5.6 Effects of a Decoction of *Nigella Sativa, Hemidesmus Indicus* and *Smilax Glabra* on Human Hepatoma HepG2 Cell Integrity

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ABSTRACT

Introduction
In Sri Lanka, a herbal decoction comprised of *Nigella sativa* (seeds), *Hemidesmus indicus* (root), and *Smilax glabra* (rhizome) has been used for many years by a particular family of indigenous medical practitioners (personal communication, Ayurvedic physician, Dr. N. Jayathilake) for the treatment of cancer, despite the lack of scientific evidence to prove or disprove its therapeutic efficacy. Recent *in vivo* investigations have demonstrated that the above decoction can offer significant protection against diethylnitrosamine induced hepatocarcinogenic changes in rats.

Objective
The aim of the present study was to investigate how changes in liver cell integrity could contribute to the anti-hepatocarcinogenic actions of the decoction comprised of *Nigella sativa, Hemidesmus indicus* and *Smilax glabra.*

Methods and materials
For experimental purpose, HepG2 cells were cultured in T25 plates ($1x10^6$ cells/ plate) in DMEM supplemented with 10% foetal bovine serum. Cells in test plates were exposed to different doses of the decoction (10µg/ml, 80µg/ml, and 160µg/ml) for a total of 72h. Cells in control plates were maintained in the same manner without exposure to the decoction. Cellular integrity in both test and control was assessed by (a). microscopic examination of cell morphology, (b). evaluation of lactate dehydrogenase (LDH) release from cells, cellular ATPase activity and intracellular reduced glutathione (GSH) status at the end of 6h, 24h, 48h and 72h incubation with or without the decoction respectively.

Results
Results demonstrate that in comparison with control cells, in the cells exposed to the decoction even at the lowest dose of 10µg/ml, there was a 12.99% increase in the leakage of cellular LDH along with a 14.79% decrease in cellular ATP'ase. Although there was a decrease in cellular GSH also, it was interesting to note that at each time point (6h, 24h, 48h and 72h), the cellular GSH level in test cells was marginally higher than the corresponding values in control cells. Reduction in cell growth was microscopically apparent even as early as 6h post-incubation.

Conclusion
The overall outcome of this study indicates a potent cytotoxicity towards human liver cancer cells once exposed to the decoction of *Nigella sative* (seeds), *Hemidesmus indicus* (root), and *Smilax glabra* (rhizome). This direct effect on cells helps confirm the results of recent *in vivo* investigations, which demonstrated a protection against chemically induced hepatocarcinogenesis in rat liver. The marginal increase in GSH subsequent to decoction exposure is supportive of a possible anti-oxidant role of the decoction, which is essential for protection against chemically induced cancers. Therefore, it may be concluded that cytotoxicity and antioxidant activity may be two mechanisms through which the DC mediates its anti-hepatocarcinogenic actions.