# 26

# Physiological Effects of Seabuckthorn (*Hippophae rhamnoides*) Fruit Pulp and Seed Oils

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## SUMMARY

Seabuckthorn (*Hippophae* L.) is a plant, fruit of which is quite rich in nutrients with medicinal properties. The medicinal value of the pplant has been documented as early as 8th Century in Tibetan System of Chinese Medicine and was recognized in the Chinese Pharmacopeia in 1977. In China and Russia, seabuckthorn berries and oils have been used as important raw materials of health products (functional foods and natural medicines) and cosmetics during the last a few decades. As the fruit is very rich in anti-oxidants like vitamin C, E, carotenoids etc., seabuckthorn seed and pulp oils have been shown to slow down the oxidation process and to stabilize the membrane structure in animal models. Electron microscopic examination showed that seabuckthorn oil protected mitochondria of liver cells from cold exposure-caused damages. Seabuckthorn seed oil has also shown antagonistic effects againt cyclophosphamide-induced suppression of specific and nonspecific immune response in mice. Seabuckthorn seed oil has been used as an adjuvant treatment to patients with different cancers receiving chemotherapy. The seed oil treatment increased the lymphocyte transforming rate and the E rosette-forming rate. In addition, the oil treatment alleviated the hemotoxic effects and the gastrointestinal disturbance caused by chemotherapy. Seabuckthorn oil was reported to be effective in treating superficial skin burns. Topical treatment with petroleum ether-extracted press cake oil significantly accelerated the epithelization and granulization of the scalded skin of rabbits. Topically applied seabuckthorn berry oil (hexane-extracted) speeded up the healing process of wound of rabbit skin, as compared to sunflower oil. Peel oil and pulp oil showed better curative effects on skin wounds in rats than seed oil. Seabuckthorn oils have also been used to treat mechanical perforation/damage of the tympanic membrane.

Placebo-controlled, double-blind studies were carried out to investigate the effects of seed and soft part oils of seabuckthorn on Atopic dermatitis (AD). AD patients took 5 g of seed oil, soft part oil or paraffin oil daily for

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4 months. During follow up, dermatitis improved in the pulp/peel oil and seed oil groups. The results suggest positive effects of α-linolenic acid on AD. Curative effects of seabuckthorn berry extracts on chemically-induced gastric ulcer in have been investigated in rats. Seabuckthorn seed oil has been used clinically to treat the chronic cervicitis. Seabuckthorn seed oil was topically used to treat children suffering from ulcerative stomatitis. Workers also used its seed oil, pulp oil and a mixture of seed and pulp oils in the treatment of esophagitis caused by irradiation therapy of different cancers in patients.

Hyperlipemia, arterial sclerosis and thrombus formation involve many aspects of lipid metabolism. Dietary lipid composition has a significant effect on the development of the disease processes. Results of animal experiments and the few clinical trials carried out have shown possible positive effects of seabuckthorn oils on this group of dieseases by influencing plasma lipid levels, LDL-oxidation, membrane lipid peroxidation, blood coagulation and immune functions. Most of the investigations on anti-cancer effects of seabuckthorn have concentrated on extracts from bark, and 5-hydroxy tryptamine has been suggested to be the main responsible component. Preliminary studies have also suggested anti-cancer effects of seabuckthorn oils. Toxicological studies have also been carried out to investigate the safety aspects of the oils, and the results have suggested no toxicity of seabuckthorn oils.

*Key words: Seabuckthorn extract, Fruit soft and seed oils, Antioxidant properties, Immune function, Wound, Burns, Scalds and irradiation dermatitis, Atopic dermatitis, Peptic ulcer, Cervicitis, Ulcerative stomatitis and Irradiation esophagitis, Plasma lipid levels, Arterial sclerosis and Thrombus formation, Cancer and safety.*

## ABBREVIATIONS

AAP: Acetaminophen; AD: Atopic dermatitis; G-6-PD: Glucose-6-phosphate dehydrase; GSH: Reduced glutathione; GSH-Px: Glutathione peroxidase; HDL: High density lipoprotein; $LD_{50}$: Half lethal dose; LDL: Low density lipoprotein; MDA: Malondialdehyde; NK: Natural killer cell; PCE: Polychromatophilic erythroblasts; $PGE_2$: Prostaglandin $E_2$; SGPT: Serum glutamic pyruvic transaminase; SGOT: Serum glutamic oxaloacetic transaminase; SOD: Superoxide dismutase.

## INTRODUCTION

Seabuckthorn berry has been used in Tibetan and Mongolian medicines for more than one thousand years in the treatment of sputum and cough, and to improve the blood circulation and the function of the digestive system. Since 1977, the seabuckthorn berry has been recorded in the Chinese Pharmacopeia. During the second half of the 20th century, numerous animal experiments and clinical studies have been carried out to investigate the physiological effects of different fractions and products such as juice and oil from the berry, especially in China and Russia.

In China and Russia, seabuckthorn berries and oils have been used as important raw materials of health products (functional foods and natural medicines) and cosmetics during the last a few decades. Recently, seabuckthorn oils are becoming more and more popular as special food supplements and ingredients in Japan, Europe and North America at a time when information on the effects of oils in clinical nutrition is also increasing in the west. The existing literatures on physiological effects of seabuckthorn are published in many different languages including English, German, French, Romanian etc, but mostly in Chinese and Russian. The aim of the authors is to make a comprehensive review of the literature so that the information is also available to the readers without sufficient skills in many of these languages.

## ANTIOXIDATIVE EFFECTS

Oxidation is a key process in the development of aging, tissue damage and many diseases. Oxidation of membrane components leads to damage of membrane structure, resulting in malfunction

or even total loss of function of cell membranes. Seabuckthorn seed and pulp oils have been shown to slow down the oxidation process and to stabilize the membrane structure in animal models (*1-3*). Malondialdehyde (MDA) levels in the tissue and cell membranes have been measured as an indicator of lipid oxidation (*1-7*). Effects of seabuckthorn oils on the activities of many enzymes involved in oxidation have also been determined (*1-10*).

Effects of seabuckthorn seed oil on the structural stability and function of cell membranes have been investigated in rats (*1, 2*). Thirty-three Wistar rats (12-week-old), randomly divided into three groups, lived for eight weeks on (A) 100 per cent basic diet (vitamin E, 5.2 mg/100 g; Se, 0.2 ppm), (B) 90 per cent basic diet plus 10 per cent seabuckthorn seed oil (vitamin E, 15.7 mg/100 g; Se, 0.2 ppm), or (C) 90 per cent basic diet plus 10 per cent seabuckthorn seed oil supplemented with sodium selenite (vitamin E, 15.7 mg/100 g; Se, 2.0 ppm) (*1, 2*). The seabuckthorn oil-supplemented diets decreased the MDA level in the cell membrane of erythrocyte ghosts by 68 per cent and in liver homogenate by 35 per cent, as compared with the 100 per cent basic diet (*1, 2*). Seabuckthorn seed oil supplementation increased the glutathione peroxidase (GSH-Px) activities in erythrocytes by 16 per cent (*1, 2*). Levels of sialic acid (by 46 per cent) and the sulfydryl group (-SH, by 73 per cent) (*2*), as well as Na, K-ATPase activity (by 44 per cent) (*1*) in the erythrocyte cell membrane, were increased by the oil supplementation. Supplementation with selenite seemed to have a synergisic effects with seabuckthorn seed oil (*1, 2*).

Antioxidative effects of seabuckthorn pulp oil and vitamin E (at the same dose as in seabuckthorn pulp oil) have been compared in rats under cold exposure (*8*) and guinea pigs (*3*). Sixty-four Wistar rats were randomly divided into four groups living on different diets for three weeks (*8*). In the positive and negative control groups, animals were given basic feed (vitamin E content, 46.2 mg/kg,). In the vitamin E supplemented group, animals were given basic feed plus vitamin E (final vitamin E content in feed, 180.2 mg/kg). In the seabuckthorn oil group, basic feed supplemented with seabuckthorn oil (20 per cent, final vitamin E content in feed, 180.2 mg/kg) was given to the animals. Animals in the positive control group were raised at room temperature (20 ± 2°C) for the whole experimental period, those in the other groups at 0-2 °C after the first week. At the end of the experimental period, MDA levels in erythrocytes in the negative control group and the vitamin E supplemented group had significantly increased compared with the positive control group (by 75 per cent, $p < 0.01$), whereas the levels in the seabuckthorn oil group were close to those of the positive control group. Cold exposure decreased activities of GSH-Px and superoxide dismutase (SOD) in blood (by 14 per cent and 20 per cent, respectively) and liver (by 26 per cent and 35 per cent, respectively) in the negative control group without a significant effect on the seabuckthorn oil supplemented group. Electron microscopic examination showed that seabuckthorn oil protected mitochondria of liver cells from cold exposure-caused damages. In an eight-week experiment carried out with guinea pigs (*3*), addition of seabuckthorn pulp oil (40 g/kg feed, vitamin E content 50 mg/kg feed) to a vitamin E free diet decreased MDA-induced erythrocyte hemolysis and the MDA level of erythrocyte cell membranes. The oil supplementation increased the level of sulfydryl groups and the activities of Na,K-ATPase and glucose-6-phosphate dehydrase (G-6-PD) of erythrocyte cell membrane (*3*). Vitamin E was less effective as compared with seabuckthorn oil, suggesting that vitamin E was not the only components in seabuckthorn pulp oil responsible for the antioxidative effects observed (*3, 8*).

Antioxidative effect and protective effect of seabuckthorn oils on the myocardium were studied in mice (*10*). Seventy ICR mice were divided into different groups, receiving oral seabuckthorn oil or negative control in different exercise programs. The serum Aspartate aminotransferase (GOT), SOD and MDA levels in heart and ultrastructure of myocardial cells were evaluated at sedentary state, immediately after exercise and 24 hours after exercise). Seabuckthorn oil increased the SOD levels

after exercise and decreased the MDA levels at sedentary state and 24 hours after exercise. In addition, the oil treatment maintained the integrity of mitochondria, endoplasmic reticulum and myofibre in the myocardium.

Lipid peroxidation induces MDA production in the cell membranes. Interactions between MDA and membrane components such as protein and phospholipids lead to changes in structure and function of the membranes. The level of the sulfydryl group (-SH) in membrane proteins reflects the level of reduced gluthathione (GSH) and reductive capacity of cells, and indicates the oxidation status of cell membranes. Se-GSH-Px metabolizes MDA and protects the cell membranes. Sialic acid is a component of glycoproteins on the surface of cell membranes. As a component of receptors, it participates in many physiological processes such as recognition, adhesion and signal transduction. The content of Sialic acid in erythrocyte membrane has been found to decrease with cell senescence and aging of man (*11, 12*). Decreased acitivities of Na, K-ATPase and Γ-6-PD are indicators of increased lipid peroxidation and –SH oxidation. The results (*1-3, 7, 10*) indicating the anti-oxidative and membrane-stabilizing effects of seabuckthorn oils are supported by the investigations suggesting protective effects of the oils against tissue damage caused by chemical intoxication (*4-7, 9*).

Three different models of chemical-induced hepatic damage have been employed in the investigation of liver-protective effects of seabuckthorn (subsp. *sinensis*) seed oil in the mice (*4*). The seed oil was given intragastrically to mice at two different doses, 2.38 g/kg and 4.75 g/kg, twice a day. The oil treatment lasted for one, two and three days, respectively, in tetrachloromethane-induced, ethanol-induced and acetaminophen (AAP)-induced models. Twenty-four hours (four hours in ethanol model) after the last oil treatment, hepatic injuries were induced by intraperitoneal injection of 0.1 per cent tetrachloromethane (10 ml/kg), peroral 50 per cent ethanol (0.15 ml/10g), or intraperitoneal injection of AAP. Sixteen hours later, serum glutamic pyruvic transaminase (SGPT) activity and hepatic MDA levels were determined. Seed oil (2.38 g/kg and 4.75 g/kg) significantly decreased the hepatic MDA levels, as compared with negative control (saline) in all the three models (by 32-35 per cent in the high dose group). Seed oil (4.75 g/kg) decreased the SGPT activity in tetrachloromethan (by 80 per cent) and AAP-induced (by 63 per cent) models, and increased hepatic GSH level (by 50 per cent) in the AAP model. The liver-protecting effects of the seed oil have also been investigated using a tetrachloromethane-induced model in the rats (*5*). Tetrachloromethane (25 per cent in corn oil, 0.2 mg/100g, intragastric) increased the activities of SGPT (by 8-fold) and serum glutamic oxaloacetic transaminase (SGOT) (by 65 per cent) and levels of MDA (by 36 per cent) and triacyglycerol (1.5 by times) in liver homogenate. The increases in SGOT activity and MDA level were completely inhibited by per oral seabuckthorn seed oil (9.5 g/kg, twice per day for three days) given before the tetrachloromethane treatment. The oil treatment suppressed SGPT activity by 50 per cent. Electron microscopic examination of the structure of hepatocyte showed less damage in endoplasmic reticula, mitochondria and nuclei in the seabuckthorn oil group, as compared to the negative control group (treated with saline).

Similar experiments have been carried out with seabuckthorn (subsp. *sinensis*) pulp oil using tetrachloromethane–and AAP-induced models in mice (*6*). Pulp oil (2.25 g/kg per oral, twice per day for three days) inhibited the increases in hepatic MDA level, induced by tetrachloromethane and AAP (*6*). Tetrachloromethane-induced increase in SGPT activity was also suppressed by the pulp oil treatment (*6*). The hepatic GSH level was higher in the seabuckthorn pulp oil group than in the negative control group in the AAP model (*6*). Oil from peels of seabuckthorn berries have shown protective effects against myocardic injuries caused by high doses of intragastric vitamin $D_3$ (*7*). Thirty-two male Wistar rats divided into three groups received per oral 2.5 per cent Tween 80 saline

solution (1 ml/day) for 12 days (normal control group), 2.5 per cent Tween 80 saline solution for 9 days, followed by 25 per cent vitamin $D_3$ emulsion (1 ml/day) for three days, or 5 per cent seabuckthorn oil emulsion (in 2.5 per cent Tween 80 saline salution, 1 ml/day) for 12 days, with 25 per cent vitamin $D_3$ emulsion added for the last three days. In the group receiving only Tween 80 solution and vitamin $D_3$, the activities of SOD and GSH-Px in myocardium homogenate were significantly decreased, and the myocardic MDA level significantly increased compared with the normal control group. The corresponding levels in the seabuckthorn oil treated group were close to those of the normal control group.

Inhalation of sulphur dioxide ($SO_2$) caused oxidative damage and resulted in decreased activity of Γ-6-PD and GSH-Px and reduced levels of GSH in brain, lung, liver, and kidney of mice (*9*). Intraperitoneal injection of seabuckthorn seed oil maintained the balance of gluthione redox system and significantly prevented the oxidative damage to lung induced by inhalation of $SO_2$. Seabuckthorn seed oil also significantly decreased the level of thiobarbituric acid-reactive substances and increased the activity of GSH-Px in lung (*9*).

Intragastric treatment of seabuckthorn extract of undefined composition for three weeks significantly suppressed the oxidative stress caused by intraperitoneal injection of nicotine, reflected in plasma vitamin E level and content of MDA and activities of antioxidative enzymes in erythrocytes (*13*).

Effects of seabuckthorn seed oil on LDL oxidation have been investigated with *in vitro* oxidation models (*14*). In the $Cu^{2+}$-catalysed oxidation model ($CuCl_2$, 50 μmol/l), LDL (0.1 μmol/l) from normal subjects was incubated in the presence of the seed oil at different concentrations (0, 0.3, 0.6, 1.2, 2.4, 4.8 per cent, v/v) at 37°C for 24 hours. In cell-catalysed oxidation models, the LDL samples (0.03 μmol/l) were incubated together with mouse abdominal macrophages or bovine endothelial cells in solutions containing the seed oil at different concentrations (0, 0.04, 0.08, 0.16, 0.32 per cent, v/v) at 37°C for 24 hours. Addition of the seed oil significantly decreased the formation of MDA and conjugated diene in the $Cu^{2+}$-catalysed oxidation system. MDA levels in the cell-catalysed oxidation system were also significantly decreased (by 18-75 per cent) by addition of the oil. The effects of seabuckthorn seed oil were dose-responsive in both types of oxidation model.

Oils from seeds and soft parts of seabuckthorn berries differ considerably in their fatty acid composition. Seed oil is rich in linoleic and aa-linolenic acids, whereas oil from soft parts contains high levels of palmtic and palmitoleic acids. Oils from both fractions are rich in tocopherols (vitamin E) and phytosterols. These compounds and the high levels of carotenoids in oils from the soft parts probably have played a major role in the anti-oxidative effects found in both types of seabuckthorn oils.

## IMMUNE FUNCTION

Effects of seabuckthorn seed oil on the immune functions have been investigated mostly with experimental models in the mice. Seabuckthorn seed oil has shown antagonistic effects againt cyclophosphamide-induced suppression of specific and nonspecific immune response in mice (*15, 16*). Zheng *et al.* (*15*) treated Kunming mice with intragastric seabuckthorn seed oil (extracted with petroleum ether) at different doses (2.5 ml/kg, 5 ml/kg, 10 ml/kg, and 20 ml/kg, per day) for seven days. On the fourth day, mice were given an intraperitoneal injection of sheep red blood cells (SRBC) and cyclophosphamide (0.06 per cent, 0.2 ml/mouse, as an immune-suppressing agent). On the eighth day, the mice were given an intraperitoneal injection of chicken red blood cells (5 per cent, 0.5 ml/mouse). Three hours later, the mice were killed and phagocytic activity of abdominal macrophages

was determined. Seabuckthorn oil at doses of 2.5 ml/kg and 5 ml/kg antagonised the suppressive effect of cyclophosphamide on phagocytic activity of abdominal macrophages ($p < 0.01$). The seed oil (2.5 ml/kg, 5 ml/kg, 10 ml/kg) treatment also increased the number of SRBC-primed spleen plasma cells. However, the seed oil at a dose of 20 ml/kg did not show any effects. Intragastric treatment with petroleum ether-extracted seed oil (2.5 g/kg, once) increased the NK activity of spleen cells in cyclophosphamide-treated mice (*16*).

Seabuckthorn seed oil has also been shown to improve the nonspecific (*17*) and specific (*18*) immune functions of normal mice. Healthy Kunming mice were randomly divided into a seabuckthorn seed oil group receiving intraperitoneally injected seabuckthorn seed oil (0.01 ml/g per day, for four days) and a control group receiving the same volume of saline solution (*17*). The seed oil treatment significantly increased the activities of acidic phosphatase and nonspecific esterase of abdominal macrophages and the intensity of luminol chemilluminiscence of macrophage cell suspension, the latter reflecting the intensity of oxidation burst of macrophages (*17*). A seabuckthorn seed oil treatment (intragastric, 0.2 ml/day for four days) increased the number of antibody-secreting cells and the titer of serum antibody compared with a water treatment in SRBC-primed Kunming mice (*18*).

Seabuckthorn seed oil was used as an adjuvant treatment to 100 patients with different cancers receiving chemotherapy (*19*). The first period of chemotherapy carried out without seabuckthorn seed oil was used as a control. During the second period of the chemotherapy, the patients took seabuckthorn seed oil, 3 x 15 ml per day (total amount taken 500-800 ml). The seed oil treatment increased the lymphocyte transforming rate and the E rosette-forming rate. In addition, the oil treatment alleviated the hemotoxic effects and the gastrointestinal disturbance caused by chemotherapy (*19*).

An intraperitonal injection of seabuckthorn berry powder solution (*20*) and an intragastric treatment with seabuckthorn juice powder (*21*) increased the phagocytic function of abdominal macrophages, the lymphocyte transforming rate and SRBC-primed formation of serotonin and antibody in serum in mice (*20, 21*). Intragastrically-given seabuckthorn berry juice increased spleen NK cell activity and secretion of interleukin-2 in normal mice (*22*). Intraperitoneal injection of seabuckthorn juice increased NK cell activity in spleen of mice inoculated with $S_{180}$ tumour cells (*23*). In addition to vitamin C and flavonoids, known to possess immune-regulating properties, lipophilic components in the juice/berry pulp probably contributed to the effects observed.

## SKIN

### Burns, Scalds and Irradiation Dermatitis

Seabuckthorn seed oil was topically used in the treatment of 32 patients with burns (12 cases of 1st degree burns, 18 cases of light 2nd degree burns, 2 cases of deep 2nd degree burns) (*24*). Thirteen patients had 10 per cent, one patient had 15 per cent, and four patients had more than 20 per cent of skin area burned. The oil treatment was followed by heat treatment of the burned skin surface. Each treatment period lasted 5-7 days. All the patients were reported to be cured in 7 days' treatment (*24*). Vlasov (*25*) compared the effects of seabuckthorn oil, "Visnevskii emulsion" (composition not defined), streptomycin, sulfanilamide emulsion, cod liver oil, potassium permanganate and antibiotics in the topical treatment of 122 patients with 1st, 2nd, and 3rd degree skin burns. Seabuckthorn oil was reported to be effective in treating superficial skin burns, but without clear advantages over other treatments.

Topical treatment with petroleum ether-extracted press cake oil significantly accelerated the epithelization and granulization of the scalded skin of rabbits (*26*). The oil treatment showed a clear anti-inflammatory effect (*26*). Lebedeva *et al.* (*27*) reported comparable effects of topically applied

seabuckthorn oil, β-sitosteryl oleate, and β-sitosteryl palmitate on the epithelization process of NaOH-produced burns on mice skin. Histological and histo-chemical studies showed that seabuckthorn oil, β-sitosteryl oleate, and β-sitosteryl palmitate accelerated fibrinogenesis and collagen formation in the wounded area, suggesting that phytosterols were probably the main components responsible for the tissue-regenerative effects of seabuckthorn oil (*28*). However, the authors (*27*) gave no information as to whether seed oil or soft part oil was used or the concentrations of β-sitosteryl oleate and β-sitosteryl palmitate preparations.

Second degree irradiation dermatitis (with inflammation, wet peeling, secretion and partial ulcer) caused by x-ray irradiation on legs of NIH female mice were treated with topically applied seabuckthorn seed oil, pulp oil and combined seed/pulp oil (*28*). After five to seven days of treatment, inflammation and secretion were reduced. After two weeks of treatment, ulcer areas were healed, and skin colour became normal. The irradiation dermatitis in the negative control group (topically treated with saline solution) worsened during the two weeks (*28*). Thirteen patients with vulvitis and perineal inflammation, and eight patients with irradiation dermatitis in the axilla (armpit) and clavicle area were treated with topically applied (three to four times a day) seabuckthorn oil (*28*). Of the patients, 85 per cent improved after treatment. The results suggested positive effects of seabuckthorn oils on the acute dermatitis caused by irradiation therapy for different cancers.

## Wounds

Topically applied seabuckthorn berry oil (hexane-extracted) speeded up the healing process of wound of rabbit skin, as compared to sunflower oil (*29*). Wounds of 78 $mm^2$ completely healed after 14 days' treatment with seabuckthorn oil. The wounds treated with sunflower oil healed after 21 days of treatment. Histological study showed that seabuckthorn oil stimulated tissue regeneration in the wound areas (*29*). Peel oil and pulp oil showed better curative effects on skin wounds in rats than seed oil (*29*).

Seabuckthorn oils have also been used to treat mechanical perforation/damage of the tympanic membrane (*30, 31, 32*). Mechanical perforations were made on the tympanic membranes of guinea pigs divided into four groups. The perforations in three groups were topically treated with 4 per cent urea solution, 1 per cent hyaluronic acid solution, or seabuckthorn oil, respectively, once every second day for 15 days, while the fourth group received no treatment (negative control group). After 4 days of treatment, 80 per cent of perforations closed in both the seabuckthorn oil group and the hyaluronic acid group, whereas in the negative control group and the urea solution group, the perforation-closing rate was only 6 per cent and 25 per cent, respectively. After 10 days and 30 days, the perforations had healed better in the seabuckthorn oil group than in the other groups ($p < 0.05$) (*32*). Patients with traumatic rupture/perforation of the tympanic membrane were treated with topically applied seabuckthorn oil (an oil-saturated cotton sheet was placed over the perforation area, and the oil was added to the cotton sheet 2-3 times a week) (*31, 32*). The rupture/perforation healed after 2-28 days of treatment (*31, 32*). In the clinical experiment of Fan *et al.* (*32*), forty-seven out of fifty-six patients healed, the average healing time being 13 days (*32*). The healing effects of sea buckhtorn oil were possibly related to acceleration of the regeneration of the epithelial tissue of the tympanic membrane (*31*).

Saturated nonbranched alcohols with even numbers of carbon atoms ($C_{22}$-$C_{28}$) have been suggested to be the major bioactive components possessing tissue-regenerative effects in the oils (*33*).

## Atopic Dermatitis

Atopic dermatitis (AD) is an inflammatory skin disease characterised by dry, itching and lichenous skin. Impaired epidermal barrier function and abnormal synthesis of eicosanoids, the mediators of

epidermal inflammation and hyperproliferation, are involved in these skin changes. Acylceramides rich in linoleic acid (18:2n-6) are essential components of the epidermal barrier system. Polyunsaturated fatty acids in phospholipids are essential components for the maintenance of the proper fluidity of cell membranes, which in turn is important for signal transduction and substance transportation. Polyunsaturated fatty acids are also precursors of eicosanoids. Abnormality in the level of polyunsaturated fatty acids has been found in plasma, skin, umbilical cord and breast milk of mothers of children at high risk of atopic diseases (*34-38*). This abnormality in AD patients is thought to be due to deficiencies in intake, incorporation and metabolism of essential fatty acids (*36, 37*).

Both evening primrose oil (rich in n-6 polyunsaturated fatty acids) and fish oil (rich in n-3 polyunsaturated fatty acids) have been shown to have a beneficial effect on AD. Seabuckthorn seed oil contains high levels of linoleic (18:2n-6, 35–43 per cent) and α-linolenic acids (18:3n-3, 20-36 per cent). Oil from soft parts of the berries is especially rich in palmitoleic acid (16:1n-7, 12–46 per cent) (*39-43*). Placebo-controlled, double-blind studies were carried out to investigate the effects of seed and soft part oils of seabuckthorn on AD (*44-47*). AD patients took 5 g of seed oil, soft part oil or paraffin oil daily for four months. During follow up, dermatitis improved in the pulp/peel oil and seed oil groups (*44*). A significant improvement was also observed in the paraffin oil group and was suggested to be due to placebo effects (*44*). Supplementation with seed oil increased the proportion of linoleic, ά-linolenic, and eicosapentaenoic (20:5n-3) acids in plasma phospholipids, and α-linolenic acid in plasma neutral lipids (*44, 46, 47*). Positive correlations were found between symptom improvement and the increase in the proportions of α-linolenic acid in plasma phospholipids and neutral lipids after 1 month of supplementation (*44*). The results suggest positive effects of α-linolenic acid on AD. The effects of α-linolenic acid were probably related to its metabolisation to eicosapentaenoic acid, which in turn resulted in competitive inhibition of the synthesis of the 4-series leukotrienes from arachidonic acid by the increased synthesis of the 5-series leukotrienes. Soft part oil treatment increased the proportion of palmitoleic acid in both plasma phospholipids and plasma neutral lipids (*44*). However, the changes in the fatty acid composition did not correlate with symptom improvements in the pulp/peel oil group (*44*).

Dietary supplementation with the two oils did not lead to significant changes in the fatty acid compositions of skin glycerophospholipids of AD patients (*45, 47*). The high level of carotenoids in the pulp oil, and vitamin E and phytosterols in both seed and pulp oils may also have contributed to the improvement of AD symptoms (*44, 45*). The supplementation with the oils did not affect the surface roughness of skin in lesion-free areas of AD patients (*47*).

## MUCOSA

### Peptic Ulcer

Peptic ulceration is a defect of the gastric or duodenal mucosa as a result of the failure of the mucosal defence mechanism against peptic digestion. Breakdown of the mucosal defence is the major reason for the development of gastric ulcer, whereas increased secretion of acid is suggested to be of more importance in the pathogenesis of duodenal ulceration (*48*). Increasing evidence suggests the causative role of *Helicobacter pylori* in the development of gastric and duodenal ulcers (*49, 50*).

#### Animal Experiments

Loginov *et al.* (*51*) reported curative effects of seabuckthorn berry extracts (intragastric administration, 1 ml/day, for 12 days) on acetic acid-induced gastric ulcer in Albino rats. The epithelialization of the ulcer edges, histological changes in the ulcer zone and the proteolytic activity

of gastric mucosa extracts were examined in evaluation of the effects of seabuckthorn oil (*51*). Zhou *et al.* (*52*) tested the effects of seabuckthorn seed oil on experimental models of gastric ulcer induced by reserpine, water-immersion and pylorus ligation in rats. In the pylorus ligation model, immediately after ligation of the pylorus, seed oil was given to the animals via duodenal injection (200 mg/kg), supplemented with one subcutaneous or intraperitoneal injection (200 mg/kg) five hours later. Fifteen hours after the supplemented injection, the animals were killed and the ulcer in the stomach was examined. Compared with the negative control (1 per cent Tween 80 water emulsion) group, ulcer formation was inhibited in the two seabuckthorn administration groups, by 80 per cent and 97 per cent, repectively. Peanut oil showed no protective effect against pylorus-ligation-induced gastric ulcer, as compared with the negative control (*52*). Intragastric administration of seabuckthorn seed oil (200 mg/kg per day for three days) showed a protective effect against water-immersion–and reserpine-induced gastric ulcers, the ulcer inhibition rate being 51.7 per cent and 68.4 per cent, respectively (*52*). Che *et al.* (*53*) reported protective and curative effects of seabuckthorn seed oil (intragastric, 2 ml/kg per day, or 5 ml/kg per day for seven days) against water–immersion-, reserpine–and acetic-acid-induced gastric ulcers in rats. Xing *et al.* (*54*) have shown both preventive and curative effects of seabuckthorn seed and pulp oils on different experimental models of gastric ulcer in rats.

Zhou (*55*) investigated the effect of seabuckthorn oil on reserpine-induced gastric ulcer in rats. After the ulcer induction, 80 rats were divided into 4 groups, receiving, daily, seabuckthorn oil, rapeseed oil, saline or cimetidine 7 days. A significant curative effect was observed with seabuckthorn oil compared with rapeseed oil and saline. The curative effect was also better than that of cimetidine. The positive effect was thought to be related to the protection of mucosa and inhibition of the gastric acid secretion (*55*). However, the author did not state whether the oil was from seeds or from the soft parts of the berries. In an experiment carried out by Jiang *et al.* with four different models of gastric ulcer in rats (*56*), seabuckthorn seed oil did not show any protective effect in water immersion and acute reserpine models. In the chronic reserpine model and the acetic acid model, seed oil showed significant curative effects (better than cimetidine) (*56*). Neither seabuckthorn pulp oil, nor vitamins A and E showed significant effects on acetic-acid-induced gastric ulcer, as compared with normal vegetable oil (*56*). Mironov *et al.* (*57*) tested the effects of intragastrically administered seabuckthorn seed, pulp and peel oils on the healing process of acetic-acid-induced gastric ulcer in rabbits. All three oils significantly accelerated the healing process, as compared to sunflower oil (*57*).

### Clinical Trials

In a clinical experiment, involving 30 cases of peptic ulcer (*58*), the patients took, orally, 12 seabuckthorn oil capsules daily for one month. A curing rate of 76.6 per cent and a total effective rate of 96.7 per cent were reported (*58*). However, the author did not give any information regarding the nature of the oil, whether it was from seeds, soft parts or whole berries. Nor was the oil content in the capsules defined. Nikitin *et al.* (*59*) used seabuckthorn oil as adjuvant therapy in the treatment of 116 peptic ulcer patients, 71 with duodenal ulcer and 45 with gastric ulcer. The authors reported that application of seabuckthorn oil relieved pain and accelerated the repair process of epithelial tissue.

### Mechanism of Effects of Seabuckthorn Oils

The possible mechanism and components behind the anti-ulcerative effect of seabuckthorn oils have not been well investigated, though widely discussed by the authors of most of the publications.

Jiang *et al.* (*56*) compared the effects of pulp oil, "salad oil" (composition not defined), β-sitosterol and β-sitosterol-β-D-glucoside on acetic-acid-induced gastric ulcer in mice. Pulp oil and β-sitosterol did not show any significant effect, whereas β-sitosterol-β-D-glucoside significantly decreased the

size of the ulcer area, as compared to the "salad oil". The authors reported the content of β-sitosterol-β-D-glucoside to be ten times higher in seed oil than in pulp oil. Thus, β-sitosterol-β-D-glucoside was suggested to be the anti-ulcer component in seabuckthorn seed oil (*56*). Jiang and Li (*60*) also reported β-sitosterol-β-D-glucoside to be the anti-ulcerative component in seabuckthorn oil. Xiao *et al.* (*61*) compared the anti-ulcerative activity of β-sitosterol and β-sitosterol-β-D-glucoside, using acetic-acid-induced and water-immersion-induced gastric ulcer models in the rats. Compared with sesame oil, both compounds showed an anti-ulcerative activity comparable to that of cimetidine. β-sitosterol was more effective than β-sitosterol-β-D-glucoside in the acetic acid model, whereas the opposite was true in the water immersion model (*61*). The effecting mechanisms of β-sitosterol and β-sitosterol-β-D-glucoside were not clear. Increasing the hydrophobicity of the mucosa surface, retarding the gastric emptying process and diluting the ulcer-inducing factors were proposed to be the possible effecting mechanisms of the two compounds (*61, 62*). Unsaponifiable components from peel and pulp of seabuckthorn berries inhibited the proteolytic activity of pepsin in mice, whereas the unsaponifiable extract from seed oil did not show this activity (*57, 63*).

There is evidence suggesting that the anti-ulcerative effects of seabuckthorn oils might be related to their antioxidative activitiy (*1-3, 8, 64-66*).

An increased level of lipid peroxidation and decreased level of tocopherols in plasma have been found of patients with gastric ulcer, as compared with healthy subjects (*64*). Seabuckthorn oil (seed oil or soft part oil not defined) treatment (intragastric) (dose not defined) of two weeks improved the ulcer symptom, decreased the MDA level and increased the level of tocopherols in plasma. Intragastric administration of seabuckthorn oil (Freon 12-extracted from dried berry residue after juice pressing) decreased the level of MDA in gastric mucosa of rats with electric-shock-induced gastric ulcer (*65*). Lipid extract from seabuckthorn berry residue (after oil pressing) (intragastric, 5 ml/kg per day for seven days) prevented the formation of ulcer in the reserpin model and speeded up the curing process of acetic-acid-induced gastric ulcer in rats (*66*). The lipid extract decreased the MDA level in the lipid secretion on the surface of gastric mucosa and SGPT activity in the acetic-acid-induced gastric ulcer model in rats (*66*). In addition, ingestion of the lipid extract maintained the level of aa-tocopherol and inhibited the countercurrent diffusion of proton in the gastric wall of rats (*66*).

Parenteral prostaglandins inhibit the secretion of gastric acid in man (*67*). The cytoprotective properties of prostaglandins have been characterized (*68*). In doses too small to inhibit gastric secretion, prostaglandins protect the gastric and intestinal mucosa from a wide range of ulcerogenic or necrotizing stress, including strong acid, strong base, ethanol, hypertonic saline, boiling water, non-steroidal anti-inflammatory agents, and prednisolone (*69-72*). In cytoprotective doses, prostaglandins are known to stimulate gastric mucus production (*73*), and to maintain the potential difference of gastric mucosa by stimulating active sodium transport (*74, 75*). Exogenous and endogenous prostaglandins, mostly of the E series have been shown to be effective in the treatment of peptic ulcer (*76*).

Investigations on the effect of seabuckthorn oils on the production of prostaglandins in the animals and human beings have not been reported. However, n-6 and n-3 polyunsaturated fatty acids in plant seed oil and fish oil have been shown to have effects on the production of prostaglandins in both animals and man (*77-81*). Grataroli *et al.* (*81*) reported a decreased production of *ex vivo* $PGE_2$ of gastric mucosa resulting from an eight-week increase in the dietary n-6/n-3 ratios of fatty acids in rats. Ingestion of fish oil was reported to reduce ethanol-induced damage to duodenal mucosa without inhibiting gastric acid secretion in healthy humans (*80*). The effect of fish oil was possibly related to regulating the formation of leukotrienes. Evening primrose oil, a source of linoleic and γ-linolenic acids, has been shown to enhance prostaglandin $E_2$ synthesis in rat antral biopsies (*78*). Using pylorus-

ligated rats, Doyle *et al.* (*77*) showed the protective effects of dihomo-γ-linolenic acids and arachidonic acid against ethanol-induced ulceration, accompanied by a significant rise in the level of intraluminal prostaglandins. Tarnawski et al. also showed that arachidonic acid was protective against aspirin-induced injuries in rat stomach (*79*).

The results (*67-81*) listed above are indirect evidence suggesting that the positive effects of seabuckthorn oils, especially seed oil, on peptic ulcer may be related to modification of the eicosanoid syntheis in gastric or duodenal mucosa.

## Cervicitis

Seabuckthorn seed oil and Shayoushuan (suppository containing seabuckthorn seed oil (50 per cent) and undefined ingredients from five herbs) have been used clinically to treat the chronic cervicitis (*82-85*). Wu et al. (*82*) treated topically 79 patients with Shayoushuan, and 50 patients with seabuckthorn seed oil. Of the patients, 70 per cent were cured by the Shayoushuan treatment, 27 per cent improved, and the total effective rate was 97 per cent (*82*). In the seabuckthorn seed oil group, 24 per cent of patients were cured, 50 per cent improved and the total effective rate was 74 per cent (*82*). The authors hypothesized that the effects of seabuckthorn seed oil are related to the content of carotenoids and vitamin E, which accelerated the epithelization process. In another experiement, Wu *et al.* (*83*) treated 140 patients suffering from chronic cervicitis with Shayoushuan, and 37 patients with Fuyanshuan (Ningxia Traditional Chinese Medicine Factory, P.R. China, composition not defined) as a positive control. In the Shayoushuan group, 65 per cent of the patients were cured after 12 days of treatment, 31 per cent improved, and the total effective rate was 97 per cent. In the positive control group, the curing rate was 43 per cent, and the total effective rate 89 per cent (*83*). Shayoushuan was more effective than the positive control ($p < 0.05$) (*83*). Wang (*85*) treated 30 patients suffering from partial erosion of the cervix with topically sprayed seabuckthorn seed oil, once a day. All the 30 cases were cured after three months of treatment (*85*).

Che *et al.* (*86*) tested the anti-inflammatory and analgesic effects of Shayoushuan on mice. The mice (40-52 in each experiment) were divided into four groups. Two groups were treated intragastrically with Shayoushouan, 1 g/kg and 1.5 g/kg, respectively, and one group with 0.2 ml 3 per cent tween 80 water emulsions (negative control), once a day for three days. The fourth group was treated only once on the third day with positive control medicines (sinomenine in body twisting and hot plate experiments, brufen in ear inflammation and peritonitis experiments, intraperitoneal injection). Compared with the negative control group, Shayoushuan, 1 g/kg and 1.5 g/kg, reduced the frequency of body twisting induced by 0.7 per cent acetic acid solution (0.1 ml/kg, ip.) ($p < 0.05$) and reduced croton-seed-oil-induced ear inflammation ($p < 0.05$, $p < 0.01$) in mice (*86*). Shayoushuan, 1.5 g/kg, ig., significantly increased the pain threshold in the hot plate experiment ($p < 0.05$) and decreased the amount of peritoneal effusion in acetic–acid-induced peritonitis ($p < 0.05$) in mice, compared with the negative control (*86*).

The occurrence of cervicitis is related to the low level of carotenoids and vitamin E in the tissues. Carotenoids and vitamin E possibly enhance the maturing and differentiation process of epithelial cells (*87, 88*). The positive effect of the seabuckthorn suppository was suggested to be related to its high content of carotenoids and vitamin E (*85*).

A pilot scale open study was carried out to study the effects of oral administration of seabuckthorn oil capsules containing a mixture of supercritical $CO_2$ extracted seed oil and berry oil (*89*). Five patients of chronic vaginal inflammation took 6 capsules (3 g oil) per day for twelve weeks. Of the five patients, three severe cases had a significant improvement in dry and itching and inflamed vaginal mucous

membranes after use of seabuckthorn oil. This was shown both in subjective symptom improvement and as clear decrease in VAS score as high as 66 per cent.

## Ulcerative Stomatitis and Irradiation Esophagitis

Seabuckthorn seed oil was topically used (3-4 times a day) to treat sixty children (4 months–12 years old) suffering from ulcerative stomatitis (*90*). The patients were treated during the same period with intramuscular injection of chymotrypsin (once a day, 800-4000 IU). All the 60 cases significantly improved after two days of treatment. Fifty-five cases were cured after 3-5 days of treatment, and two severe cases were cured after 8 days of treatment (*90*). Seabuckthorn seed oil was also topically used to treat 35 stomatitis patients with leukemia (*91*). A small piece of cotton soaked in the oil was placed on the surface of the ulcer area and renewed every 4-6 hours. Thirty stomatitis patients were treated with Xibitai anti-ulcer membrane (the composition was not defined) as a positive control group. All the patients took vitamin $B_2$ orally during the treatment period. After 3 days, the stomatitis of 6 patients in the oil group was cured, but none in the positive control group ($p< 0.05$). After 5 days, 19 patients (54.2 per cent) were cured in the oil group, but only 1 (3.3 per cent) in the positive control group ($p < 0.05$). After 7 days of treatment, the total effective rate was 100 per cent in the oil group and 73 per cent in the positive control group (*91*).

Li *et al.* (*92*) tested the effects of seabuckthorn seed oil and compound seabuckthorn seed oil containing other herbal components on experimental models of irradiation esophagitis in SD rats. After irradiation, 60 rats, randomly divided into 6 groups, received, twice a day, 1) no treatment (negative control), 2) 1 ml seabuckthorn oil orally, 3) seabuckthorn oil compound, 4) terramycin, 5) Jingan II (composition not defined), and 6) preparations containg the same herbal components as treatment 3) without seabuckthorn oil. The treatment started on the fifth day after the irradiation and lasted eight days. The effects of different treatments were compared in terms of activity and survival rate of the animals in different groups during the first five days of treatment. In addition, the pathological and histochemical changes in the epithelium of the esophagus after seven days of treatment were investigated. In the negative control group, inflammation and hyperemia of esophagus mucosa were observed, the esophagus wall became thin and smooth, covered by secretion, and ulcer areas were clearly seen. In the compound seabuckthorn oil group, slight mucosa inflammation was found on the esophagus wall, the wrinkled wall structure was clear, there was slight secretion, but no ulcer was seen. There was less epithelial damage caused by irradiation in the compound seabuckthorn oil group, as compared to the other groups (92).

Zhang *et al.* (*27*) also used seabuckthorn seed oil, pulp oil and a mixture of seed and pulp oils in the treatment of esophagitis caused by irradiation therapy of different cancers in 22 patients. The oil was taken orally, three times per day for three days. Nineteen (86.4 per cent) patients improved after the treatment (*27*). The authors suggested that carotenoids and vitamin E in the oils might be responsible for the curing effects (*27*). The antibacterial (*27*) and tissue-regenerative effects (*89*) were also discussed. A synergic effect between the other herbal components and seabuckthorn oil was also highlighted (*92*).

Dry mouth (xerostomia) is a common clinical complaint affecting up to 40 per cent of adults, mainly women and the elderly. Dry mouth, often a symptom of salivary gland dysfunction, provokes unpleasant oral symptoms such as burning mouth, difficulty in speaking, chewing and swallowing. Oral treatment with seabuckthorn oil (a mixture of pulp oil and seed oil) capsules (5 g oil per day) for four weeks effectively relieved the dry mouth symptoms and improved the general condition of mouth mucosa (93).

The authors are referred to a comprehensive review on the effect of seabuckthorn oils on the mucous membranes (89).

## EFFECTS ON PLASMA LIPID LEVELS, ARTERIAL SCLEROSIS AND THROMBUS FORMATION

Hyperlipemia, arterial sclerosis and thrombus formation involve many aspects of lipid metabolism. Dietary lipid composition has a significant effect on the development of the disease processes. Results of animal experiments and the few clinical trials carried out have shown possible positive effects of seabuckthorn oils on this group of dieseases by influencing plasma lipid levels (*94, 95*), LDL-oxidation (*13*), membrane lipid peroxidation (*1-3*), blood coagulation (*96, 97*) and immune functions (*15-18*).

Seabuckthorn oil (produced by Qin Yong Seabuckthorn Ltd., China) was used in the treatment of 52 cases of hyperlipemia (*94*). Each patient took 5 ml of seabuckthorn oil, three times per day. The oil treatment decreased the plasma triacylglycerol and total cholesterol level, and increased plasma HDL-cholesterol level. The treatment period needed to observe these effects varied from 10 days to 45 days, depending on the patients (*94*). Effects of the oil on hyperlipemia and atherosclerosis were also investigated by the same group using an experimental model (*94*). Intragastric treatment with seabuckthorn oil (0.3-0.5 ml) for 2-3 hours before the induction of hyperlipemia, using 75 per cent egg yolk emulsion, decreased serum triacyglycerol and total cholesterol levels of mice, as compared to the negative control (composition not defined) (*94*). Intragastric seabuckthorn oil (2 ml/chicken, twice per day for four weeks) decreased the serum triacylglycerol and total cholesterol levels and alleviated atherosclerosis formation in the breast artery in chickens living on high cholesterol diet (*94*). However, the authors gave no information about whether the oil used was seed oil or pulp/peel oil nor about the oil extraction method. In addition, original data of plama lipid levels and determination methods were not presented in the publication. Nor was the length of the whole treatment period defined. The missing information left the conclusion of this publication open to further investigation.

Patients with hyperlipemia and/or coronary heart disease (36 cases of hyperlipemia, 16 cases of coronary heart disease with hyperlipemia and 9 cases of coronary heart disease with normal plama lipid level) were treated with oral administration of concentrated seabuckthorn juice (8.3 times concentrate of the original pressed juice) (*95*). Each patient took the concentrated juice 13 ml/day. The administration period lasted for 60 days for patients without coronary heart disease, and 90 days for patients with coronary heart disease. The treatment decreased the levels of plasma triacylglycerol and cholesterol of hyperlipemia patients (TAG, from 270 ± 108 mg per cent to 179.4 ± 70.4 mg per cent; cholesterol, from 274.3 ± 26.4 mg per cent to 233.3 ± 37.5 mg per cent, before treatment and after treatment) without affecting the corresponding levels in patients with normal plasma lipid levels. The juice treatment alleviated coronary heart disease symptoms in 19 of the 26 patients treated (*95*).

A small-scale preliminary cross-over study was conducted to investigate the effects of supercritical $CO_2$-extracted seabuckthorn whole berry oil on some risk factors of cardiovascular disease (*96*). Twelve healthy men with normal levels of plasma lipids took seabuckthorn berry oil and fractionated coconut oil (control) 5 g per day for a period of 4 weeks in random order with a wash-out period of 4-8 weeks between the two oils. Seabuckthorn oil supplementation reduced the adenosine-5'-diphosphate-induced platelet aggregation reaction rate ($p < 0.05$) and the maximum aggregation (aggregation percentage at 4 min, $p < 0.01$), as compared to the fractionated coconut oil. The oil supplementation did not show any significant effect on the plasma lipid profiles of the normalipidemic men.

Twelve rabbits, randomly divided into two groups, were given intragastrically 2.5 ml/kg 20 per cent seabuckthorn seed oil emulsion or 5 per cent gum arabic gel (*97*). Two hours before and after the

oil treatment, bleeding time and coagulation time, prothrombin time, prothrombin consumption time, Factor V, Factor VII, and Kaolin partial thromboplastin time were determined. Seabuckthorn oil treatment significantly increased the blood Kaolin partial thromboplastin time (from 47.0 ± 5.7 seconds to 62.5 ± 6.5 seconds, $p < 0.01$) and decreased fibrinogen content in plasma (by 30 per cent, $p < 0.05$). The influence on the other factors was not statistically significant. The oil treatment also delayed the electrode-stimulated thrombus formation in common carotid arteries of rabbits (*97*).

Seabuckthorn oil capsules (0.25 x 6 g oil, three times per day) and Seabuckthorn Saimaitong capsules containing seabuckthorn oil (0.51 X 6 g oil and other components, three times per day) were used in the treatment of 80 cases of ischemic apoplexy (*98*). In seabuckthorn oil group, 77.7 per cent of patients (23 out of 30) improved, and 17 per cent of the patients were cured. In the Seabuckthorn Maisaitong group, 94 per cent (47 out of 50) of the patients improved, and 25 per cent of the patients were cured. The better effect observed with the Seabuckthorn Maisaitong capsule was possibly due to a synergisic effect between seabuckthorn oil and the other herbal components. The authors give no information concerning whether the seabuckthorn oil used was from the seeds of other parts of the berries. No information was given concerning the length of the treatment period either.

A seabuckthorn chocolate (normal chocolate supplemented with seabuckthorn seed oil) was tested for its effect on the hyperlipemia and atherosclerosis using animal models (*99*). Thirty-six male quails were randomly divided into three groups, fed on a high lipid diet (containing 0.5 per cent cholesterol and 2.5 per cent lard), a high lipid diet plus 10 per cent seabuckthorn chocolate, or high lipid diet plus 10 per cent normal chocolate for three months. The levels of plasma total cholesterol, HDL-cholesterol, and triacylglycerols were determined before, and two and three months after the beginning of the experiment. Compared with the starting point, the plasma lipid levels in all the three groups significantly increased after two and three months of treatment, no significant difference found among the groups. The seabuckthorn chocolate significantly decreased liver total cholesterol level, as well as the severity and frequency of atherosclerosis formation in the common artery, as compared with the normal chocolate. However, the authors did not give the concentration of seabuckthorn oil in the seabuckthorn chocolate.

Possible effects of seabuckthorn seed oil and vitamin E on the atherosclerosis were investigated using the main arterial smooth muscle cells cultured in high lipid content culture media (*100*). Addition of seabuckthorn seed oil (10 mmg/ml) to the culture media decreased the number of dead cells after five days of cell culture. Examination with scanning electron microscope showed less damage to cell membranes in seabuckthorn oil-added cell culture, as compared to the negative control (high lipid content culture without seabuckthorn oil). MDA content was decreased by 39 per cent in cells cultured with seabuckthorn seed oil. The compensatory increase in SOD activity in the cells induced by high lipid culture media was decreased (by 28 per cent) by the addtion of seabuckthorn oil. Addition of vitamin E to the cell culture showed silimar effects to those observed with seabuckthorn seed oil. The authors suggested that the protective effects of seabuckthorn oil and vitamin E were due to their antioxidative capacities (*100*).

Seabuckthorn juice contains up to 1 per cent oil and is rich in both lipophilic and hydrophilic antioxidants. The effect of sea buyckthorn juice on plasma lipids, LDL oxidation, platelet aggregation, and plasma soluble adhesion protein were studied in 20 healthy men taking seabuckthorn juice or placebo for 8 weeks (*101*). Seabuckthorn juice increased plasma HDL levels (by 20 per cent) and at the same time decreased the susceptibility of LDL to oxidation. No statistically significant effect was found of the juice on platelet aggregation, plasma intercellular cell adhesion molecule 1, or the level of total and LDL cholesterol (*101*).

## ANTICARCINOGENIC AND ANTICANCER EFFECTS

Most of the investigations on anti-cancer effects of seabuckthorn have concentrated on extracts from bark, and 5-hydroxy tryptamine has been suggested to be the main responsible component (*102*). Preliminary studies have also suggested anti-cancer effects of seabuckthorn oils (*103-108*).

*In vivo* studies carried out with rats bearing M-1 sarcoma, did not show any suppressive effects of orally given seabuckthorn oil (source not defined, 0.2 ml per day for ten days) on the growth of the tumor (*103, 104*). However, the oil treatment was reported to alleviate the hematological damage caused by treatment with antitumor agents (*103, 104*).

Intraperitoneal injection of seabuckthorn seed oil has been reported to suppress the growth of S180 and B16 tumors in mice (*105*). Intraperitoneal injection of oil from seabuckthorn press residue (125-500 mg/kg per day for five days) elongated the living period of mice pre-inoculated with S180 cells. The effect has been reported to be dose-responsive (*106*). *In vitro* study also suggested a cytotoxic effect of the oil on human leukemia cell line K562 (*106*). Additon of petroleum ether-extracted seabuckthorn seed oil to the culture media at a concentration of 0.005-1.0 mg/ml inhibited DNA synthesis of Leukemia 7712 cells, the inhibition correlating negatively with the concentration of seabuckthorn oil in the media (*107*).

Three latest animal studies (*13, 109, 110*) suggested great anti-mutagenic and anti-carcinogenic potential of seabuckthorn berry. Feeding rats with seabuckthorn berry extract (HRe-1) for three weeks significantly reduced nicotine-induced lipid oxidation (*13*). Oral application of an alcoholic extract (RH-3) from whole berries of seabuckthorn provided 80 per cent protection against radiation-induced mortality in mice (*109*). In vitro study showed that RH-3 inhibited radiation–and tert-Bu hydroperoxide-induced DNA damage in a dose-dependent manner (*109*). The study also suggested that RH-3 protects the DNA by compacting chromatin into a more radiation resistant structure (*109*).

The genetic toxicological effects of $SO_2$ were investigated by $SO_2$ inhalation simulating $SO_2$ pollution in air (*110*). The inductive effect of $SO_2$ on micronuclei formation in polychromatophilic erythroblasts (PCE) of mouse bone marrow and protective effect of seabuckthorn seed oil were studied *in vivo* (*110*). Short-term and long-term inhalation of $SO_2$ increased the frequency of micronuclei in PCE cells in a dose-dependent manner. Seabuckthorn seed oil showed a significant suppressive effect on the genetic damage caused by $SO_2$ (*110*).

## SAFETY ASPECTS

Toxicological studies have also been carried out to investigate the safety aspects of the oils, and the results have suggested no toxicity of seabuckthorn oils (*111-116*).

The mutagenicity and teratogenicity of oil from the press residue of seabuckthorn berries have been investigated (*111, 112*). In comparison with corn oil, oral administration of seabuckthorn oil (9 g/kg and 18 g/kg, per day) for 5 days did not increase the proportion of micronucleus cells in the bone marrow or the percentage of teratogenic sperm in mice (*111*). The oil given orally to female rats on the sixth day of fertilization (9 g/kg and 18 g/kg, for five days) did not show any teratogenic effect on the embryos, as compared with corn oil (*112*).

Evidence exists suggesting that seabuckthorn seed oil does not have any carcinogenic effects in mice, nor does it damage the template of DNA replication in human lymphocytes (*113*).

The acute and chronic toxicities of seabuckthorn seed oil have been investigated in mice and rats by giving the oil orally to the animals (*114*). The $LD_{50}$ was higher than 60 ml/kg and 20 ml/kg for mice

and rats, respectively. In the chronic toxicity test, the oil was given to rats at three different dosages, 2 ml/kg, 5 ml/kg, and 10 ml/kg, twice a day, for three month. The side effects observed (in the highest dose group) were loose stools, yellowing of hair (originally white), and decreased appetite. No significant effects were found on the general activity, body weight, blood or the function of liver and kidney of the animals. Histological examination of tissues of heart, liver, spleen, lung, kidney and testis did not show any harmful effect of the oil treatment.

The toxicity (acute toxicity, irritating effects on skin and mucosa and allergic effect on skin) of local application of seabuckthorn seed oil has been investigated in the animals (*115*). In the acute toxicity test, the seed oil was topically applied to healthy and wounded skin on the dorsa of rabbits 4 times in the same day (at three-hour intervals, two doses were used, 2.5 ml/kg and 10 ml/kg). The local and systemic changes in the skin and general activities was followed for seven days and compared with those treated with control (saline) solutions in the same way. None of the animals died, and no significant difference was found between the seed oil treated and the control solution treated groups. The result suggested that topical application of seabuckthorn seed oil did not have any acute toxic effect on the healthy or wounded skin surface of rabbits (*115*). At skin irritation test, the seed oil was topically used on normal and wounded skin of guinea pigs for 7 days (1 ml per day, once) and the skin was followed up for 7 days after the last oil treatment. No irritation symptom (erthyma, inflammation) was observed (*115*). In mucosa irritation test, seabuckthorn seed oil was applied to the eyes (0.1 ml) and vaginal mucosa (1 ml) of rabbits, once a day for 7 days. The animals were followed up for 7 days after the last treatment. No irritation of mucosa was observed in the oil-treated group (*115*). Repeated application of the seed oil on the skin surface of guinea pigs did not induce any allergic reaction (*115*).

## CONCLUSION

Table 26.1 presents a summary of the claims of physiological effects of seabuckthorn oils in the selected publications. Of these investigations, most have been carried out with experimental models in animals; clinical observations are of a limited number. The positive effects of the oils on the lipid peroxidation, immune fuction, mucosa and skin are obvious. More clinical trials should be carried out in the future to prove these positive effects in human beings. In addition, further investigations are needed to identify the more active principals and understand the effecting mechanisms.

Animal experiments and *in vitro* studies have also suggested anticancer and anticarcinogenic effects of the oils, although results of clinical trials are scarce. This presents an important area for studies in the future. The oils also seem to have an effect on the risk factors of cardiovascular diseases, such as inhibiting platelet aggregation and LDL-oxidation. The influence of the oils on the plasma lipid levels and the formation of atherosclerosis and thrombus are clearly open to further investigations.

Inaccuracies are found in the publications reviewed. In many papers, the authors did not define the extracting method, or composition, in some cases not even the raw materials, of the oils tested. In many animal experiments, the strains of the animals were not defined. In the clinical experiments, the basic information required by the quality criteria in human experimental nutrition research (*118*), such as the study design, the patients and their diet is generally scanty. Moreover, accurate information on control medicines is missing in some studies. This lack of information makes it difficult to interpret the results. Many claims of these investigations need to be confirmed with well-designed animal experiments and clinical trials. In addition, our knowedge of the biochemical mechanisms of the effects of seabuckthorn oil is still very limited, although components such as phytosterols, tocopherols and carotenoids have been highlighted to be the bioactive components of the oils. The role of palmitoleic acid in the observed effects of seabuckthorn oils is also an interesting topic for further investigations.

**Table 26.1: A Summary of the Claims on Effects of Seabuckthorn Oils in the Existing Publications**

| *Oils* | *Claims* | *Type of Experiment* | *Reference* | *Comments* |
|---|---|---|---|---|
| Seed oil, Pulp oil | **ANTIOXIDATION**<br>☆ Protect cell membrane from lipid peroxidation<br>☆ Conclusions supported by results<br>☆ Maintain membrane structure and functions<br>☆ Effects better than vitamin E | Animal experiments with rats and guinea pigs | *1–10, 13, 14* | ☆ Experiments well designed and well described<br>☆ Promising results<br>☆ Oil compositions not well defined<br>☆ *in vivo* human studies missing |
| Pulp oil, Seed oil, Peel oil | **CHEMICAL INTOXICATION**<br>☆ Protect heart and liver against chemical-induced damage<br>☆ Suppress lipid peroxidation and maintain the normal functions of cells | Animal experiments with mice and rats | *4-7, 10, 13* | |
| Seed oil | **IMMUNE FUNCTION**<br>☆ Antagonizes the effects of immune suppressor<br>☆ improves specific and nonspecific immune functions<br>☆ Alleviates the immune suppression caused by chemotherapy of cancer patients | Mostly animal experiments with mice,<br><br><br>Cancer patients | *15-19* | ☆ Experiments well described<br>☆ Preliminary results<br>☆ Conclusions supported by results<br>☆ Oil composition not defined<br>☆ Effecting mechanisms not investigated<br>☆ Clinical studies missing |
| Seed oil, Whole berry oil | **CARDIOVASCULAR DISEASE**<br>☆ Decrease the levels of plasma total cholesterol and triacylglycerols and increase plasma HDL-cholesterol level<br>☆ Most experiments not properly described<br>☆ Inhibit platelet aggregation<br>☆ Retard formation of sclerosis and thrombus<br>☆ Suppress LDL-oxidation | Clinical trials,<br>Animal experiments,<br>Animal experiment<br>Clinical trial<br>*In vitro* study<br>*In vitro* study | *14, 94-101* | ☆ Limited number of well-designed study<br>☆ Original data not presented (in some studies)<br>☆ Some tested oils not defined<br>☆ The claims to be confirmed by further investigations |
| Seed oil, Oil from press residue, Whole berry oil | **SKIN**<br>☆ Speed up healing of burns, wounds and scalds on skin<br>☆ Promote tissue regeneration<br>☆ Cure irradiation dermatitis<br>☆ Anti-inflammatory and analgesic effects<br>☆ Improve the fatty acid composition of plasma lipids and symptoms of topic dermatitis | Clinical trial,<br>Animal experiments with mice, rats, rabbits and guinea pigs | *24-33, 44-47, 117, 120* | ☆ Promising results<br>☆ Further studies needed many experiments not well described<br>☆ Oils (source and composition) not defined in some experiments<br>☆ Incredible curing effect on skin burns reported in some studies needs confirmation |

*Contd...*

**Table 26.1–Contd...**

| *Oils* | *Claims* | *Type of Experiment* | *Reference* | *Comments* |
|---|---|---|---|---|
| Seed oil, Berry oil, Oil from press residue | **GASTRIC AND DUODENAL ULCERS**<br>☆ Protective and curative effects on experimental gastric ulcer and duodenal ulcer.<br>☆ Increase the hydrophobicity of the surface of gastric mucosa<br>☆ Retard gastric emptying process<br>☆ Inhibit gastric secretion<br>☆ Dilute ulcer-inducing factors<br>☆ Sitosterol and sitosterol glycoside are suggested to be among the major anti-ulcerative components<br>☆ Antioxidants including tocopherols and tocotrienols and carotenoids possibly also responsible components | Mostly animal experiments, a few clinical trials | *51-61, 63-66, 89* | ☆ Clear protective effects on gastric and ntestinal mucosa<br>☆ Curative effects to be confirmed<br>☆ Experimental design, source and isolation methods and composition of the oils not well described in some investigations<br>☆ Biochemical mechanism at molecular levels of the observed effects still to be investigated |
| Seed oil | **CERVICITIS**<br>☆ Curing effect of topically applied seed oil on cervicitis<br>☆ Anti-inflammatory effects | Clinical practice | *82-86, 89* | ☆ Most of the publications better described as case reports than as scientific publications<br>☆ Results promising<br>☆ Patients with divergent physical backgrounds included in single studies<br>☆ Oil composition not defined<br>☆ Further studies are needed to confirm the claims and to investigate the mechanisms |
| Seed oil, Pulp oil | **MOUTH MUCOSA, STOMATITIS AND ESOPHAGITIS**<br>☆ Topical application of seed oil speeds up the recovery process of stomatitis<br>☆ Intragastric treatment with seed oil and pulp oil promote the reparation of irradiation esophagitis.<br>☆ Sjögren's syndrome, dry mouth | Clinical trials, Animal experiments | 28, 89-93 | |

*Contd...*

**Table 26.1–Contd...**

| *Oils* | *Claims* | *Type of Experiment* | *Reference* | *Comments* |
|---|---|---|---|---|
| Seed oil | **CANCER**<br>☆ Antimutagenic effects<br>☆ Inhibitive effect on growth of tumor cells | Animal experiments, *in vitro* studies | *9, 10, 13, 102-110* | ☆ Some experiemnts well designed and described<br>☆ Results promising<br>☆ Oil compositions not defined or not well defined<br>☆ Further investigations needed to test the effects on different tumor cell lines and to understand the mechanism<br>☆ Clinical results scarce |
| Seed oil, Pulp oil, Oil from press residue | **SAFETY**<br>☆ Intragastric administration of the oils showed no toxic effects on nervous system, blood or internal organs in acute or chronic toxicological tests<br>☆ No mutagenicity or terogenicity was observed after short period of ingestion of oil from press residue<br>☆ Ingestion of seed oil did not show carcinogenic effects in mice or damaging effects on human DNA<br>☆ No toxic, irritating or allergic effects observed in topical application of seed oil on mucosa, or skin | Animal experiments with mice, rats, cats, dogs, guinea pigs. | *111-116* | ☆ In most studies conclusions supported by results<br>☆ Some slight yellowing of hair found after ingestion of seed oil in mice<br>☆ A loose stool complained of by some subjects after use of the oils<br>☆ No toxic effects suggested |

Chinese and Russian scientists are the pioneers in the field of seabuckthorn research. Co-operation between Western, Chinese, Russian and other scientists will lead to the best use being made of the exisiting knowlege and to the most efficient progress in the field.

At the end, the readers are referred to three concise but still comprehensive reviews recently published by the authors (89, 119, 120) for a quick going-through of the health effects of seabuckthorn lipids.

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