CELL CYTOTOXICITY TESTING OF KERICHO HERBAL COMPOUND

Laboratory Technicians: Rency Lel and Susan Ramdahin
Supervisors/Investigators: Elijah Songok , PhD and Blake Ball, PhD

This compound was presented by Paul Chepkwony, PhD of Kericho County and Dr. Mitch Medina, PhD and Maria Jaylo, MD as a probable anti-HIV/AIDS Compound. Our preliminary investigations were to be in two steps:

i. Compound cell cytotoxicity studies
ii. In vitro HIV Inhibition studies with the confirmed safe concentrations of the compound

We have carried out the first part of the investigations. The second part requires a longer duration (at least 18 months) to work on various cell lines and various HIV isolates. This second part will also require a formal scientific proposal to be submitted to the KEMRI Ethical Review committee for approval. Similarly any outcome that shall arise from the inhibition testing will have to be approved by the KEMRI Publication committee. This will ensure that any results that shall arise will have the seal of KEMRI.

We hence only present here the results of the compound cell cytotoxicity tests. We however caution that these preliminary data shall not be used in any way for publication, circulation or marketing of the product. It is for the information of the three investigators only.

The Kericho compound was tested with peripheral blood mononuclear cells (PBMCs) derived from donor blood samples. The cytotoxicity tests was done in parallel and compared with the outcome from Bovine Serum Albumin (BSA) and a human plant food compound, Soya bean, as controls.

Step 1: Dissolving of the compounds

BSA that was used was in liquid form and hence didn't need to be dissolved. It was at a concentration of 2mg/ml and before every experiment it was reconstituted to the required concentration using media or PBS (Phosphate buffered salt) then filtered using a syringe and a filter.

Soya was dissolved in PBS but it left some particles and so DMSO was added and heated in water bath to aid in dissolution. The dissolved soya was filtered then aliquoted to a final stock concentration of 100µg/120µl.

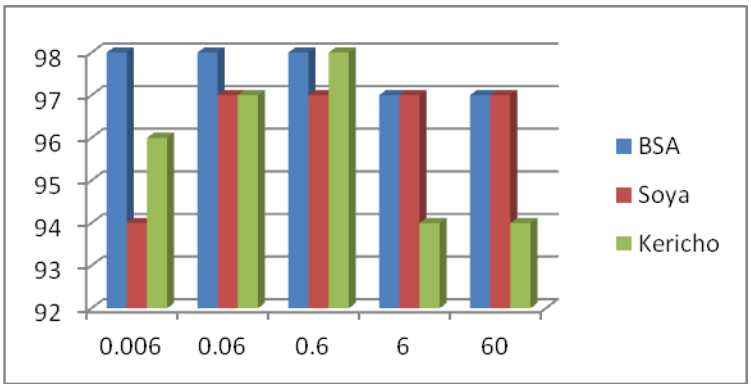
The Kericho compound was dissolved in PBS but it jelled (became jelly) and so DMSO was added and similar to above heated in water bath. It fairly dissolved and was filtered and aliquoted to a final stock concentration of 100µg/120µl.

Step 2: Cytotoxicity studies.

PBMCS were isolated from whole donor blood using the ficoll paque method . Recovered cells were washed, resuspended, counted using tryphan blue. In a 48 well plate, media and cells derived from PBMCs were cultured in different concentrations of the above compounds and then incubate for five days at 37ºC, 5 % CO_2 . On a daily basis, 350 µl of cell suspension was pippeted out from every well (until day 5) and cell viability staining was performed with 20 µl of the solution mixed with 20 µl of trypan blue and counted to find out the number of live cells. For detecting selective toxicity, the count was express as % . **i.e % of viable cells = (number of live cells) / (total number of cells) × 100**

Results

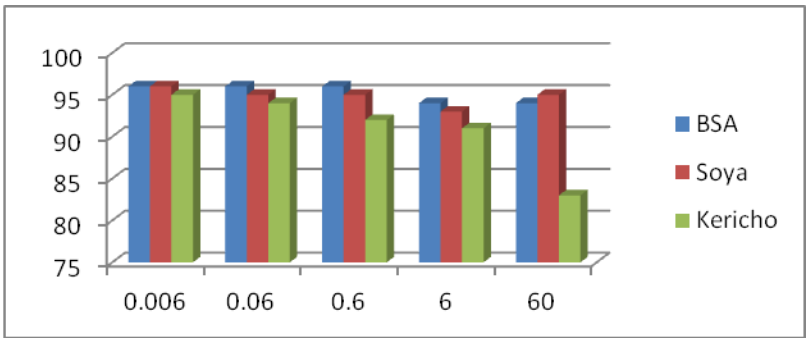
Figure 1: Cell viability Day 3



Compound concentration in ug/ml

At 60 ug/ml, the highest concentration, the cell viability is a little bit lower in all compounds as compared to viability at the other concentrations. At 0.006 ug/ml, the lowest concentration, the cell viability is a higher for BSA. For soya and Kericho compound the viability is a little lower at this concentration compared to the others but this does not mean they are toxic to the cells since viability was still above 90%.

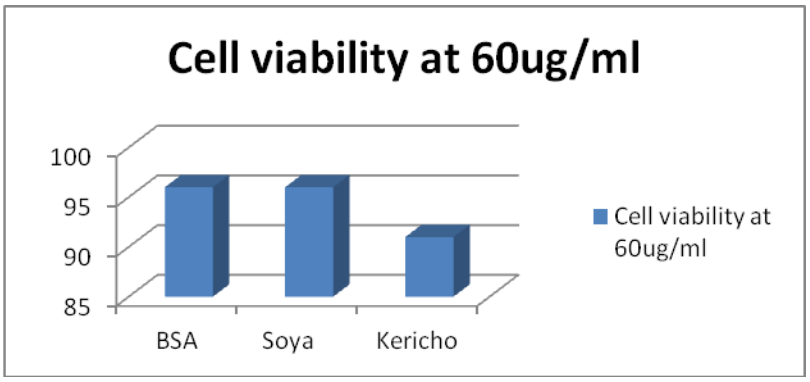
Figure 2 : Cell viability Day 4



Compound concentration in ug/ml

Since the compounds were not toxic to the cells, the viability shows that they continued to multiply every other day with a slight difference in viability when compared to the different compounds

Figure 3: Cell viability Day 5



The cells continued to grow even in day 5 and all of them had viability above 90% including the Kericho compound even though its viability may seem lower than the rest of the compounds.

Conclusion

At a concentration of 60ug/ml, the Kericho compound was non cytotoxic to cells. We recommend to move forward with in vitro HIV inhibition studies.