SUMMARY

The present study evaluates cytoprotective, antioxidant, immunomodulatory and adaptogenic potentials of seabuckthorn (*Hippophae rhamnoides* L.). The study was initially performed *in-vitro* using immune cells such as lymphocytes and macrophages. Immune cells are the first line of defense against invading microorganisms and are sensitive to changes in oxidant-antioxidant balance because of the production of ROS as part of their normal physiological function. These cells offer an excellent model system to study the antioxidant and immunomodulatory activity of herbs. Water and alcoholic extracts of leaves and fruits of seabuckthorn were evaluated for cytoprotective activity in doses ranging from 1µg/ml to 10mg/ml. The alcoholic leaf and fruit extracts at the concentration of 500µg/ml provided optimal protection and inhibited chromium induced cytotoxicity significantly. Chromium (VI) as potassium dichromate and Sodium Nitro Prusside (SNP) were used to induce oxidative damage. The SBT extracts protected the immune cells against chromium and SNP induced cytotoxicity, free radical production, DNA fragmentation, apoptosis and maintained the antioxidant status similar to control cells. The extracts also stimulated lymphocyte proliferation even in the absence of stimulants like LPS/Con A indicating a significant immunomodulatory activity. The leaf extract showed higher cytoprotective, antioxidant and immunomodulatory activity than the fruit extract. The leaf extract alone stimulated interleukins IL-2 and γ-IFN production indicating the stimulation of cell mediated immune response.
To validate the results obtained in the *in-vitro* study, the antioxidant and immunomodulatory activity of the leaf extract was also evaluated in animal model using male albino rats. The animals were divided into various groups comprising six rats in each group. Three different concentrations of the leaf extract were given orally, 50, 100 and 250 mg/Kg body weight with the help of a gastric canula and the control group was maintained on saline containing 0.1% Tween-80. Oxidative stress was induced in rats by force-feeding of 1ml potassium dichromate equivalent to a dose of 30mg/Kg BW of chromium (VI) for 30 days. Food and water intake by the animals were monitored daily and body weight was measured weekly. The animals were then sacrificed by cervical dislocation and weight of various organs of the body was determined. Blood samples of the animals were taken and analyzed for GSH, MDA and antioxidant enzymes such as GPx and SOD. CPK, SGPT and SGOT activity in the serum were also determined. The results showed that 100 and 250mg/Kg BW leaf extract protected the animals from chromium induced oxidative stress. Administration of leaf extract maintained the antioxidants GSH, GPx and SOD levels identical to the values observed in control untreated animals. The leaf extract was also found to be hepatoprotective and maintained the SGPT and SGOT activity comparable to that of control animals. The histopathological studies of liver, kidney and spleen also showed that the extract was able to provide protection to the animals against from chromium induced toxicity. The leaf extract alone stimulated S-RBC induced delayed type hypersensitivity response but did not affect the antibody levels. This is in confirmation with our *in-vitro* observations. The leaf extract alone stimulated IL-2, γ-IFN production in the absence of concanavalin A but did not have any significant effect on IL-4 production. These observations demonstrate that the leaf extract of
seabuckthorn has significant immunomodulatory activity and specifically activates the cell-mediated immune response.

Antistress and adaptogenic activity of the leaf extract was evaluated using cold, hypoxia and restrain (CHR) multistress model. The animals were exposed to hypoxia in a decompression chamber maintained at 5°C and low oxygen tension of 428 mm Hg. The overnight fasted rats were administered 100mg/Kg BW of leaf extract with the help of a gastric canula. The rats were kept in a restrainer and a rectal probe was inserted 2 cm past the rectum. The colonic temperature of the rats was monitored continuously every minute using an Iso-Thermex recorder. When the animals attained a rectal temperature of 23°C (Tr 23), they were taken out of the chamber and allowed to recover to normal colonic temperature at atmospheric pressure in a room maintained at 32±1°C. The alcoholic leaf extract of seabuckthorn was found to have a significant adaptogenic activity, as revealed by a delay in attaining the Tr 23°C and a faster recovery pattern as compared to control animals. There was a significant increase in MDA levels in plasma and significant decrease in erythrocyte GSH levels in animals exposed to CHR stress. However, administration of leaf extract maintained the MDA and GSH levels similar to the values observed in control animals.

These observations suggest that seabuckthorn leaf and fruit have a significant cytoprotective, antioxidant, immunomodulatory and adaptogenic activity. The seabuckthorn leaf and fruit extracts can be developed as plant drug or nutraceutical to increase the antioxidant status and strengthen the immune system which in turn may be useful in enhancing the adaptogenic competency of the organisms subjected to multiple environmental stress.