Short compilation of physiological effects of Chlorella vulgaris – micro algae, documented in the medical literature

Introduction

Chlorella vulgaris is a unicellular eucariotic alga, which is growing both under phototrophic and mixotrophic conditions mainly in freshwater. The taxonomic criteria for determination of the genus Chlorella were established by *Fott* et al. [1].
With a cell – diameter of about 5 µm this spherical micro alga in its size is comparable to the red blood cells. Especially striking on Chlorella is the high reproduction rate. One mother cell is dividing into 4 daughter cells within 16 – 20 hours, which are dividing again within the next 16 – 20 hours in the same way. That means Chlorella vulgaris possesses one of the highest potential for production of biomass per square and time unit of all known plants. Taking into account the unique nutrition and health properties it is easy to understand that both conditions for cultivation and application get investigated nowadays also in regions, which are not traditionally known for consumption of micro algae.
Chlorella contains high amounts of chlorophyll, amino acids, nucleic acids, enzymes, fats, vitamins, fibres and minerals. With up to 4 % of chlorophyll in dry mass Chlorella vulgaris has the highest chlorophyll content of all known nutrients.
The range of amino acids (about 40 to 50 % in dry mass) covers the 20 proteinogenic amino acids including the 8 essentials for human beings. Fats synthesised by Chlorella vulgaris (10 to 15 % in d.m.) consists of more than 80 % unsaturated fatty acids with α - linoleic acid as major part to more than 30 %.
Among vitamins the high amount of B – group vitamins is especially worth mentioning. Furthermore, also such vitamins with antioxidant properties as vitamin C, vitamin A and β-Carotene as its precursor are contained in high amounts in Chlorella vulgaris.

The hypothesis that a sufficient supply with unsaturated fatty acids and antioxidants human beings protect for coronary diseases is widely accepted. But the health supporting and protecting effects of chlorella cannot only be explained by the content of vitamins, fats and amino acids alone, particularly as the intake is small at normal application (for adults nearly 3 gram per day are recommended).
That's why a lot of research was focussed in the last years on the identification of physiological effective components. The main attention was paid to a mixture of nucleic acids, peptides and enzymes, obtainable by extraction of Chlorella cells by hot or cold water. This mixture is also designated as "Chlorella Grow Factor" in popular-science literature. The extract as well as some of the single components, isolated from the extract show distinct health stimulating effects, mostly explainable by stimulating the immune system.
In the following compilation basic physiological effects reported in the medical literature are summarized. Only the reports on exact investigations were taken into consideration and the focus was directed on colligative effects. Compounds and components of relatively high concentration in Chlorella were evaluated separately. Due to the very complex composition of Chlorella the major part of the observed effects cannot contributed to definite separated components. Therefore, the systematisation reflects according to pathogenic actions or to indicated symptoms.

1. Interaction of Chlorella vulgaris with the immune system

1.1 Stabilization against bacterial infections

Basing on observations, that persons consuming micro algae regularly seems to be more resistant against infection diseases, in the middle of the sixties in Japan first systematically research was done on this phenomenon. A field study with a group of approximately 1000 Japanese marine soldiers over a period of 95 days showed the surprising result that the

soldiers in the trial group, which got 2 g Chlorella vulgaris each day, had a significant lower risk (25 %) to catch a cold (*KASHIWA* et al. [2]).

In 1973 *KOJIMA* et al. demonstrated the immune stimulation effect of Chlorella. Rats were injected with Chlorella extracts and 24 hours later injected with carbon particles. They observed that the carbon particles concentration in the blood decreased more rapidly in the Chlorella treated trial group. Examination of the rats tissues proved that the macrophages were much more active in the rats treated with Chlorella than those in the control group.

TANAKA et al. observed in 1986 that the resistance against Escherichia coli inoculated intraperitoneally into mice was enhanced by intraperitoneal, intravenous or subcutaneous administration of a water-soluble, high-molecular-weight fraction extracted from Chlorella vulgaris (**CVE**). The elimination of bacteria from the spleen of **CVE** – treated mice was increased, and this enhanced elimination have been related to the acceleration of superoxide generation and chemokinesis in polymorphonuclear leukocytes by **CVE** – treatment. The enhancing effect was detected with doses of about 2 mg / kg and when doses were administered 1,4 or 7 days before the infection [4].
Oral administration of **CVE** shows similar effects, which give some evidence for stimulating the unspecific cellular defence. HASEGAWA et al. fed male fisher rats with 1000 mg **CVE** / kg for 14 days**.** The rats were inoculated with $2{,}7*10^8$ Escherichia coli intraperitoneally. The bacteria numbers increased during 1 – 6 h and reached the peak after 6 h in both control and **CVE** – administered group. In both groups the bacteria numbers decreased to an undetectable level within 24 h. In the **CVE** – administered group, the numbers of viable bacteria in each organ (spleen, liver, peritoneal cavity and blood) were remarkably lower than those in the control group. Whereas, the leukocyte numbers, especially polymorphonuclear leukocytes, in the peritoneal cavity and peripheral blood maintained higher levels in the **CVE** – administered group [5].
After oral administration of **CVE** in mice (20 mg / mouse, 10 consecutive days) the resistance against an intraperitoneal infection with Listeria monocytogenes was improved. The numbers of bacteria in the **CVE** – administered group were significantly lower both in the peritoneal cavity and spleen than those in the control group. FCM analysis revealed that γ/δ + Thy 1.2+ cells in the nonadherent peritoneal exudates cells (PEC) and spleen from **CVE** – administered mice increased more prominently in number at the early stage on day 3 or 5 after infection as compared with those in control mice. The proportion of TCR α/β + Thy1.2+ T cells in the nonadherent PEC of the control group increased from 13 % on day 0 to 49 % at the late stage on day 10 after infection, whereas the proportion in **CVE** treated mice increased to 64 % on this stage in association with augmentation of DTH response to Listeria.

The results suggest that **CVE** – administration effectively augment cell-mediated immunity against Listeria through the increment of γ/δ + T cells in the early phase and the increment of α/β + T cells in the late phase of listerial infection (*HASEGAWA* et al. [6]).
Also preventive oral administration of Chlorella vulgaris biomass (**CVB**) exhibits effects on immune situation. *DANTAS* et al. have demonstrated those effects on Natural Killer cells (NK cells) activity of mice infected with a sublethal dose of viable Listeria monocytogenes. The treatment with Chlorella vulgaris produced a significant increase on NK – cells activity both in non-infected and infected animals compared to the animals that received only vehicle (water). When **CVB** was administered in infected mice, there was an additional increase in NK cells activity which was significantly higher than that found in the only infected group. Moreover, **CVB** treatment (50 and 500 mg/kg) of mice infected with a dose of $3*10^5$ bacteria / animal, which was lethal for all the non treated controls, produced a dose response protection which led to a 20 % and 55 % survival rate, respectively [7].

Furthermore *DANTAS* et al. found, that this kind of protection is due, at least in part, to increased granulocyte-macrophage colony – forming unit in the bone marrow and an increase in colony stimulating activity of the serum as compared to the control group [8].

Organisms which have a poor immune system for instance by application of immune suppressants may be also protected by administration of Chlorella vulgaris or **CVE**.
In case of administration of **CVE** *KONISHI* et al. [9] and *HASEGAWA* et al. [10] observed acceleration of recovery of polymorph nuclear leucocytes in the peripheral blood in mice or rats made neutropenic by cyclophosphamide. The number of granulocyte/ monocyte - progenitor cells in the spleen increased rapidly. In contrast to the non CVE treated mice the **CVE** - animals showed an enhanced resistance against intraperitoneal E. coli infection. It seems to be probable, that **CVE** activates both mature leukocytes and haematopoietic progenitor cells in the bone marrow.
Further investigations of *KONISHI* et al. [11] back up this hypothesis. "The subcutaneous administration of an acidic glycoprotein prepared from **CVE** in 5-fluorouracil (5FU) treated mice showed protective effects on myelosuppression and indigenous infection.

The administration of the glycoprotein greatly reduced the mortality of non-tumour bearing mice given a high dose of 5FU, and could increase the LD_{50} – value of 5FU for these mice. Normally after 5FU treatment, indigenous infection developed as a result of the impairment of the host defence system. The glycoprotein reduced the incidence of indigenous infections and this effect was attributable to the acceleration of recovery from 5FU – induced myelosuppression. Early recovery of haematopoietic progenitor cells, or cells responding to interleukin-3 or granulocyte / macrophage – colony – stimulating factor, was observed in the bone marrow of glycoprotein treated mice. When tumour bearing mice were given the glycoprotein during treatment with 5FU, the glycoprotein prolonged the survival of mice without affecting the antitumour activity of 5FU. In addition, the glycoprotein was itself shown to exert an antitumour effect. These results suggested that the glycoprotein may be beneficial for the alleviation of side – effects in cancer chemotherapy without affecting the antitumour activity of the chemotherapeutic agent."
In terms of this effects it is obvious to examine the effects of **Chlorella** on immune compromised hosts.

HASEGAWA et al. proposed that preventive administration of **CVE** might be effective in the treatment of opportunistic infection in retrovirus –induced immunodeficient patients. He showed that oral administration of **CVE** restored the capacity of mice with murine acquired immunodeficiency syndrome (by infection with LP-BM5 murine leukaemia virus) to eliminate Listeria monocytogenes in association with improvement of the deteriorated immune response to Listeria monocytogenes. DTH response to Listeria monocytogenes in **CVE** – treated mice was significant higher than those in the control group [12].

The authors hypothesized that through augmentation of helper T cell type1 responses producing γ - interferon, the latter activates macrophages to produce interleukin 12 and enhance on this way the host defence against Listeria. Both the higher secretion of γ - interferon and the higher contents of cytokine are detectable (*HASEGAWA* et al. [13, 14].)

1.2. Protection against virus infections

IBUSHUKI et al. evaluated the host mediated antiviral effect of **CVE** in ICR mice against murine cytomegalovirus (MCMV) infection. Mice treated with 10 mg **CVE** on day 3 and 1 before virus challenge survived the infection. The protective effect of **CVE** was shown by a decrease in the infectious viruses replicated in the target organs of the **CVE** treated mice. **CVE** also protected mice from histopathological damage of the target organs due to MCMV infection. Both the serum interferon level and 2`5`-oligoadenylate (2-5) synthetase activity

were elevated and higher than those in the control mice. The natural killer activity of spleen cells, which is otherwise deteriorated by lethal MCMV – infection, was remarkably augmented in **CVE** treated mice. Especially remarkable is the fact that neither virocidal nor virostatic activity of **CVE** on MCMV was seen in vitro. **CVE**-induced resistance seems to be host mediated [15].

1.3 Anti-tumour effects

The previous quoted literature show that via administration of Chlorella vulgaris, both as algae (**CVB**) and algae extract (**CVE**), a series of positive immune stimulatory effects were induced. It seems to be that via activated haematopoiesis and accelerated progenitor cell differentiation the cell mediated immunity increased, accompanied by increasing macrophage activity. That's why it is to be expected that anti-tumour effects mainly occur via stimulating the body's own defences. But recent investigations showed that Chlorella vulgaris produces also substances like sterols [16] and glycerolglycolipides [17] with direct anti-tumour activity.
Both as oral administration of **CVB** (*TANAKA* et al. [18, 19]) and intraperitoneal injection of **CVE** (KONISHI et al. [20]) in mice, inoculated with Meth-A-tumour cells, the survival times were strikingly prolonged. **CVB** and **CVE** treated mice showed antigen-specific concomitant immunity, mediated by cytostatic T – cells but not by cytotoxic T – cells. Natural killer cells seemed not to contribute to anti-tumour resistance in this system.
NODA et al. succeeded to show that a high molecular glycoprotein, witch can be isolated from the **CVE** in high amounts , bears this anti-tumour effect.

For the screening experiments, 5 * 10^6 methylcholanthrene induced Meth A fibrosarcoma cells of BALB / c origin were inoculated subcutaneous into the right and left flank of 8 – 12 week old mice. Each glycoprotein fraction (2 / 10 / 50 mg / kg) was injected into the right flank tumour 5 times every two days from day 2 to evaluate the anti-tumour activity against both tumours 8, 10 and 12 days after tumour inoculation. Anti tumour activity was determined as the product of the longest and shortest diameters of growing ellipsoid tumours over the skin. It was possible to identify the fraction of glycoprotein which inhibits the growing of tumours totally. (doses of 10 mg /kg each injection). The most active substance was found to be a glycoprotein with a molecular weight of 63.000 amu. It contains 65 % carbohydrates, mainly D – galactose, and 35 % protein. The protein moiety was determined and contains 15 amino acids. It was shown, that the protein moiety is responsibly for the anti-tumour activity [21]. The anti tumour activity was stable after autoclaving at 121° C for 30 min and even after treatment with 1M HCl at 80°C for 1 h the anti-tumour activity doesn't decrease.
The observed anti-tumour effect is comparable to the effects of some other already established biological response modifiers like OK – 432 (*OKAMOTO* et al., prepared from Streptococcus pyogenes [22]) and PSK (*TSUKAGOSHI* et al., prepared from Coriolus versicolor) and sometimes stronger than that of the standard dose of OK – 432 (*NODA* et al.[24]).
Biological response modifiers isolated from plant tissues and bacterial products show non-specific and T – cell mediated antitumour effects. The effect induced by glycoprotein fractions extracted from Chlorella vulgaris might depend on a T cell-mediated mechanism in an antigen specific manner [24, 19].

TANAKA et al. showed that the described glycoprotein exhibits the anti tumour effect against both spontaneous and experimentally induced metastasis in mice. The anti – metastatic immunopotentiation was observed in euthymic mice but not in athymic nude mice. This fact is also an indication for a T – cell mediated mechanism . It seems to be that the glycoprotein extract induces T - cell activation in peripheral lymph nodes in tumour bearing mice [25].

1.4. Repair of radiation damages

With respect to the above mentioned activation of haematopoietic progenitor cells and the observed effects in cyclophosphamide treated rats is it obvious to investigate the effects of **CVE / CVB** on organisms, damaged by radiation.
ROTKOVSKA et al. showed that after subcutaneous, intraperitoneal and intramuscular injection of **CVE** the number of haematopoietic cells in the bone marrow and spleen of mice increased, as did their after irradiation. Irradiation with a lethal dose of gamma-rays 24 hours after injection of **CVE** was survived by a larger number of treated mice and rats as untreated ones. On the first day after administration **CVE** protects against brief and prolonged action of irradiation [26].
The observed resistance against irradiation is accompanied by an increasing number of spleen colony –forming units in the bone marrow and spleen and their increasing proliferation activity. The amount of granulocyte – macrophage colony forming cells in the bone marrow grows and the colony – stimulating activity of the blood serum of mice is elevated at an early period after injection of the substance. The recovery of the colony – forming units and granulocyte – macrophage colony –forming cells pools in femoral bone marrow after irradiation proceeds at faster rate in **CVE** – treated animals than in control groups (*VACEK* et al., [27], see also *DANTAS* [7]).
Comparable protection against radiation damage is also possibly by oral administration of **CVB**. Both feeding of **CVB** (400 mg / kg) once, twice or thrice a day for 28 days and acute administration within 0,4 hr after irradiation afforded significant radioprotection (*SARMA* et al.,[28]).
Investigations about the effect of **CVB** doses and administration time on protection against gamma-irradiation showed optimal results when **CVB** (500 mg / kg) was fed 1 hr before or immediately after irradiation. $LD_{50/30}$ for **CVB** pre- and post-treated mice were 8,66 and 9,0 Gy, respectively compared to the control value of 7.8 Gy (*SINGH* et al., [29]).

The above mentioned immune stabilisation and protection effects open interesting possibilities for application of **CVB / CVE** as precaution against and therapies of malignant tumours.

In a two-year study of 20 patients with malignant gliomas, *MERCHANT* et al. [30] added **CVB** and **CVE** to patient's diets to observe what effects might be on their immune system, quality of life and length of survival. **CVB / CVE** was given in addition to the normal treatment by radiation, chemotherapy and medication such as anticonvulsants and corticosteroids. They found that the immune system of patients, compromised by radiation, chemotherapy and medication, reached nearly the normal level by **CVB / CVE** – administration.

1.5. Unspecific effects

The following articles describe some effects obtainable by **CVB / CVE** – application by there symptoms.
Topical application of **CVB** (500 mg / kg . day) during peri-, post- or peri- and post-initiational stages of 7,12-dimethylbenz[a]anthracene induced papillomagenesis, significantly modulated the tumour burden to 5.00, 4.33 and 3.94 (5.88 control group), the cumulative numbers of papillomas to 90, 78 and 67 (106 control group) and the percent incidence of mice bearing papillomas to 94, 90 and 89 (control group 100). **CVB** treating alone or during the different initiational stages significantly elevated the sulfhydryl- and glutathione S-transferase levels in the liver and in skin tissues (*SINGH* et al., [31]).
The significant increase in the hepatic levels of sulfhydryl- and glutathione S-transferase is also detectable in fetal and neonatal systems after 14 days treatment with **CVB** of gestating or lactating mice. The modulation in the levels of hepatic drug metabolising enzymes suggests a chemopreventive potential of CVB via perinatal passage of active constituents and/or metabolites (SINGH et al., [32]).

For assessment of this results it is also necessary to take into consideration that by application of **CVB** high amounts of Chlorophyll are administered. Chlorophyll possesses anti-genotoxic (*NEGISHI* et al., [33];[34]) and anti-inflammatory (*SINGH* et al., [35]) properties. Because of the small particle size of Chlorella the application of Chlorophyll takes place in a highly active and highly available manner.
TANAKA et al. [36] showed that oral administration of **CVB** causes clear prophylactic effects in water-immersion restraint, stress-induced and in cystamine-induced ulcer models.
MARCHANT et al. [37] reported in a pilot study on positive effects by nutritional supplementation by **CVB** for patients with fibromyalgia syndrom.

2. Cardiovascular diseases

Some empirical investigations show that Chlorella may have the ability to reduce high blood pressure and cholesterol levels and to prevent arteriosclerosis. Reasons for this effects may be the high amount of unsaturated fatty acids in combination with antioxidants like chlorophyll as well as the unique balance of nutrients in **CVB**.
OKAMOTO et al. reported that when hypertensive rats were treated with **CVE**, their blood pressure showed a decrease of 63 mm Hg one hour after intravenous administration and of 47 mm Hg two hours after intraperitoneal administration. The blood pressure of normotensive rats also showed a fall of 32 mm Hg one hour after administration [38].

The anti-lipidemic and anti-arteriosclerotic action of **CVB** were investigated by SANO et al., using male Japanese White rabbits. A ten-week load of high cholesterol diet remarkably increased serum total cholesterol and beta-lipoprotein cholesterol levels in serum, causing aortic arteriomatous lesion. In the Chlorella group which was administered a high-cholesterol diet containing 1 % powdered Chlorella vulgaris, the increase of total and beta-lipoprotein cholesterol level was suppressed. Further, the development of aortic arteriomatous lesions was significantly inhibited. Clofibrat used as positive control in this experiment, did not show any inhibitory effect [39]. Similar effects were observed after oral administration of CVE in cholesterol fed rats.

The increase of serum lipids were inhibited by feeding of **CVE** and **CVB** almost at the same degree. Faecal excretion of steroids (cholesterol, deoxycholic and lithocholic acid) were also increased. The authors concluded that the feeding of **CVB / CVE** inhibits the absorption of exogenous steroids and promotes turnover of bile acids in liver to suppress the increase of serum cholesterol level caused by administration of high cholesterol diet (*SANO* et al., [40]).

3. Detoxification

Chlorella vulgaris possesses a three layer cell wall, mainly consisting of cellulose and chitin. This wall shows strong adsorptive properties against xenobiotica, for example organic toxins like dioxin or heavy metals like mercury, cadmium or lead. That means Chlorella has the ability to bind heavy metals, pesticides and toxins and carry these substances safely out of the body.
Although in popular science articles are often reporting on the ability of Chlorella to remove mercury from the body, there were no clues in medical specialist journals about this fact. On the contrary, on the use of Chlorella for decontamination of heavy metal polluted sewage in environmental technology papers is often reported (for instance [41]; [42]; [43]). Accordingly, in the stomach and in the intestinal tract similar adsorptive processes at chlorella surfaces are thinkable so that it seems to be possible to use **CVB** for faecal excretion of mercury out of the body.
For cadmium such processes are documented. *HAGINO* et al. found that chlorella increased the excretion of cadmium in human beings. Both faecal and renal cadmium excretion

accelerate 3 to 7 fold by **CVB** – administration in individuals suffering from cadmium poisoning (Itai – Itai – disease) [44].
On acceleration of Dioxin – excretion in dioxin fed rats by administration of **CVB** (10 % Chlorella diet) report *MORITA* et al. [45].
PORE et al. published a study in which chlorella administered to rats increased the rate of detoxification of chlordecone. It was shown that chlorella caused the toxin removal from the body more than twice as fast as the control group.

4. Prevention of deficiency diseases

Because of the unique nutritional properties and the high biological valency of Chlorella vulgaris it is impossibly to get deficiency symptoms even on exclusively consumption of this micro algae.
With the recommended daily uptake of 3 g CVB for a normal weighted human being, certainly the body will get some limiting amino- and fatty acids, but it is impossible to supply the body with all limiting components totally by administration of 3 g a day.
The same is value for vitamins and minerals, that means in this case of application Chlorella vulgaris acts only as food supplement, which helps to prevent nutritional deficiencies.
In this connection the theory is widely accepted, that the collective effect of a continuous and complex basic supply with micronutrients plays an important role in inhibition of carcinogeneses by chemoprotection (*MARCHANT* et al.[47]).
One exception is by the high concentration of vitamin B_{12} in Chlorella vulgaris (nearly 2,5 mg / kg). Already on administration of the recommended 3 g of **CVB**, the body gets several times as much it needs daily. This fact is especially interesting for vegetarians and those who are anaemic. *RAUMA* et al. showed in a two-year study, that strict vegetarians can be supplied sufficiently with bio-available vitamin B_{12} by oral administration of **CVB** [48].
MATSUURA et al. showed that rats with iron deficient anemia recover by **CVB** – administration [49].

5. Literature

[1] *Fott, B., Novakova, M.:* Studies in Phytology;
Academia, Prag 1969, pp 10 - 74

[2] *Kashiwa, Y., Tanaka, Y.:* Effect of Chlorella on the changes in the body weight and rate of catching cold of the 1966 training fleet crew;
Reported at the **Japan Medical Science Meeting, Nagoya, Japan 1966**

[3] *Kojima, M., Kasajima, T., Imai, S., Kobayashi, M., Dobashi, M., Uemura, T.:* A new Chlorella polysaccharide and its accelerating effect on the phagocytic activity of the reticuloendothelial system;
Recent Adv. R.E.S. Res. 1973, 13, 101

[4] *Tanaka K., Koga, T., Konishi, F., Nakamura, M., Mitsuyama, M., Himeno, K., Nomoto, K.:* Augmentation of host defense by a unicellular green alga, Chlorella vulgaris, to Escherichia coli infection;
Infect. Immun. 1986, 53 (2), 267

[5] *Hasegawa, T., Tanaka, K., Ueno, K., Ueno, S., Okuda, M., Yoshikai, Y., Nomoto, K.:* Augmention of the resistance against Escherichia coli by oral administration of hot water extract of Chlorella vulgaris in rats;
Int. J. Immunopharmacol. 1989, 11(8), 971

[6] *Hasegawa, T., Okuda, M., Nomoto, K., Yoshikai, Y.:* Augmention of the resistance against Listeria monocytogenes by oral administration of a hot water extract of Chlorella vulgaris in mice;
Immunopharmacol. Immunotoxicol. 1994, 16(2), 191

[7] *Dantas, DC., Kaneno, R., Queiroz, ML.:* The effect of Chlorella vulgaris in the protection of mice infected with Listeria moncytogenes. Role of natural killer cells. **Immunopharmacol. Immunotoxicol. 1999, 21(3), 609**

[8] *Dantas, DC., Queiroz, ML.:* Effects of chlorella vulgaris on bone marrow pregenitor cells of mice infected with Listeria monocytogenes.
Int. J. Immunopharmacol. 1999, 21(8), 499

[9] *Konishi, F., Tanaka, K., Kumamoto, S., Hasegawa, T., Okuda, M., Yano, I., Yoshikai, Y., Nomoto, K.:* Enhanced resistance against Escherichia coli infection by subcutaneous administration of the hot-water extract of Chlorella vulgaris in cyclophosphamid –treated mice.
Cancer Imminol. Immunother. 1990; 32(11) 1

[10] *Hasegawa, T., Yoshikai, Y., Okuda, M., Nomoto, K.:* Accelerated restoration of the leukocyte number and augmented resistance against Escherichia coli in cyclophosphamid – treated rats orally administerd with a hot water extract of Chlorella vulgaris;
Int. J. Immunopharmacol. 1990, 12(8), 883

[11] *Konishi, F., Mitsuyama, M., Okuda, M., Tanaka, K., Hasegawa, T., Nomoto, K.:* Protective effect of an acidic glycoprotein obtained from culture of Chlorella vulgaris against myelosuppression by 5-fluorouracil;
Cancer Immunol. Immunother. 1996, 42, 268

[12] *Hasegawa, T., Okuda, M., Hiromatsu, K., Nomoto, K., Yoshikai, Y.:* Hot water extracts of Chlorella vulgaris reduce opportunistic infection with Listeria monocytogenes in C57BL/6 mice infected with LP-BM% murine leukemia viruses; Int. J. **Immunopharmacol. 1995, 17(6), 505**

[13] *Hasegawa, T., Kimura, Y., Hiromatsu, K., Kobayashi, N., Yamada, A., Makino, M., Okuda, M., Sano, T., Nomoto, K., Yoshikai, K.:* Effect of hot water extract of Chlorella vulgaris on cytokine expression patterns in mice with murine acquired immunodefiency syndrome after infection with Listeria monocytogenes; **Immunopharmacology 1997, 35(3), 273**

[14] *Hasegawa, T., Ito, K., Ueno, S., Kumamoto, S., Ando, Y., Yamada, A., Nomato, K., Yaunobu Y.:* Oral administration of hot water extracts of Chlorella vulgaris reduces IgE production against milk casein in mice;
Int. J. Immunopharmacol. 1999, 21(5), 311

[15] *Ibusuki, K., Minamishima, Y.:* Effect of Chlorella vulgaris extracts on murine cytomegalovirus infections;
Nat. Immun. Cell Growth. Regul. 1990, 9(2), 121

[16] *Yasukawa, K., Akihisa, T., Kanno, H., Kaminaga, T., Izumida, M., Sakoh, T., Tamura, T., Takido, M.:* Inhibitory effects of sterols isolated from Chlorella vulgaris on 12-0-tetradecanoylphorbol-13-acetate – induced inflammation and tumor promotion in mouse skin;
Biol. Pharm. Bull. 1996, 19(4), 573

[17] *Morimoto, T., Nagatsu, A., Muratami, N., Sakakibara, J., Tokuda, H., Nishino, H., Iwashima, A.:* Anti-tumor-promoting glyceroglycolipids from the green alga, Chlorella vulgaris;
Phytochemistry 1995, 40(5), 1433

[18] *Tanaka, K., Konishi, F, Hemeno, K., Taniguchi, K., Nomoto, K.:* Augmentation of antitumor resistance by stran of unicellular green algae, Chlorella vulgaris; Cancer **Immunol. Immunother. 1984, 17(2), 90**

[19] *Tanaka, K., Tomita, Y., Tsuruta, M., Konishi, F., Okuda, M., Himeno, K., Nomoto, K.:* Oral administration of Chlorella vulgaris augments concomitant antitumor immunity;
Immunopharmacol. Immunotoxicol. 1990, 12(2), 277

[20] *Konishi, F., Tanaka, K. Hiemeno, K., Taniguchi, K., Nomoto, K.:* Antitumor effect induced by a hot water extract of Chlorella vulgaris (CE): resistance to Meth-A tumor growth mediated by CE – induced polumorphonuclear leukocytes;
Cancer Immunol. Immunother. 1985, 19(2), 73

[21] *Noda, K., Ohno, N., Tanaka, K., Kamiya, N., Okuda, M., Yadomae, T., Nomoto, K., Shoyama, Y.:* A water-soluble antitumor glycoprotein from Chlorella vulgaris;
Planta Med. 1996, 62(5), 423

[22] *Okamoto, M., Kaji, R., Kasetani, H., Yoshida, H., Saito, M., Sato, M.:* Purification and characterisation of interferon-gamma-inducing molecule of OK-432, a penicillin-killed streptococcal preparation, by monoclonal antibody neutralizing interferon-gamma-inducing activity of OK-432;
J. Immunother 1993, 13(4) 232

[23] *Tsukagoshi, S., Hashimoto, Y., Fujii, G., Kobayashi, H., Nomoto, K., Orita, K.:* Krestin (PSK);
Cancer Treat. Rev. 1984, 11(2), 131

[24] *Noda, K., Ohno, N., Tanaka, K., Okuda, M., Yadomae, T., Nomoto, K., Shoyama, Y.:* A new type of Biological Response Modifier from Chlorella vulgaris which needs protein moiety to show an antitumour activity;
Phytotherapy Research 1998, 12(5) 309

[25] *Tanaka, K., Yamada, A., Noda, K. Hasegawa, T., Okuda, M., Shoyama, Y., Nomoto, K.:* A novel glycoprotein obtained from Chlorella vulgaris strain CK22 shows antimetastatic immunopotentiation;
Cancer Immunol. Immunother. 1998, 45(6), 313

[26] *Rotkovska, D., Vacek, A., Bartonickova, A.:* The radioprotective aqueous extract from chlorococcal freshwater algae (Chlorella kessleri) in mice and rats;
Strahlenther. Onkol. 1989, 165(11), 813

[27] *Vacek, A., Rotkovska, D., Bartonickova, A.:* Radioprotektion of hemopoiesis conferred by aqueous extract from chlorococcal algae (Ivastimul) administered to mice before irradiation;
Exp. Hematol. 1990, 18(3), 234

[28] *Sarma, L., Tiku, AB., Kesavan, PC., Ogaki, M.:* Evaluation of radioprotektive action of mutant (E-25) form of Chlorella vulgaris in mice;
J. Radiat. Res. 1993, 34(4), 277

[29] *Singh, SP., Tiku, AB., Kesavan, PC.:* Post-exposure radioprotection by Chlorella vulgaris (E-25) in mice;
Indian J. Exp. Boil. 1995, 33(8), 612

[30] *Merchant, R., Rice, C., Young, H.:* Dietary Chlorella pyrenoidosa for patients with malignant glioma: effects of immuno-competence, quality of life, and survival; **Phytotherapy Research 1990, 4(6) 220**

[31] *Singh, A., Singh, SP., Bamezai, R.:* Inhibitory potential of chlorella vulgaris(E-25) on mouse skin papillomagenesis and xenobiotic detoxikation system;
Anticancer Res. 1999, 19(3A), 1887

[32] *Singh, A., Singh, SP., Bamezai, R.:* Perinatal influence of Chlorella vulgaris (E-25) on hepatic drug metabolizing enzymes and lipid peroxidation:
Anticancer Res. 1998, 18(3A), 1509

[33] *Negishi, T., Arimoto, S., Nishizaki, C., Hayatsu, H.:* Inhibitory effect of chlorophyll on the gentoxoicity of 3-amino-1-methyl-5H-pyrido[4,3]indole (Trp-P-2); **Carcinogenesis 1989, 10(1), 145**

[34] *Negishi, T., Rai, H., Hayatsu, H.:* Antigenotoxic activity of natural chlorophylls; **Mutat. Res. 1997, 376(1-2), 97**

[35] *Singh, A., Singh, SP., Bamezai, R.:* Modulatory influence of Chlorophyllin on the mouse skin papillomagenesis and xenobiotic detoxication system:
Carcinogenesis 1996, 17(7), 1459

[36] *Tanaka, K., Yamada, A., Noda,K., Shoyama, Y., Kubo, C., Nomoto, K.:* Oral administration of a unicellular green algae, Chlorella vulgaris, prevents stress-induced ulcer:
Planta Med. 1997, 63(5), 465

[37] *Merchant, R., Carmback, C. A., Wise, Ch. M.:* Nutritional supplementation with Chlorella pyrenoidosa for patients with fibromyalgia syndrome: A pilot study; **Phytotherapy Research 2000, 14, 167**

[38] *Okamoto, K., Iizuka, Y., Murakami, T., Miyake, H., Suzuki, T.:* Effects of chlorella alkali extract on blood pressure in SHR:
Jpn. Heart J. 1978, 19(4), 622

[39] *Sano,T., Tanaka, Y.:* Effect of dried, powdered Chlorella vulgaris on experimental atherosclerosis and alimentary hypercholesterolemia in cholesterol-fed rabbits;
Artery 1987, 14(2), 76

[40] *Sano, T., Kumamoto, Y., Kamiya, N., Okuda, M., Tanaka, Y.:* Effect of lipophilic extract of chlorella vulgaris on alimentary hyperlipide cholesterol-fed rats;
Artery 1988, 15(4), 217

[41] *Travieso, L., Canizares, RO., Borja, R., Dominguez, AR., Dupeyron, R., Valient, R.:* Heavy metal removal by microalgae ;
Bull. Environ. Contam. Toxicol. 1999, 62(2), 144

[42] *Chong, AM., Wong, YS., Tam, NF.:* Performance of different microalgal species in removing nickel and zinc from industrial wastewater:
Chemosphere 2000, 41(1-2), 251

[43] *Krihnamurthy, S., Frederick, RM.:* Using biopolymers to remove heavy metals from soil and water;
Govt. Reports Announcements & Index (GRA&I), 1994, 13

[44] *Hagino, N., Ichimura, S.:* Effect of clorella on fecal and urinary cadmium excretion in "Itai – Itai" disease:
Nippon Eiseigaku Zasshi 1975, 30(1)77

[45] *Morita, K., Matsueda, T., Iida, T., Hasegawa, T.:* Chlorella accelerates dioxin excretion in rats:
J. Nutr. 1999, 129(9), 1731

[46] *Pore, RS.:* Detoxification of Chlordecone poisoned rats with chlorella and chlorella derived sporopollenin;
Drug. Chem. Toxicol. 1984, 7(1), 57

[47] *Marktl, W.:* Micronutrients in cancer prevention;
Forsch. Komplementarmed. 1999, 6(5), 248

[48] *Rauma, AL., Torronen, R., Hanninen, O., Mykkanen, H.:* Vitamin B_{12} status of long-term adherents of a strict uncooked vegan diet ("living food diet") is compromised;
J. Nutr. 1995, 125(10), 2511

[49] *Matsuura, E., Nemoto, T., Hozumi, H., Izumi, K., Sait, Y., Ispida, H., Fukimbara, T., Kawahara, H.:* Effect of chlorella on rats with iron deficient anemia;
Kitasato Arch. Exp. Med. 1991, 64(4), 193