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Investigation of the effects of a traditional Sri Lankan Medicine on Hepatocarcinogenesis

by

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Investigation of the effects of a traditional Sri Lankan Medicine on hepatocarcinogenesis

S. S. Iddamaldeniya

ABSTRACT

Hepatocellular carcinoma is among the eight leading causes of cancer deaths worldwide with a clear tendency to increase further. Therapeutic possibilities for this are very limited and prognosis is usually poor.

Several plant-based treatments are being recommended for cancer patients by traditional medical practitioners of Sri Lanka. However, none of these has been subjected to scientifically controlled investigation to validate their anti-cancer potential. One of these is a decoction comprised of *Nigella sativa* seeds, *Hemidesmus indicus* root and *Smilax glabra* rhizome.

In the present investigation protection against Diethylnitrosamine (DEN) –induced hepatocarcinogenesis was investigated in Wistar rats by the decoction using the medium term bioassay system of Ito, based on a two-step model of hepatocarcinogenesis. In previous studies, garlic has been shown to protect, rat liver against DEN-induced carcinogenesis. Therefore, it was used as a positive control in the present study.

Objectives of the investigation were to determine,

1. whether a decoction of *Nigella sativa*, *Hemidesmus indicus* and *Smilax glabra* inhibits glutathione S-transferase (GST-P) expression in rat liver.

2. whether this decoction has any anti-tumour potential.

3. whether this decoction has any toxic side effects.

4. the mechanism/s of action/s by which the decoction mediates its anti-tumour activity.

Four studies (Studies 1,2,3, and 4) were conducted to achieve the above four objectives. In study 1, the short-term effects of the decoction on GST-P+ expression in rat hepatocytes were investigated. Carcinogenic potential in study 1 was scored by comparing the number, area and staining intensity of GST-P positive foci and number of cells/cm^2 of the foci in the livers of rats treated with the decoction (test 1 and test 2) or garlic (positive control, control 2) for 10 weeks with those of the corresponding group (control 1) of rats given DEN and distilled water. Decoction dose 1 (4g/ kg body weight/day) corresponding to the normal therapeutic dose, was administered to the test group of rats, while dose 2, was given to the test 2 group of rats, provided a higher dose (6 g/kg body weight/day).

Treatment with decoction dose 1 reduced significantly, (a) the number and area of GST-P positive foci, (b) number of cells/cm^2 of foci, (c) staining intensity of GST-P positive foci ($P<0.01$) compared with animals in control 1. Treatment with decoction dose 2 resulted in a further significant reduction in the above parameters ($P<0.001$).

The reduction mediated by dose 2 was similar to that produced by garlic (20 mg/kg body weight/day).

In study 2, the effects of long-term treatment (for 9 months) of the decoction on tumour development were investigated. Two groups were used- DEN only treated group (DEN-control) and DEN+ decoction treated group (6g/kg body weight/day; test study). During the post mortem of rats after nine months, one hepatocellular adenoma (HA) was found in the DEN control group. Haematoxylin and Eosin staining of liver sections confirmed the HA and revealed altered hepatocyte nodules which may progress to HA. DEN and decoction treated group showed no HA. There were also very few nodules of altered hepatocytes which precedes HA. Reticulin stain was done to confirm the HA and to see whether it has progressed up to the hepatocellular carcinoma (HCC). DEN control group showed well-preserved reticulin framework of normal liver, which confirms that HA, has not yet progressed up to HCC. Test group showed expected normal liver reticular pattern.

Toxic effects of the decoction were investigated in study 3. Treatment with decoction dose 1 or dose 2 for three months had no adverse effects on the liver function (as assessed by its effects on serum levels of alanine and aspartate aminotrasnferase and alkaline phosphatase). Histopathological studies indicated that no significant histological changes had occurred in any of the major body organs (liver, kidney, lung, heart, stomach and duodenum) investigated.

No significant changes in haematological parameters (red blood cell count, white blood cell count, haemoglobin concentration, packed cell volume, mean corpuscular haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin concentration) were observed during treatment with this decoction. The investigations also demonstrated that the decoction did not possess anti- ovulatory, anti-implantation, and anti-spermatocytogenic properties.

The LD_{50} study showed that even at a dose of 40 times the highest dose (6 g/kg body weight/day), used in other experiments of the study, the decoction did not cause any mortality. Long-term (3 months) treatment with the decoction did not cause any changes in average feed consumption, average body weight gain, and body weight : liver weight ratios and the general behaviour of the animals.

In study 4, preliminary investigations were carried out to determine possible mechanism of action by which the decoction mediates its anti-cancer effects. Tests done for anti-oxidant activity showed that the dcoction significantly increases the activity of blood glutathione peroxidase and superoxide dismutase ($P<0.05$) with little radical scavenging activity. Studies on immunomodulatory activity indicated that the decoction could stimulate production of T lymphocytes and NK cells (CD8 and NK receptors) although the results were not statistically significant.

Overall results indicate that the decoction comprised of *N. sativa* seeds, *H. indicus* root and *S. glabra* rhizome can protect against chemically induced hepatocarcinogenesis with no significant toxic effects even when it was

administered for a period of three months. Antioxidant activity and immunomodulation are two possible mechanisms by which the decoction mediates its anti-carcinogenic activity.