. fragilis* was conducted.

Results: The results of the assessments showed that 26 of 50 (52.0%) patients and 36 of 46 (78.3%) healthy controls were positive for *D. fragilis*. The difference between the groups was significant (p=0.007; OR: 0,301).

Conclusion: Our findings contribute to recent reports in which *D. fragilis* was found more common in healthy controls. We now

plan to assess the role of *D. fragilis* in immune modulation and its interactions with human microbiota in a larger scale.

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Detection and subtype identification of Blastocystis in a hospital setting from southeasternIndia

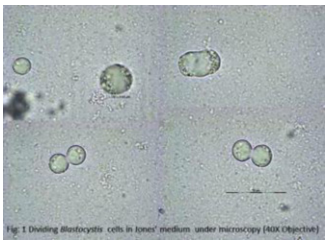
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Background: *Blastocystis* was identified almost 100 years back but its clinical significance is controversial. An estimate suggests that *Blastocystis* inhabit large intestine of more than 1 billion human worldwide. Based on the ribosomal lineages different species of *Blastocystis* are designated as various subtypes (ST) with extensive inter and intra subtype genetic diversity. Due to its polymorphic nature identification by microscopy is obscure. However, in India, data pertaining to *Blastocystis* were chiefly derived from direct stool microscopy. In this study we employed microscopy, culture and PCR for the detection of *Blastocystis* from stool samples. Further, subtyping of representative samples were carried out to identify the subtypes available in this region.

Methods & Materials: It is a cross sectional analytical study approved by JIPMER Institute Ethics Committee. All the stool samples were screened by routine microscopic investigations and they were subjected to *in vitro* propagation in Jones' medium. Fecal DNA was extracted by using QIAamp DNA stool mini kit (Qiagen, Germany) following manufacturer's instructions and stored at -20°C. Further, extracted DNA was quantified and subjected to PCR, which targets initial 600 bp barcoding region of 18SSU rDNA of *Blastocystis*. PCR products were visualized on 1.5% agarose gel and representative positive amplicons were sequenced for subtype analysis. Sequence results obtained from both the strands were assembled and subtype analysis was performed by using following database http://www.pubmlst.org/blastocystis/

Results: A total of 173 stool samples were screened for *Blastocystis*. PCR detected the maximum number of *Blastocystis* (n = 77, 44%) followed by culture (n = 48, 28%) and Microscopy (n = 25, 14%). The Sequencing results of the representative PCR amplicons confirmed the presence of *Blastocystis* ST3 allele 34 (n = 9) and ST1 allele 4 in (n = 5).



Conclusion: In comparison with stool microscopy and culture, *Blastocystis* specific PCR is an excellent diagnostic tool in terms of sensitivity, specificity and subtype identification. However, in resource poor settings Jones' medium (xenic culture) could be used as an alternative diagnostic modality for the detection of *Blastocystis* from stool. Subtyping results indicate ST3 predominance. However, large number of samples needs to be subtyped to reveal the association of particular ST with particular clinical manifestations.

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MicroRNA mediated immune regulation of T helper cell differentiation and plasticity during visceral leishmaniasis infection: A computational approach

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Background: Visceral leishmaniasis (VL) is a tropical neglected disease caused by *Leishmania donovani*, results in significant mortality in Indian subcontinent. The protective immune response to *Leishmania* parasites is mediated by proliferation and differentiation of IFN-γ secreting $CD4^+$ T helper (Th1) cells while IL-4 dependent $CD4^+$ T helper (Th2) cell leads to aggravate VL pathogenesis. The plasticity of T cell proliferation and differentiation depends on microRNA mediated gene regulation which leads Th1/Th2 or Th17/Treg type of immune response during human VL.

Methods & Materials: MicroRNAs participates in T cell proliferation and differentiation in human VL. This study depicts the identification of target immune signaling molecule and transcription factors, which play role in T-cell proliferation and differentiation followed by the identification of miRNA controlling their gene expression using three web servers viz., TargetScan, mirPath and miRDB.

Results: The present study provides the *in silico* evidences that seed region present in the microRNAs miR-29-a, miR-29b and miR29c have the putative binding site in the 3'-UTR region of TBX21 transcription factor of $CD4^+$ T helper (Th1), which may suppress the Th1 specific protective immune response. Development of Th2 type specific immune response can be suppressed by binding of miR-135 microRNA over the 3'-UTR region of GATA-3 transcription factor of Th2 specific $CD4^+$ T helper cells. Interestingly, miR-21 and miR-24