SKIN HEALTH

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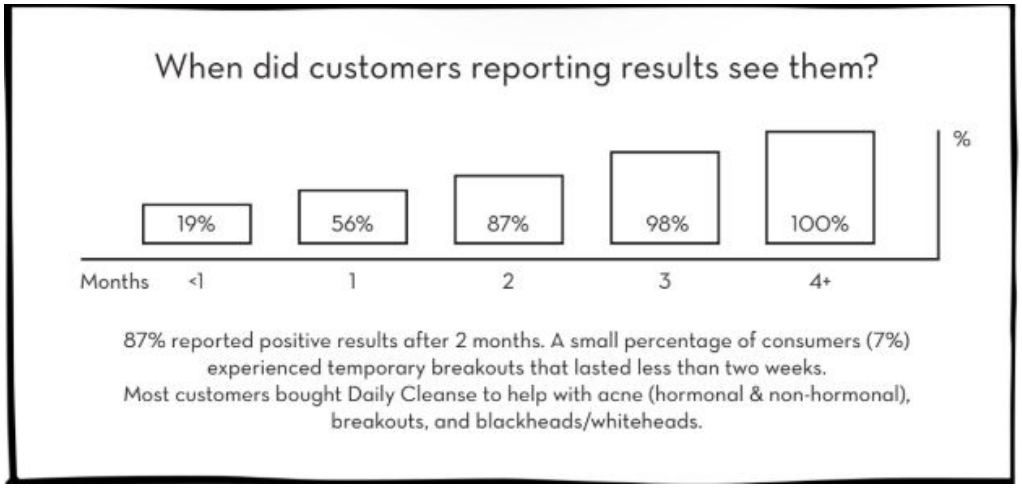
THE ROLE OF HERBAL SUPPLEMENTS IN THE SKIN & BODY'S DETOXIFICATION PROCESS

Abstract:
Daily Cleanse® was formulated to help support the body's natural detoxification processes and skin utilizing a variety of herbs, minerals and compounds that support detox. The liver is one of our primary detoxifying organs, and requires antioxidants and certain compounds for toxin conjugation to work at its highest level. The bowels, kidneys, lungs, lymphatic system and skin are important pathways for toxin removal. When detox pathways don't function optimally, the body suffers. The body also needs minerals as coenzymes for biochemical reactions to support both skin and overall health. Since skin is the body's largest organ, it can show signs of poorly functioning detox pathways such as breakouts and other skin concerns.

Intro:
Based on 600 survey responses sent to HUM customers who purchased Daily Cleanse for at least 3 months: 80% of people who used Daily Cleanse for 6-8 weeks reported improved skin, 43% reported improved self-confidence and 44% said it helped them lead a healthier lifestyle.

Table 1.



***Data was collected via customers who were taking Daily Cleanse for 3 months or more. Table 1 illustrates when users experienced results. 80% reported improved skin. Those who did not see results were omitted from table 1.*

How the Body Detoxifies:

Many different organs support detoxification in the body. The liver pulls toxins out of the body, conjugates them, and prepares them for excretion. The bowel and the kidneys eliminate conjugated toxins via feces and urine. The respiratory system filters out toxins and the lymphatic system protects the body. Lastly, skin expels conjugated toxins via sweat.

<u>Liver</u>

Liver detoxification consists of three phases. Phase one converts toxins into free radicals, phase two conjugates compounds for removal, and phase three actually transports conjugated toxins for removal. These conjugated toxins are shuttled to different areas of the body to be excreted mainly by feces, urine and sweat.

In phase one of liver detox, toxins convert into free radicals. These free radicals can damage the skin when found in high levels, and antioxidants are then needed to neutralize their impact. Chlorella, spirulina, alpha-lipoic acid, beetroot, red clover, green tea, and Oregon grape help strengthen endogenous antioxidant defenses, and thus protects cellular components from oxidation. Milk thistle has been found to increase glutathione (a powerful antioxidant that supports liver health) and impact liver cell regeneration.

In phase two of liver detox, compounds are needed to donate structural components for conjugation. Daily Cleanse contains Methylsulfonylmethane (MSM) is a sulfur-containing compound that produces a significant increase in plasma glutathione level due to its serving action as a sulfur donating agent for synthesis of new cysteine, a rate limiting precursor of glutathione production (1).

In phase three of liver detox, conjugated toxins transfer to the colon, kidneys, lungs and skin.

Excretion

Once conjugated toxins are prepared for elimination, they will be excreted through feces, urine, breath and sweat. Daily Cleanse includes ingredients that support the health of the organs needed for effective detox. Chlorella promotes healthy epithelial structure (2) and supports the body's endogenous antioxidant defense system (3), which helps keep the colon healthy. Alpha-lipoic acid, spirulina, and milk thistle promote healthy inflammatory responses (4,5,6) which keeps organs functioning at optimal capacity. Dandelion enhances the kidneys eliminatory function (7). The formula supports healthy respiratory system by supporting the body's oxidant-
antioxidant balance with alpha-lipoic acid, milk thistle, MSM and spirulina (8,9,10,11). The lymphatic system is supported by alpha-lipoic acid, MSM, green tea, milk thistle and spirulina which aid in the production of immune cells (12,13,14,15,16).

The body also undergoes many biochemical reactions that are crucial for healthy skin. It requires coenzymes like copper, manganese, selenium and zinc, which are important ingredients in the Daily Cleanse formula. It has also been reported that patients suffering from acne vulgaris appear to have lower plasma zinc levels than their healthy counterparts. (17,18)

Ingredients that support detox:

Chlorella

Although clinical studies are lacking thus far, another promising dietary supplement in the complementary treatment of acne vulgaris appears to be the single-celled green algae, chlorella. Experimental research has shown that several chlorella species inhibit lipase enzymes, free radical (ROS) and pro-inflammatory cytokines production, such as TNF-alpha, while also showing potent anti-acne activity in a test-tube setting. (18)

Experimental research also indicates that chlorophyll supports body's detoxification processes by inhibiting the activity of numerous dietary and environmental mutagens such as aflatoxins (Jubert et al. 2009), cigarette smoke, coal dust, diesel emission particles (Pratt, et al. 2007), and gamma-radiation and photosensitization (19).

Spirulina

Spirulina has become popularly known as a superfood due to the great diversity and concentration of nutrients it contains. It is rich in protein containing 63% and is rich in B12, folic acid, iron and calcium, Spirulina's concentrated nutrition makes it an ideal food supplement for people of all ages and lifestyles (20).

Research on Spirulina's health benefits has been far ranging. The antioxidant and anti-inflammatory effects have been documented in the literature. Other areas of research on Spirulina are varied; among many potential health applications researched are the protection of the liver, kidneys and the improvement of blood quality via removing heavy metals from the body (21).

Studies have demonstrated its hepatoprotective effects of Spirulina. Carotenoids extracted from a mixture of hexane alcohol:isopropyl alcohol (1:1 vol/vol) in two microalgae, among these S. platensis were mixed with olive oil and were administered orally to Wistar rats at a dose of 100 µg/kg bw/d (in terms of carotenoids). The degree of hepatoprotection was measured by means of estimating biochemical parameters, such as serum transaminases [serum glutamate oxaloacetate transaminase (GOT) and serum glutamate pyruvate transaminase (GPT)], serum ALP, total albumin, and total protein. The results were compared with those of a control group, a CCl4-induced hepatic damage group, and a group treated with synthetic β-carotene at the same dose. The protein content of the CCl4-treated group, which received a normal diet, showed a significant decrease (3.92 mg/mL), while the carotenoids from spirulina raised this value to 6.32 mg/mL. The CCl4-treated group showed higher transaminase activity (128.68 units/mL GPT and 171.52 units/mL GOT). However, the activity of GPT was 76.83 units/mL with spirulina. For serum ALP, the standard beta-carotene value was 81.52 units/mL, compared with 84.46 units/mL for the CCl4-treated group; however, natural algal carotenoids yielded 44.73 units/mL. Similarly, the authors observed this same decrease in the parameter corresponding to total albumin. In summary, their results indicated that the carotenoids derived from spirulina had greater antihepatotoxic effects, compared to synthetic beta-carotene (22).

Alpha-Lipoic Acid

In vitro and animal studies suggest lipoic acid supplementation might be a beneficial component in the treatment of heavy metal toxicity, particularly toxicity involving lead, cadmium, mercury, or copper. In one study an intraperitoneal injection of 25 mg/kg ALA given to rats for seven days was able to significantly alter the oxidative stress induced by lead toxicity. Another study demonstrated ALA, at concentrations of 5 mM, was able to protect rat hepatocytes from cadmium toxicity (200 µM) by preventing decreases in total glutathione and increases in lipid peroxidation. Furthermore, a study on mercury intoxication revealed an injection of 10mg/kg/day ALA in rats inoculated with 1 mg/kg/day mercuric chloride prevented damage to nerve tissue caused by lipid peroxidation. Long-Evans Cinnamon rats have a genetic defect that causes them to accumulate copper in the liver – in a manner similar to patients with Wilson's disease – and spontaneously develop acute hepatitis. ALA has been shown to protect these rats from developing hepatitis. ALA appears to improve tissue redox status in metal toxicity and during chelation with dithiol compounds, including dimercaptosuccinic acid (DMSA). Anecdotal reports note the use of lipoic acid may improve the clearance of toxic metals (23).

Beet root

Beetroot supplementation might serve as a useful strategy to strengthen endogenous antioxidant defenses, helping to protect cellular components from oxidative damage. Under normal metabolic conditions, the biological environment of a cell is considered to be in a state of redox balance, or in other words, equilibrium exists between reducing (antioxidants) and oxidizing (pro-oxidants) agents. Molecules capable of oxidation are commonly known as reactive oxygen and nitrogen species (RONS) and are continuously generated in cellular metabolism. At these low concentrations, RONS play an important role in a diverse multitude of

cellular and biochemical processes, including gene expression, cell proliferation, apoptosis and muscular contraction. However, excess exposure of a cell to exogenously generated RONS (UV radiation, xenobiotics) or endogenously synthesized RONS (aberrant cell metabolism, inflammation), can overwhelm the cells antioxidant defenses, causing an imbalance in redox homeostasis, which gives rise to the condition typically referred to as oxidative stress. This imbalance may overwhelm the endogenous antioxidant defense network leaving DNA, carbohydrate, protein and lipid structures susceptible to oxidation and functional impairments (24).

Beetroot juice was shown to protect against N-nitrosodimethylamine (NDEA)- induced liver injury and increases the activity of phase II enzymes, suggesting the activation of the nuclear factor erythroid-2-related factor 2 (Nrf2)-antioxidant response element (ARE) pathway. The aim of the present study was to further explore the mechanism of the activity of beetroot by evaluating the cytoprotective effects of its major component. The influence of betanin (BET) on the activation of Nrf2 and the expression of GSTA, GSTP, GSTM, GSTT, NQO1 and HO-1 was assessed in two hepatic cell lines: non-tumour THLE-2 and hepatoma-derived HepG2 cell lines. The level of the tumour suppressor p53 in both cell lines and the methylation of GSTP in HepG2 cells were also evaluated. Treatment of both cell lines with 2, 10 and 20 μm of BET resulted in the translocation of Nrf2 from the cytosol to the nucleus. The mRNA and nuclear protein levels of Nrf2 and the binding of Nrf2 to ARE sequences were increased only in the THLE-2 cells and were accompanied by the phosphorylation of serine/threonine kinase (AKT), c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK). BET also significantly increased the mRNA and protein levels of GSTP, GSTT, GSTM and NQO1 in these cells. Conversely, besides the translocation of Nrf2 from the cytosol to the nucleus, BET did not modulate any of the other parameters measured in the HepG2 cells. BET did not change the methylation of GSTP1 in these cells either. These results indicate that BET through the activation of Nrf2 and subsequent induction of the expression of genes controlled by this factor may exert its hepatoprotective and anticarcinogenic effects. Moreover, the activation of mitogen-activated protein kinases may be responsible for the activation of Nrf2 in the THLE-2 cells (25).

Milk Thistle

Silybum marianum (milk thistle) has been used for centuries as an herbal medicine for the treatment of liver disease. Its use for liver disorders dates back to Pliny the Elder, a Roman naturalist, who described milk thistle as being “excellent for carrying off bile.” Silymarin’s hepatoprotective effects are accomplished via several mechanisms including antioxidation, inhibition of lipid peroxidation, enhanced liver detoxification via inhibition of Phase I detoxification and enhanced glucuronidation, and protection of glutathione depletion. Studies have also shown silymarin exhibits several anti-inflammatory effects, including inhibition of leukotriene and prostaglandin synthesis, Kupffer cell inhibition, mast cell stabilization, and inhibition of neutrophil migration. In addition, silymarin has been shown to increase hepatocyte protein synthesis, thereby promoting hepatic tissue regeneration.

Animal studies have also demonstrated silybin reduces the conversion of hepatic stellate cells into myofibroblasts, slowing or even reversing fibrosis. Clinical studies conducted in Hungary also demonstrated silymarin to have immunomodulatory effects on the diseased liver (26).

MSM

The effect of MSM pretreatment on acetaminophen induced liver damage was investigated in male Sprague Dawley rats pretreated with 100 mg/kg MSM for one week. On day seven rats were received acetaminophen (850 mg/kg, intraperitoneal). Twenty-four hours later, blood samples were taken to determine serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Tissue samples of liver were also taken for the determination of the levels of malondialdehyde (MDA); total glutathione (GSH), superoxide dismutase (SOD), and myeloperoxidase (MPO) activity together with histopathological observations. High dose of acetaminophen administration caused a significant decrease in the GSH level of the liver tissue, which was accompanied with a decrease in SOD activity and increases in tissue MDA level and MPO activity. Serum ALT, AST levels were also found elevated in the acetaminophen-treated group. Pretreatment with MSM for one week was significantly attenuated all of these biochemical indices. The findings suggest that MSM pretreatment could alleviate hepatic injury induced by acetaminophen intoxication, may be through its sulfur donating and antioxidant effects (27).

This study evaluated the effect of methylsulfonylmethane (MSM) on carbon tetrachloride (CCl_4)-induced acute liver injury in rats. A single injection of CCl_4 (2ml/kg, i.p.) increased serum aminotransferases (ALT and AST) activities. In addition, CCl_4 treatment led to elevation of hepatic malondialdehyde (MDA) content as well as decrease in superoxide dismutase (SOD) and catalase (CAT) activities. Furthermore, cytochrome P450 2E1 (CYP2E1) content was suppressed while proinflammatory cytokines tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels increased in liver tissue after CCl_4 administration. We showed that acute CCl_4-induced damage was accompanied by a rise in Bax/Bcl2 ratio indicating apoptosis. Pre-treatment with MSM (400 mg/kg) inhibited the increases of serum ALT and AST activities, decreased hepatic MDA, TNF-α, IL-6 and Bax/Bcl2 ratio compared to CCl_4 treated group. On the other hand, MSM raised SOD and CAT activities as well as CYP2E1 level in liver tissues. The present study shows that MSM possesses a hepatoprotective effect against CCl_4-induced liver injury in rats. This protective effect might be through its antioxidant, anti-inflammatory and antiapoptotic properties (28).

Green Tea

Green tea polyphenols have been shown to act as metal chelators, preventing the metal-catalyzed formation of radical species, antioxidant enzyme modulators, and scavengers of free radicals, including the hydroxyl radical (•OH), superoxide anion (O_2^-), nitric oxide (NO), and peroxynitrite ($ONOO^-$). These antioxidant activities are considered to be closely related to their protective effects against various health deteriorations (29).

The aim of the present study was to investigate the hepatoprotective effect of green tea extract (GTE) against the hepatic fibrosis induced by carbon tetrachloride (CCl_4), ethanol, and dual

exposure to CCl4 plus ethanol in rats. In particular, an investigation of the three-dimensional architecture was conducted using scanning electron microscopy. Various techniques revealed that hepatic fibrosis with intermingled fibers was located between cells in the CCl4, ethanol and combined CCl4 plus ethanol groups. The hepatic fibrosis differed among the ethanol, CCl4 and CCl4 plus ethanol groups in terms of the type, thickness and distribution of fibers. The fibrotic lesions virtually disappeared in all the groups after 25 days of treatment with GTE, returning the architecture of the liver tissue to its normal status. The rats were also found to regain normal body weight and fur color, which had earlier been discolored due to weight loss. The autopsy results also showed that the animal livers returned to the normal shape and color. GTE demonstrated the same clear action in attenuating the hepatofibrosis for all three inducing treatments, by impairing collagen fibers, eliminating lipid peroxidation and returning the liver architecture to normal. GTE presents a safe therapeutic strategy for hepaticfibrosis (30).

Spirulina-Zinc

The present placebo-controlled double-blind study was conducted to evaluate the effectiveness of spirulina extract plus zinc in the treatment of chronic arsenic poisoning. Forty-one patients of chronic arsenic poisoning were randomly treated orally by either placebo (17 patients) or spirulina extract (250 mg) plus zinc (2 mg) (24 patients) twice daily for 16 weeks. Each patient was supplied with arsenic-safe drinking water by installing a locally made water filter at household level. Effectiveness of spirulina extract plus zinc was evaluated by comparing changes in skin manifestations (clinical scores), arsenic contents in urine and hair, between the placebo- and spirulina extract plus zinc-treated groups. The concentrations of total arsenic in water (without filtration) of placebo- and spirulina extract plus zinc-treated groups were 150.1 +/- 18.3 and 161.7 +/- 23.9 microg/l, respectively. Intake of these high concentrations of arsenic lead to increased excretion of arsenic in urine (72.1 +/- 14.5 microg/l in placebo-treated group and 78.4 +/- 19.1 microg/l in spirulina plus zinc-treated group). After 2 weeks of using filtered water, there were significant reduction of both arsenic intake through water and urinary arsenic excretion (8.3 +/- 3.6 microg/l and 18.4 +/- 7.3 microg/l in placebo group; 9.7 +/- 5.4 microg/l and 21.6 +/- 5.8 microg/l) in spirulina extract plus zinc-treated group. There was a sharp increase in urinary excretion of arsenic (138 +/- 43.6 microg/l) at 4 weeks following spirulina plus zinc administration and the effect was continued for another 2 weeks. Spirulina extract plus zinc removed 47.1% arsenic from scalp hair. Spirulina extract had no major adverse effect that required physician's attention. The clinical scores (median) for melanosis before and after treatment with placebo was not statistically significant ($p > 0.05$), whereas in spirulina extract plus zinc-treated group it was statistically significant ($p < 0.01$). In cases of keratosis, the median clinical scores before and after treatment was not statistically significant ($p > 0.05$) in placebo-treated group. In spirulina extract plus zinc-treated group, the clinical scores for keratosis before and after treatment was statistically significant ($p < 0.05$). Results show that spirulina extract (250 mg) plus zinc (2 mg) twice daily for 16 weeks may be useful for the treatment of chronic arsenic poisoning with melanosis and keratosis (31).

Oregon Grape

Roots and stem-bark of Mahonia aquifolium (Oregon grape) (Berberidaceae) are effectively used in the treatment of skin inflammatory conditions. In the present study, the effect of Mahonia aquifolium crude extract and its two representative alkaloid fractions containing protoberberine and bisbenzylisoquinoline (BBIQ) alkaloids on activity of 12-lipoxygenase (12-LOX), was studied. The reactivity with 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a free stable radical, was evaluated to elucidate the rate of possible lipid-derived radical scavenging in the mechanism of
the enzyme inhibition. The results indicate that although the direct radical scavenging mechanism cannot be ruled out in the lipoxygenase inhibition by Mahonia aquifolium and its constituents, other mechanisms based on specific interaction between enzyme and alkaloids could play the critical role in the lipoxygenase inhibition rather than non-specific reactivity with free radicals (32).

Red Clover

In order to examine the antioxidant properties of five different extracts of Trifolium pratense L. (Leguminosae) leaves, various assays which measure free radical scavenging ability were carried out: 1,1-diphenyl-2-picrylhydrazyl, hydroxyl, superoxide anion and nitric oxide radical scavenger capacity tests and lipid peroxidation assay. In all of the tests, only the H2O and (to some extent) the EtOAc extracts showed a potent antioxidant effect compared with BHT and BHA, well-known synthetic antioxidants. In addition, in vivo experiments were conducted with antioxidant systems (activities of GSHPx, GSHR, Px, CAT, XOD, GSH content and intensity of LPx) in liver homogenate and blood of mice after their treatment with extracts of T. pratense leaves, or in combination with CCl4. Besides, in the extracts examined the total phenolic and flavonoid amounts were also determined, together with presence of the selected flavonoids: quercetin, luteolin, apigenin, naringenin and kaempferol, which were studied using a HPLC-DAD technique. HPLC-DAD analysis showed a noticeable content of natural products according to which the examined Trifolium pratense species could well be regarded as a promising new source of bioactive natural compounds, which can be used both as a food supplement and a remedy (33).

Ingredients that Support Healthy Skin

The formula supports healthy skin by enhancing the antioxidant defense and promoting healthy anti-inflammatory processes (Alpha-Lipoic Acid, Milk Thistle, Spirulina), enhancing cutaneous microvascular function and promoting the skin oxygenation (Green Tea), and supporting healthy skin structure (Red Clover).

Alpha-Lipoic Acid

This investigation was carried out to study possible effects of Alpha Lipoic Acid (ALA) on smoking-induced rat skin injury. 28 Spraque-Dawley female rats were allocated into three groups: control group (n = 8), smoking group (n = 10; 12 cigarettes/day, 8 weeks) and smoking + ALA group (n = 10; 12 cigarettes/day + 100 mg/kg, 8 weeks). Experiment group animals were

sacrificed under anesthesia with 10% ketamine + 2% xylasine at the end of second mounts and then skin examples were taken from the epigastric area. Histochemical (Haematoxylin-Eosin and Masson's trichrome, immunohistochemical (TNF-α) and biochemical analysis (CAT, MDA and protein carbonylation) were performed on these skin tissues. Histologically, skin was distinguished normal structure in the control group. In the smoking group, collagen bundles and hair follicle degradation/reduction, sweat gland degeneration, mononuclear cell infiltration in dermis were encountered. In ALA-treated group, all of these changes were improved ($p < 0.05$). Collagen bundles structures were appearance more regular than the smoking group. Immunohistologically, intense staining was observed in the smoking group, while very weak staining was observed in control group, weak staining was observed in the ALA-treated group. Biochemically; The CAT activity compared to cigarette group with control was raised high and in ALA group was higher compared to both groups, but not significant ($p > 0.05$). MDA; which is an indicator of lipid peroxidation was significantly higher in cigarette group than in control group ($p < 0.05$) and was significantly lower in ALA group than cigarette ($p < 0.05$). Protein carbonylation was higher in cigarette group than the control group but not in the non-significant ($p > 0.05$). In the ALA it was significantly lower compared to the control group and cigarette ($p < 0.05$). Based on biochemical and histopathological determinations, the study showed that cigarette smoke can cause degenerative effects on skin tissues in rats. However, ALA has a curative effect on cigarette-induced injuries on the skin tissues by anti-oxidative and anti-inflammatory effects (34).

Green Tea

The aim of this study was to evaluate the short-term effects of green tea consumption on microvascular functioning in both an older and younger population. Fifteen young [24 (4.0)] and fifteen older [61 (4.0)] participants, consumed two cups of green tea daily for 14days. We used Laser Doppler Flowmetry (LDF) to assess cutaneous microvascular function and Transcutaneous Oxygen monitoring (TcPO2) to assess skin oxygen tension. Systolic and diastolic blood pressure were also assessed on both visits. We observed significant improvements in axon-mediated microvascular vasodilation for the younger group [1.6 (0.59) vs 2.05 (0.72), $p<0.05$] and the older group [1.25 (0.58) vs 1.65 (0.5) $p<0.05$]. Improvements in skin oxygen tension were also noted for both groups in both noted TcPO2 measures (i.e. 1.25 (0.58) vs 1.65 (0.5) ($p<0.05$), for ΔTcPO2max for the older group, between visits) respectively. Improvements were also observed for systolic blood pressure in both the younger [120 (10) vs 112 (10), $p<0.05$] and older group [129 (12) v 124 (11), $p<0.001$]. In conclusion, we observed statistically significant improvements in microvascular function and skin oxygen tension. Our results suggest that green tea may prove beneficial as a dietary element in lifestyle interventions aiming to lower cardiovascular disease risk, in both older and younger populations (35).

Milk Thistle

Silibinin, a major polyphenol in milk thistle, has been reported to have multiple pharmacological activities; therefore, there is an urgent need to understand how silibinin works on inflammation-associated skin diseases. We herein designed silibinin on 12-O-tetradecanoylphorbol-13-acetate (TPA)-stimulated skin inflammation to test its inhibitory

effects. It was demonstrated that silibinin, applied topically onto mouse ears following TPA stimulation, effectively down-regulated the expressions of TPA-induced interleukin-1β (IL-1β), interleukin-6 (IL-6), necrosis factor-alpha (TNF-α) and cyclooxygenase-2 (COX-2) in a dose-dependent manner. Further mechanistic investigations indicated that silibinin suppressed the expression of IκB kinase (IKK) by inhibiting the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) signaling pathway, and thereby suppressing TPA-stimulated nuclear factor-κB (NF-κB) activation. Promisingly, silibinin, used for transdermal application, may be a potent naturally occurring anti-inflammatory agent for the prevention of inflammation-associated skin diseases (36).

Red Clover

Estrogens have a profound influence on skin. The relative hypoestrogenism that accompanies menopause exacerbates the deleterious effects of both intrinsic and environmental aging. Estrogens improve skin in many ways. Among these, they increase collagen content, skin thickness and improve skin moisture. There is evidence that diets with high levels of phytoestrogenic isoflavones are associated with a low incidence of menopausal symptoms and osteoporosis. Plant extracts such as red clover, which contain high levels of isoflavones, have been used to reduce menopausal symptoms and have been shown to reduce bone loss in healthy women. In this study to investigate the effects of red clover isoflavones on skin aging, the histology of the skin, skin thickness and the amount of total collagen was determined by a colorimetric method, were studied in ovariectomized rats after treatment for 14 weeks with a red clover extract standardized to contain 11% isoflavones determined by HPLC. In ovariectomized rats the thickness and keratinization of the epidermis were reduced; glands were less in number and vascularity was poor; the distribution and morphology of the collagen bundles and elastic fibers were altered. Whereas the skin of ovariectomized rats treated with red clover isoflavones (20 and 40 mg of total isoflavones daily for 14 weeks) appeared well organized with a normal epidermis with uniform thickness and regular keratinization; vascularity, collagen and elastic fibers were well developed. The amount of collagen significantly increased in the treated group in comparison with the control group. These findings suggest that red clover isoflavones are effective in reducing skin aging induced by estrogen deprivation (37).

Spirulina

Reactive oxygen species produced in response to UVR are important in skin tumor development. We have previously reported that deficiency of the Ogg1 gene, encoding the repair enzyme for 8-oxo-7,8-dihydroguanine (8-oxoG), increases skin tumor incidence in mice upon repetitive UVB exposure and modulation of UVB-induced inflammatory response. Spirulina platensis is used as a human food supplement because it contains abundant nutritional and antioxidant components. Therefore, we investigated the inhibitory effects of S. platensis on UVB-induced skin tumor development in Ogg1 knockout-(KO) mice and the wild-type (WT) counterpart. Dietary S. platensis suppressed tumor induction and development in both genotypes compared with our previous data without S. platensis. Induction of erythema and ear swelling, one of the hallmarks of UVB-induced inflammatory responses, was suppressed in the skin of Ogg1-KO mice and albino hairless mice fed with dietary S. platensis. Compared with

untreated mice, S. platensis-administered mice showed significantly reduced 8-oxoG formation in the skin after UVB exposure. Moreover, we found that S. platensis effectively down regulated the signal proteins p38 mitogen-activated protein kinase, stress-activated protein kinase/c-Jun N-terminal kinase, and extracellular signal-regulated kinase after UVB exposure especially in Ogg1-KO mice. Our results suggest that S. platensis exerts antitumor effects against UVB irradiation in the skin through its anti-inflammatory and antioxidant effects (38).

Ingredients to Detox the Bowels:

Alpha-Lipoic Acid

Ulcerative colitis affects many people worldwide. Inflammation and oxidative stress play a vital role in its pathogenesis. Previously, we reported that ulcerative colitis leads to systemic genotoxicity in mice. The present study was aimed at elucidating the role of α-lipoic acid in ulcerative colitis-associated local and systemic damage in mice. Experimental colitis was induced using 3%w/v dextran sulfate sodium in drinking water for 2 cycles. α-Lipoic acid was administered in a co-treatment (20, 40, 80 mg/kg bw) and post-treatment (80 mg/kg bw) schedule. Various biochemical parameters, histological evaluation, comet and micronucleus assays, immunohistochemistry and western blot analysis were employed to evaluate the effect of α-lipoic acid in mice with ulcerative colitis. The protective effect of α-lipoic acid was mediated through the modulation of nuclear factor kappa B, cyclooxygenase-2, interleukin 17, signal transducer and activator of transcription 3, nuclear erythroid 2-related factor 2, NADPH: quinone oxidoreductase-1, matrix metalloproteinase-9 and connective tissue growth factor. Further, ulcerative colitis led to an increased gut permeability, plasma lipopolysaccharide level, systemic inflammation and genotoxicity in mice, which was reduced with α-lipoic acid treatment. The present study identifies the underlying mechanisms involved in α-lipoic acid-mediated protection against ulcerative colitis and the associated systemic damage in mice (39).

Chlorella

Endotoxemia has long been documented in obstructive jaundice, and altered intestinal barrier function is considered to be one of the important mechanisms for this phenomenon. The aim of this study was to investigate the role of different microalgae (Chlorella sp. and Spirulina sp.) extracts in intestinal barrier function and oxidative stress in experimentally jaundiced rats. A total of 60 male Wistar rats were randomly divided into four groups of 15 each: I, sham operated; II, bile duct ligation (BDL); III, BDL+Chlorella sp.; IV, BDL+Spirulina sp. Rats were fed rat chow or microalgae extracts supplemented enteral diet ten days after sham operation or BDL. Main outcome measures were endotoxin concentrations in plasma, evidence of bacterial translocation (BT) in mesenteric lymph nodes (MLNs) and liver, oxidative stress, and histology. Compared to the group I, a significant increase in contaminated MLNs, liver, and spleen samples and increased endotoxemia were noted in group II ($P<0.01$) but were significant reduced in group III ($P<0.05$). There was no significant difference in BT rate between the group II and group IV ($P>0.05$). Moreover, Chlorella sp. administration protected in jaundiced rats against oxidative stress, as demonstrated by reduction of intestinal lipid peroxidation, increase of the antioxidant reduced glutathione (GSH), and decrease of the oxidized glutathione

(GSSG). The intestinal mucosa in control rats was atrophic with significantly decreased villous density and total mucosal thickness. Chlorella sp. caused a significant reduction in villous atrophy compared with controls. Chlorella sp. microalgae supplemented enteral diet has significant protective effects on intestinal mucosa barrier in obstructive jaundice, and reduces intestinal translocation of bacteria and endotoxin (40).

Milk Thistle

The flavonolignan silibinin, the major biologically active compound of milk thistle (Silybum marianum), has been shown to possess anticancer properties in a variety of epithelial cancers. The present study investigated the potential of silibinin as a chemopreventive agent in colon carcinogenesis. The rat azoxymethane (AOM)-induced colon carcinogenesis model was used because of its molecular and clinical similarities to sporadic human colorectal cancer. One week after AOM injection (post-initiation), Wistar rats received daily intragastric feeding of 300 mg silibinin/kg body weight per day until their sacrifice after 7 weeks of treatment. Silibinin-treated rats exhibited a 2-fold reduction in the number of AOM-induced hyperproliferative crypts and aberrant crypt foci in the colon compared to AOM- injected control rats receiving the vehicle. Silibinin-induced apoptosis in the colon mucosal cells was demonstrated by flow cytometry after propidium iodide staining and by colorimetric measurement of caspase-3 activity. Mechanisms involved in silibinin-induced apoptosis included the downregulation of the anti-apoptotic protein Bcl-2 and upregulation of the pro-apoptotic protein Bax, inverting the Bcl- 2/Bax ratio to <1. This modulation already takes place at the mRNA expression level as shown by real-time RT-PCR. Furthermore, silibinin treatment significantly ($P<0.01$) decreased the genetic expression of biomarkers of the inflammatory response such as IL1β, TNFα and their downstream target MMP7, all of them shown to be upregulated during colon carcinogenesis. The downregulation of MMP7 protein was confirmed by western blot analysis. The present findings show the ability of silibinin to shift the disturbed balance between cell renewal and cell death in colon carcinogenesis in rats previously injected with the carcinogen AOM. Silibinin administered via intragastric feeding exhibited potent pro-apoptotic, anti-inflammatory and multi-targeted effects at the molecular level. The effective reduction of preneoplastic lesions by silibinin supports its use as a natural agent for colon cancer chemoprevention (41).

Spirulina

To evaluate the beneficial effects of spirulina on the treatment of experimental colitis in Wistar rats weighing 200-300 g. Experimental colitis was created during anesthesia using the trinitrobenzene sulfonic (TNBS) acid. The rats were randomly divided into 3 groups. In the group 1 (sham; n = 8), saline was administered via oral gavage 7 days after 1 ml of rectal saline was administered. In group 2 (experimental colitis + spirulina; n = 8), 2 g/kg spirulina was administered via oral gavage 7 days after the rectal 1 ml TNBS was administered. In group 3 (experimental colitis; n = 8), enema was administered via oral gavage 7 days after the rectal 1 ml TNBS was administered. Eight days after the instigation of TNBS colitis, the rats were sacrificed and blood and tissue samples were taken. Histopathologic and immunohistochemical evaluations were conducted, and malondialdehyde (MDA), advanced oxidation protein products (AOPP), catalase (CAT), total antioxidant status (TAS), and glutathione (GSH) levels were

determined. Inflammation on mucosa and submucosa, hemorrhage, necrosis, cellular infiltration and crypt abscess formation, immunoreactivity and tissue MDA levels were decreased in the experimental colitis + spirulina group when compared to the experimental colitis group ($p < 0.05$). The results of the present study indicate the beneficial effects of spirulina on TNBS-induced inflammatory bowel disease. (42).

Ingredients that Support Kidney Detox:

Alpha-Lipoic Acid

The purpose of this study is to determine the antioxidant and anti-inflammatory effects of alpha lipoic acid (ALA) on methotrexate (MTX) induced kidney injury in rats. Thirty-two rats were equally divided into four groups; control, ALA, MTX and MTX with ALA groups. A single dose of MTX (20 mg/kg) was administered to make kidney injury to groups 3 and 4, intraperitoneally. The ALA was administered intraperitoneally in groups 2 and 4 and the other groups received saline injection for five days. On the sixth day the blood samples and kidney tissues were obtained for the measurement of TNF-α, IL-1β, malondialdehyde, glutathione, myeloperoxidase and sodium potassium-adenosine triphosphatase levels and histological examination. Administration of MTX caused a decrease in tissue GSH, and Na+, K+- ATPase activity significantly. A significant increase in tissue MDA and MPO activities were also seen. The pro-inflammatory cytokines (TNF-α, IL-β) were increased in the MTX group significantly. ALA treatment reversed all biochemical indices as well as histopathological alterations induced by MTX administration. MTX made oxidative damage on kidneys of rat and it was partially prevented by anti-inflammatory and antioxidant effects of ALA treatment (43).

Beet Root

The present investigation was designed to investigate the protective effect of (Beta vulgaris L.) beetroot ethanolic extract (BVEE) on gentamicin-induced nephrotoxicity and to elucidate the potential mechanism. Serum specific kidney function parameters (urea, uric acid, total protein, creatinine, and histopathology of kidney tissue) were evaluated to access gentamicin-induced nephrotoxicity. The oxidative/nitrosative stress (Lipid peroxidation, MDA, NP-SH, Catalase, and nitric oxide levels) was assessed. The inflammatory response (TNF-α, IL-6, MPO, NF-κB (p65), and NF-κB (p65) DNA binding) and apoptotic marker (Caspase-3, Bax, and Bcl-2) were also evaluated. BVEE (250 and 500 mg/kg) treatment along with gentamicin restored/increased the renal endogenous antioxidant status. Gentamicin-induced increased renal inflammatory cytokines (TNF-α and IL-6), nuclear protein expression of NF-κB (p65), NF-κB-DNA binding activity, myeloperoxidase (MPO) activity, and nitric oxide level were significantly down regulated upon BVEE treatment. In addition, BVEE treatment significantly reduced the amount of cleaved caspase 3 and Bax, protein expression and increased the Bcl-17-2 protein expression. BVEE treatment also ameliorated the extent of histologic injury and reduced inflammatory infiltration in renal tubules. These findings suggest that BVEE treatment attenuates renal dysfunction and structural damage through the reduction of oxidative stress, inflammation, and apoptosis in the Kidney (44).

Dandelion Leaf

Taraxacum officinale (L.) Weber (Asteraceae) has been extensively employed as a diuretic in traditional folk medicine and in modern phytotherapy in Europe, Asia, and the Americas without prior clinical trial substantiation. In this pilot study, a high-quality fresh leaf hydroethanolic extract of the medicinal plant T. officinale (dandelion) was ingested by volunteers to investigate whether an increased urinary frequency and volume would result. Volume of urinary output and fluid intake were recorded by subjects. Baseline values for urinary frequency and excretion ratio (urination volume:fluid intake) were established 2 days prior to dandelion dosing (8mL TID) and monitored throughout a 1-day dosing period and 24 hours post dosing. For the entire population (n1/417) there was a significant ($p<0.05$) increase in the frequency of urination in the 5-hour period after the first dose. There was also a significant ($p<0.001$) increase in the excretion ratio in the 5-hour period after the second dose of extract. The third dose failed to change any of the measured parameters. Based on these first human data, T. officinale ethanolic extract shows promise as a diuretic in humans (45).

Green Tea

Green tea, prepared from the leaves of Camellia sinensis L., is a beverage that is popular worldwide. Polyphenols in green tea have been receiving much attention as potential compounds for the maintenance of human health due to their varied biological activity and low toxicity. In particular, the contribution of antioxidant activity to the prevention of diseases caused by oxidative stress has been focused upon. Therefore, in this study, we investigated the effects of (−)-epigallocatechin 3-O-gallate and (−)-epigallocatechin 3-O-gallate, which account for a large fraction of the components of green tea polyphenol, on oxidative stress related renal disease. Our observations suggest that green tea polyphenols have a beneficial effect on pathological states related to oxidative stress of the kidney (46).

Milk Thistle

Potential of silymarin in protection of cisplatin-induced renal cell death without compromising effect on anticancer activity of cisplatin was demonstrated in this study. Cisplatin-induced cell death was evaluated in human proximal tubular HK-2, lung carcinoma H460, and melanoma G361 cells using MTT, Hoechst 33342, and propidium iodide assays. Cisplatin induced both apoptosis and necrosis in HK-2 cells and caused a decrease in cell viability by ~40% and 60% at the doses of 25 and 100 μM, respectively. Pretreatment with 25-200 μM of silymarin significantly protected against cisplatin-induced cell death in a dose-dependent manner. In contrast, pretreatment of silymarin (25-100 μM) caused no significant change on cisplatin-induced cell death in H460 cells but significantly potentiated cisplatin-induced apoptosis in G361 cells. These findings reveal the selectivity of silymarin in protection of renal cells from cisplatin-induced cell death and could be beneficial for the development of this considerately safe compound as a renoprotective agent (47).

Spirulina

This study was conducted to investigate the possible protective effect of Spirulina platensis against chromium-induced nephrotoxicity. A total of 36 adult male Sprague-Dawley rats were

divided into 4 equal groups (Gps). Gp1 served as control, rats of Gps 2, 3, and 4 were exposed to Spirulina platensis (300 mg/kg b.wt per os) and sodium dichromate dihydrate (SDD) via drinking water at concentration of 520mg /l respectively. Chromium administration caused alterations in the renal function markers as evidenced by significant increase of blood urea and creatinine levels accompanied with significant increase in kidney's chromium residues and MDA level as well as decreased catalase activity and glutathione content in kidney tissue. Histologically, Cr provoked deleterious changes including: vascular congestion, wide spread tubular epithelium necrobiotic changes, atrophy of glomerular tuft and proliferative hyperplasia. The latter was accompanied with positive PCNA expression in kidney tissues as well as DNA ploidy interpretation of major cellular population of degenerated cells, appearance of tetraploid cells, high proliferation index and high DNA index. Morphometrical measurements revealed marked glomerular and tubular lumen alterations. On contrary, spirulina co-treatment with Cr significantly restored the histopathological changes, antioxidants and renal function markers and all the previously mentioned changes as well (48).

Ingredients that Support Lung Detox:

Alpha-Lipoic Acid

Oxidative stress is believed to be an important factor in the pathogenesis of acute lung injury (ALI). The aim of this study was to investigate the possible protective role of alpha-lipoic acid (α-LA) on oleic acid (OA)-induced ALI in rats. A total of thirty-five rats were divided into five groups in the study. Group 1 served as a control group. Rats in Group 2 (α-LA) were administered α-LA intraperitoneally at a dose of 100 mg/kg body weight (BW). Rats in Group 3 (OA) were administered OA intravenously at a dose of 100 mg/kg BW. In Group 4 (pre-OA-α-LA), α-LA was given 15 minutes prior to OA infusion, and in Group 5 (post-OA-α-LA), α-LA was given two hours after OA infusion. Four hours after the OA infusion, rats were decapitated. Blood samples were collected to measure serum levels of malondialdehyde (MDA) and glutathione (GSH), and the levels of activity for superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). Lung tissue samples were taken for histopathological examination. Exposure to OA resulted in increases in serum MDA levels ($p<0.001$), as well as histopathological lesions in lung tissue, and decreases in CAT ($p<0.05$), GSH-Px ($p<0.05$) activities and GSH ($p<0.05$) levels. On the other hand, MDA levels were decreased significantly ($p<0.001$), while CAT ($p<0.05$), GSH-Px ($p<0.01$) activities and GSH ($p<0.05$) levels were increased significantly in the pre-OA-α-LA group compared with the OA group. α-LA was found to lessen oxidative stress and to have positive effects on antioxidants in cases of OA-induced ALI. In conclusion, α-LA appears to have protective effects against ALI and potential for the prevention of ALI (49).

Milk Thistle

Cigarette smoke (CS), which increases inflammation and oxidative stress, is a major risk factor for the development of COPD. In this study, we investigated the effects of silymarin, a polyphenolic flavonoid isolated from the seeds and fruits of milk thistle, on CS-induced airway inflammation and oxidative stress in mice and the possible mechanisms. BALB/c mice were

exposed to CS for 2 h twice daily, 6 days per week for 4 weeks. Silymarin (25, 50 mg/kg·day) was administered intraperitoneally 1 hour before CS exposure. Bronchoalveolar lavage fluid (BALF) was acquired for cell counting and the detection of pro-inflammatory cytokine levels. Lung tissue was collected for histological examination, myeloperoxidase (MPO) activity assay, superoxide dismutase (SOD) activities, and malondialdehyde (MDA) levels. The phosphorylation of ERK and p38 was evaluated by Western blotting. Pretreatment with silymarin significantly attenuated CS-induced thickening of the airway epithelium, peribronchial inflammatory cell infiltration, and lumen obstruction. The numbers of total cells, macrophages, and neutrophils, along with the MPO activity (a marker of neutrophil accumulation) in BALF, were remarkably decreased by silymarin in CS-exposed mice (all $p<0.05$). In addition, silymarin pretreatment dampened the secretion of TNF-α, IL-1β, and IL-8 in BALF. High-dose silymarin (50mg/kg·day) administration also prevented CS-induced elevation in MDA levels and decrease in SOD activities ($p<0.05$). Furthermore, the CS-induced phosphorylation of ERK and p38 was also attenuated by silymarin ($p<0.05$). These results suggest that silymarin attenuated inflammation and oxidative stress induced by cigarette smoke. The anti-inflammatory effect might partly act through the mitogen-activated protein kinases (MAPK) pathway (50).

The application of bleomycin is limited due to its side effects including lung toxicity. Silymarin is a flavonoid complex isolated from milk thistle [Silybum marianum L. (Asteraceae)] which has been identified as an antioxidant and anti-inflammatory compound. This study evaluates the effect of silymarin on oxidative and inflammatory parameters in the lungs of mice exposed to bleomycin. BALB/c mice were divided into four groups of control, bleomycin (1.5 U/kg), bleomycin plus silymarin (50 and 100 mg/kg). After bleomycin administration, mice received 10 d intraperitoneal silymarin treatment. On 10th day, blood and lung samples were collected for measurement of oxidative and inflammatory factors. Silymarin led to a decrease in lung lipid peroxidation (0.19 and 0.17 nmol/mg protein) in bleomycin-injected animals. Glutathione-S-transferase (GST) which was inhibited by bleomycin (32.4 nmol/min/mg protein) induced by higher dose of silymarin (41 nmol/min/mg protein). Silymarin caused an elevation in glutathione (GSH): 2.6 and 3.1 μmol/g lung compare with bleomycin-injected animals 1.8 μmol/g lung. Catalase (CAT) was increased due to high dose of silymarin (65.7 μmol/min/ml protein) compare with bleomycin treated-mice. Myeloperoxidase (MPO) which was induced due to bleomycin ($p < 0.05$) reduced again by high dose of silymarin (0.51 U/min/mg protein). Bleomycin led to an increase in TNF-α and interleukin-6 (IL-6) (7.9 and 11.8 pg/ml). These parameters were reduced by silymarin ($p < 0.05$). Silymarin attenuated bleomycin induced-pulmonary toxicity. This protective effect may be due to the ability of silymarin in keeping oxidant-antioxidant balance and regulating of inflammatory mediator release (51).

MSM

Methylsulfonylmethane (MSM) is a natural organosulfur compound that exhibits antioxidative and anti-inflammatory effects. This study was carried out to investigate the effect of MSM on paraquat (PQ)-induced acute lung and liver injury in mice. A single dose of PQ (50 mg/kg, i.p.) induced acute lung and liver toxicity. Mice were treated with MSM (500 mg/kg/day, i.p.) for 5 days. At the end of the experiment, animals were euthanized, and lung and liver tissues were

collected for histological and biochemical analysis. Tissue samples were used to determine malondialdehyde (MDA), myeloperoxidase (MPO), catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), and tumor necrosis factor-α (TNF-α) levels. Blood samples were used to measure plasma alanine transaminase (ALT), γ-glutamyl transferase (GGT), and alkaline phosphatase (ALP). Histological examination indicated that MSM decreased lung and liver damage caused by PQ. Biochemical results showed that MSM treatment significantly reduced tissue levels of MDA, MPO, and TNF-α, while increased the levels of SOD, CAT, and GSH compared with PQ group. MSM treatment also significantly reduced plasma levels of ALT, GGT, and ALP. These findings suggest that MSM as a natural product attenuates PQ- induced pulmonary and hepatic oxidative injury (52).

Spirulina

Oxidative stress is intimately associated with many diseases, including chronic obstructive pulmonary disease (COPD). Study objectives include a comparison of oxidative stress, antioxidant status, and lipid profile between COPD patients and controls and evaluation of the effect of spirulina intervention on oxidative stress, antioxidant status, and lipid profile of COPD patients. 30 patients with COPD and 20 controls with no respiratory problems were selected. Global Initiative for Chronic Obstructive Lung Disease criteria were served as the basis of COPD diagnosis. The serum content of malondialdehyde (MDA), lipid hydroperoxide, glutathione (GSH), vitamin C, cholesterol, triglyceride (TG), and high density lipoprotein (HDL) was measured. The activity of superoxide dismutase (SOD), catalase (CAT), and glutathione-s-transferase (GST) was also measured. Two different doses, (500 × 2) mg and (500 × 4) mg spirulina, were given to two groups, each of which comprises 15 COPD patients. All targeted blood parameters have significant difference (P =0.000) between COPD patients and controls except triglyceride (TG). Spirulina intake for 30 and 60 days at (500 × 2) mg dose has significantly reduced serum content of MDA, lipid hydroperoxide, and cholesterol (P = 0.000) while increasing GSH, Vit C level (P = 0.000), and the activity of SOD (P = 0.000) and GST (P = 0.038). At the same time, spirulina intake for 30 and 60 days at (500 × 4) mg dose has favorable significant effect (P = 0.000) on all targeted blood parameters except for HDL (P = 0.163) (53).

Ingredients for Lymphatic Detox:

Alpha-Lipoic Acid

The objective of this investigation was to explore possible molecular changes for role of a high-fat diet (HFD)-induced oxidative stress in splenic lymphocytes, and whether a dietary lipoic acid (LA) supplement could attenuate these changes. Male C57BL/6 mice were fed one of three diets 10 weeks and outcome measures centered on parameters of oxidative stress and lymphocytes apoptosis in spleen. Two-dimensional gel electrophoresis was used to compare the proteomes of splenic lymphocytes with three dietary groups. Differentially expressed spots whose expression altered over three fold were identified by MALDI-TOF MS. In this study, HFD resulted in oxidative stress in mice spleen, and significantly increased apoptotic percentages of

splenic lymphocytes. Bioinformatic evaluation results of MALDI-TOF MS showed that 20 differentially expressed protein spots were known to be involved in many processes associated with cell function, such as cytoskeleton, energy metabolism and oxidative stress, signal transduction and cell defense. In conclusion, these results indicate that HFD-induced oxidative stress could lead to the functional decline of splenic lymphocytes, and LA supplement attenuates the alterations of protein expression to maintain the basic biological processes (54).

Green tea

The most common drinking beverage in large portion of the world is Camellia sinensis (green tea). In the present study, we evaluated the adjuvant effect of green tea and tea polyphenols to particulate and non-particulate antigens. BALB/c mice were immunized with particulate and non-particulate antigens. Modulation of immunoglobulin-secreting splenocytes, IgG-mediated and IgM-mediated immunity, was evaluated by hemolytic plaque assay and enzyme-linked immunosorbent assay, respectively. Dose-dependent response of tea polyphenols was also assayed. Phenolic content was measured in crude preparations of green tea. We observed a stimulatory effect of green tea preparations on humoral immune response mediated by the increased number of antibody-secreted cells in spleen. A significant increase in IgM-mediated and IgG-mediated immune response to non-particulate antigen was also observed in green tea-treated animals. A dose-dependent adjuvant effect was seen in the case of tea polyphenols for a longer period of time compared with crude tea preparations. This study indicates polyphenols as major constituents responsible for the enhanced and sustained adjuvant activity of green tea. We suggest that tea polyphenols might be considered for real-life evaluation during adjuvant-mediated vaccination trial programs (55).

Milk Thistle

The effect of silibinin on antigen-specific antibody production and T-cell cytokine expression was investigated. BALB/c mice were either left untreated or administered daily with vehicle (VH; saline) and/or silibinin (200 or 400 mg/kg) by gavage for 3 consecutive days prior to sensitization with ovalbumin (OVA). The antibody production in the serum and T-cell-derived cytokine expression by splenocytes were determined 7 days post OVA sensitization. Our results demonstrated that the production of OVA-specific serum IgE and total IgE was significantly attenuated by silibinin treatment, whereas OVA-specific IgG(2a) was markedly enhanced. In parallel with the differential modulation of the production of IgG(2a) and IgE, treatment of OVA-sensitized mice with silibinin markedly increased and decreased the production of IFN-gamma and IL-4, respectively, by splenocytes cultured in the presence of OVA. Together, these results suggest that silibinin treatment polarizes the Th1/Th2 immune balance toward the Th1-dominant direction, which may be beneficial against IgE-mediated allergy (56).

MSM

In the present investigation, autoimmune strain MRL/lpr, C3H/lpr, and male BXSB mice were placed on a continuous treatment regimen with 3% DMSO or 3% DMSO2 in the drinking water, ad libitum, commencing at 1 to 2 months of age, before spontaneous disease development could be detected. This represented doses of 8- 10 g/kg/day of DMSO and 6-8 g/kg/day of

DMSO2. Both compounds were observed to extend the mean life span of MRL/lpr mice from 5 1/2 months to over 10 months of age. All strains showed decreased antinuclear antibody responses and significant diminution of lymphadenopathy, splenomegaly, and anemia development. Serum IgG levels and spleen IgM antibody plaque formation, however, did not differ from control values. There was no indication of involvement of systemic immunosuppressive or antiproliferative effects, and treated animals were observed to remain healthy and vigorous with no signs of toxicity. These results demonstrate that high doses of both DMSO and its major in vivo metabolite, DMSO2, provide significant protection against the development of murine autoimmune lymphoproliferative disease (57).

Spirulina

Anti-inflammatory effects of blue-green algae (BGA), i.e., Nostoc commune var. Sphaeroides Kützing (NO) and Spirulina Platensis (SP), were compared in RAW 264.7 and mouse bone marrow-derived macrophages (BMM) as well as splenocytes from apolipoprotein E knockout (apoE−/−) mice fed BGA. When macrophages pretreated with 100 μg/ml NO lipid extract (NOE) or SP lipid extract (SPE) were activated by lipopolysaccharide (LPS), expression and secretion of pro-inflammatory cytokines, such as tumor necrosis factor α (TNFα), interleukin 1β (IL-1β), and IL-6, were significantly repressed. NOE and SPE also significantly repressed the expression of TNFα and IL-1β in BMM. LPS-induced secretion of IL-6 was lower in splenocytes from apoE−/− fed an atherogenic diet containing 5% NO or SP for 12 weeks. In RAW 264.7 macrophages, NOE and SPE markedly decreased nuclear translocation of NF-κB. The degree of repression of pro-inflammatory gene expression by algal extracts was much stronger than that of SN50, an inhibitor of NF-κB nuclear translocation. Trichostatin A, a pan histone deacetylase inhibitor, increased basal expression of IL-1β and attenuated the repression of the gene expression by SPE. SPE significantly down-regulated mRNA abundance of 11 HDAC isoforms, consequently increasing acetylated histone 3 levels. NOE and SPE repress pro-inflammatory cytokine expression and secretion in macrophages and splenocytes via inhibition of NF-κB pathway. Histone acetylation state is likely involved in the inhibition (58).

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