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An investigation of the anti-carcinogenic mechanisms of a Sri Lankan herbal remedy and its ability to protect against radiation induced tissue injury

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Abstract

Decoction comprised of *Nigella sativa* (seeds), *Hemidesmus indicus* (roots) and *Smilax glabra* (rhizome) has traditionally been used to treat malignancies of various origins. Recent *in-vivo* and *in-vitro* studies have confirmed that the above decoction is effective against HCC, the commonest primary malignancy in the liver which accounts for highest mortality rate. However, the exact mechanism/s by which the decoction mediates its anti-hepatocarcinogenic activity is poorly understood. Use of herbs and their derivatives has also been reported to be beneficial for the patients suffering from severe adverse side effects during radiotherapy. Therefore the present study was carried out with the aims of investigating (a) the possible mechanisms by which the decoction mediates its claimed anti-hepatocarcinogenic activity, and (b) its ability to protect against radiation induced cellular damages.

Anti-carcinogenic mechanisms were evaluated by assessing the effects of the decoction on (a) oxidative stress, (b) cell signaling and host immune response, (c) malignant cell proliferation, and (d) expression of key genes associated with carcinogenesis. Possible radioprotection mediated by the decoction was evaluated by examining the protection against cytogenetic damages induced by bleomycin, a known radiomimetic drug, in human peripheral blood lymphocytes *in vitro*.

Results showed that the decoction could mediate a potent anti-oxidant effect by; (a) stimulating both enzymic (GPx, SOD, and GST activity) and non-enzymic (GSH) anti-oxidant defenses in rats bearing DEN-induced early hepatocarcinogenic changes *in vivo*, and (b) scavenging of nitric oxide (NO) and Diphenyl-2-picrylhyrazyl (DPPH) *in vitro*.

Free radical induced activation of NF-κB plays a key role in cell proliferation, apoptosis and modulation of inflammation by activating an array of its down-stream

targets. In the present study, it was observed that oral administration of the decoction could mediate a significant inhibition of hepatic NF-κB activity through hepatic IKKα/β suppression in C3H mice bearing early hepatocarcinogenic changes. Such inactivation was further confirmed by the down-regulation of two key down-stream targets of NF-κB transcription factor; TNFα and IL-6. These two cytokines actively participate in co-ordination of the host inflammatory response and are potent stimulators of NF-κB activation. Therefore, inhibition of these cytokines is considered to be vital in breaking the cytokine mediated NF-κB activation cycle, thus restricting the expression of its downstream targets associated with signal transduction, cell cycle regulation, apoptosis and inflammation.

Generalized inhibition of inflammation mediated by the decoction was also evident by (a) significant reduction in carrageenan induced rat paw oedema formation, (b) human red cell membrane stabilization, and (c) inhibition of NO production by carrageenan induced rat peritoneal cell infiltrate. Reduction in $CD4^{+}$ clonal expansion in response to oral administration of the decoction also supports the immunomodulatory potential of the decoction.

Cellular oxidative stress induced mutations lead to expression of functionally defective genetic products. Altered and/or defective expression of onco-suppressor, $p53^{wt}$ and cell cycle regulator, $p21^{WAF1}$ are considered to be more critical during carcinogenesis. Upregulation of hepatic $p53^{wt}$ and $p21^{WAF1}$ expression in C3H mice bearing early hepatocarcinogenic changes by the oral administration of the decoction observed in the present study strongly justify its claimed anti-hepatocarcinogenic activity. Human hepatoma (HepG2) cells treated with the decoction *in-vitro* also showed a significant; (a) morphological changes under microscope, (b) overall reduction in cell activity, (c) reduction in cell survival, and (d) cellular necrosis.

Therefore, it is reasonable to hypothesize that the restriction of malignant cell survival and proliferation could be mediated by $p53^{wt}$ and $p21^{WAF1}$ over-expression in the presence of the decoction.

Claimed anti-oxidant role of the decoction may influence its observed protection against cytogenetic damages mediated by radiomimetic drug; bleomycin, in human peripheral blood lymphocytes. This protection was evident by a significant reduction in (a) chromosomal aberration, (b) formation of micronuclei, and (c) γ-H2AX foci yield.

Overall findings of the study suggest that the decoction mediates its anti-hepatocarcinogenic effects by modulating multiple targets that are closely associated with carcinogenesis. Further the ability of the decoction to modulate expression of key genes associated with carcinogenesis produce a new insight with the possible molecular mechanisms by which the decoction may be of benefit in the therapy of hepatocellular cancer. The decoction also has the ability to protect against radiation induced cytogenetic damages, thus provides an added advantage for cancer the patients who are treated with radiotherapy. Therefore, the present study strongly suggests for further investigation of the decoction in search for novel chemotherapeutic leads.