Permit Title : Laboratory trial on inhibition of Ghanaian medicinal drug (*cryptolepis sanguinolenta*) on hepatitis b virus replication in human liver cell.

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Permit Duration : Three (3) years

Background

The type 1 interferon (IFN-1) response signalling pathway of the innate immune system plays a vital role in antiviral and anticancer immunosurveillance (Fuertes *et al.*, 2013; Robertsen, 2018). Cognizance of these biological effects of the endogenous IFNs, recombinant IFNs have been clinically approved and have successfully been used in the treatment of cancers, viral infections, and immunological disorders (Bekisz *et al.*, 2010; Chi *et al.*, 2017). However, in low and middle-income countries, IFN-based therapies are limited by the unavailability and high cost (Rosenthal & Graham, 2016). Due to these limitations associated with recombinant IFNs, there is a huge demand for small molecules or natural products that are cheaper, available and could substitute for or mimic endogenous IFNs (Khiar *et al.*, 2017).

Cryptolepine is the main bioactive alkaloid derived from a West African medicinal PLANT/TISSUE *Cryptolepis sanguinolenta* (Ansah & Mensah, 2013). The aqueous root extract of *Cryptolepis sanguinolenta* has been used for years as a tonic that is often taken daily with no reported toxicity and hence, has been used in Ghana for the treatment of malaria (Bugyei *et al.*, 2010). Besides its plasmodiocidal activity, it has been reported that cryptolepine has anticancer properties via topoisomerase II inhibition and induction of DNA damage (Pal & Katiyar, 2016), has anti-inflammatory effects via the suppression of the NF-κB signalling (Olajide *et al.*, 2009). This study is aimed to establish the effect of cryptolepine on the antiviral and anticancer IFN-1 pathway.

Specific aim 1: *To determine the cytotoxic effects of cryptolepine effects on the human hepatoma cell line HepG2 and subline HepG2.2.15.* Cryptolepine is cytotoxic at high dose and hence, its maximum non-toxic concentration to the cells will be assessed using MTT-based assay at 24, 48 and 72 hours post cryptolepine treatment.

Specific aim 2: *To establish the mechanistic effects of cryptolepine on the IFN-1 signalling pathway*. Dual luciferase reporter gene assay will be used in the cells where the IFN-1 pathway will be switched on in the presence or absence of the cryptolepine. The ISRE reporter assay will be used to assess the regulatory effects of the cryptolepine on the pathway. To establish the mechanisms by which the cryptolepine regulates the pathway, the cells would be cultured and treated appropriately as above. RT-qPCR and western blot will be used to assess the key signalling elements at the transcript and protein level respectively.

Specific aim 3: *To investigate the effects of cryptolepine alone or in combination with interferon on viral replication using the hepatitis B virus as a model.* HepG2.2.15 cells would be treated with or without cryptolepine and interferon Extracellular viral markers such as HBsAg and HBeAg will be assessed by ELISA. Also, viral DNA will be quantified by qPCR.

Expected outcome: The study is expected to generate the baseline data to potentially repurpose C. sanguinolenta aqueous root extract for the treatment of chronic viral hepatitis which could help more than 3 million people in Ghana and over 100 million people in sub-Saharan Africa.