1
Medical Aspects and Applications of Humic Substances

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AA	Arachidonic acid
BFD	Blackfoot disease
BOP	Catechol oxidation product
CC_{50}	Half-maximal cytotoxic concentration

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CMV	Cytomegalovirus
2,5-DHBQOP	2,5-Dihydroxybenzoquinone oxidation product
2,5-DHPOP	2,5-Dihydroxyphenylacetic acid oxidation product
3,4-DHPOP	3,4-Dihydroxyphenylacetic acid oxidation product
2,5-DHTOP	2,5-Dihydroxytoluene oxidation product
3,4-DHTOP	3,4-Dihydroxytoluene oxidation product
CHOP	Chlorogenic acid oxidation product
DNA	Deoxyribonucleic acid
GENOP	Gentisinic acid oxidation product
HA	Humic acids
HS	Humic substances
HSV-1	Herpes simplex virus type 1
HSV-2	Herpes simplex virus type 2
HYDROP	Hydroquinone oxidation product
HYKOP	Hydrocaffeic acid oxidation product
IC_{50}	Half-maximal inhibition concentration
IL-1	Interleukin-1
KOP	Caffeic acid oxidation product
M.W.	Molecular weight
MX	Mutagen 'X'
POP	Protocatechuic acid oxidation product
RSV	Respiratory syncytial virus
TNF-α	Tumor necrosis factor-alpha
UV	Ultraviolet

1 Introduction

Humic substances (HS) comprising one of the largest reservoirs of carbon in nature may originate from different sources. For example, they can be formed as final products of biosynthetic pathways in micro-organisms, degradation and transformation products in plants, synthetic oxidation products of phenolic compounds and as polymers resulting from roasting processes, e.g. coffee roasting. Surprisingly, besides their brown color, which is responsible for the high UV absorption of HS, polymers of the HS type have several features in common that enable them to interact with other biopolymers as well as with low-molecular weight organic and inorganic compounds and, in particular, with metals, thus forming chelate complexes. Fractal structures, neighboring carboxyl and hydroxyl groups, reduction-oxidation and association-dissociation potentials are some of the most important features of HS as they cause HS to be important biogeochemical components of the Earth's surface. Besides their traditional use as fuel and organic fertilizers, HS are substrates for medical preparations, and also starting materials in the synthesis of specialized industrial products.

In this chapter, we will focus on medical and veterinary-medical applications of HS, and follow this with a discussion of several aspects of environmental health. Finally, we will look ahead to the possibilities of preparing novel biopolymers of the HS type, and to their potential use and application.

2
Historical Outline

The balneotherapeutic use of peat represents the most significant medical application of HS with regard to volume, therapeutic spectrum and tradition. Heavily degraded high moor peat, which is abundant in HS, has been used therapeutically long ago in Babylonia and the Roman Empire, where the inhabitants already recognized the healing effects of mud (Priegnitz, 1986). As health clinics' specialties, mud baths were offered in Europe in the early 19th century. Traditional indications for mud therapy are gynecological and rheumatic diseases (Baatz, 1988; Kleinschmidt, 1988; Kovarik, 1988; Lent, 1988). Beside mud baths which consist of peat pulp, baths with suspended peat material as well as drinking cures were also applied, the latter especially in case of gastric, intestinal or hepatic diseases (Kallus, 1964). The most frequent indications of peat therapy currently offered by health clinics in Germany are summarized in Table 1.

These conditions comprise various disorders of the musculoskeletal and gynecological systems, as well as skin diseases. Acute inflammatory and infectious diseases as well as malign tumors are usually regarded as contraindications. The primary effect of high-temperature peat therapy is the unique depth hyperthermia, which improves blood circulation and regeneration processes in the patient being treated. Depth warming is caused by the special physical consistency of the mud bath. As therapeutic effects have also been reported for low-temperature peat therapy (Balasheva and Gadzhi, 1971), HS (and possibly other chemical compounds) are also strongly suggested to participate in the healing effect by both chemical and biochemical effects. In a recent study, Bellometti et al. (1997) were able to show a favorable influence of mud bath therapy on osteoarthritis, a rheumatic condition characterized by the progressive destruction of cartilage. It was shown that peat therapy influenced the state of chondrocytes as well as the level of inflammation markers interleukin-1 (IL-1)

Tab. 1 Selected indications of peat therapy in the effect of which humic substances are probably involved

Diseases	*Indications*	*Major therapeutic effects*
Musculoskeletal diseases	Degenerative and deforming arthroses Gout Spondylopathies, e.g. Morbus Bechterew, Osteoporosis Muscular rheumatism Rheumatoid arthritis (polyarthritis) Rehabilitation after operations and accidents	Depth hyperthermia improves blood circulation and regeneration processes
Gynecological diseases	Chronic inflammatory diseases Hormonal imbalances Low back pain Adhesions Sterility Climacteric complaints	Depth hyperthermia Estrogenic effect and/or support of endogenous estrogen production Prophylaxis of thrombosis through release of tissue-type plasminogen activator (profibrinolytic effect)
Skin diseases	Chronic eczema Neurodermatitis Psoriasis	Activation of skin metabolism and regeneration processes, improvement of blood circulation

and tumor necrosis factor alpha (TNF-α). Iubitskaia and Ivanov (1999) demonstrated clearly a substantial contribution of HA to the balneotherapy of osteoarthritis patients. Using sodium humate (instead of peat) they observed analgesic, anti-inflammatory and lipid modulatory effects. Moreover, due to the low concentration of the HA preparation and the lack of effects of other mud factors, sodium humate procedures proved to be well tolerated by the patients.

The question of whether, and to what extent, HS are transferred to the patient during the mud bath has still to be answered. Application conditions such as temperature, ionic strength and pH value are thought to influence the balneotherapeutic effect.

3
Pharmacological Effects of Humic Substances with Potential Use in Medicine

In spite of the predominantly positive experience with balneological peat therapy, only limited knowledge is available of the physiologic and pharmacological effects of peat components. Nevertheless, our understanding of the biologic effects of HS with regard to their antiviral activity, interactions with isolated enzymes, effects on blood coagulation and fibrinolysis, estrogenic activity as well as toxicologically significant interactions with environmentally harmful substances has been considerably extended during the last decades. In this section, we will discuss some of these effects with regard to their potential therapeutic uses.

3.1
Antiviral Activity

Studies on the antiviral effect of HS were initiated after the successful combat against foot-and-mouth disease by means of peat dust-containing litter (Schultz, 1962, 1965). Preliminary in-vitro studies with Coxsackie A9 virus, influenza A virus and herpes simplex virus type 1 (HSV-1) have already shown that HS are effective against both naked and enveloped DNA viruses (Klöcking and Sprößig, 1972, 1975; Thiel et al., 1977). The same is true for synthetic HA derived from polyphenolic compounds, which in part are superior to natural HA in their effect (Thiel et al., 1976, 1981; Klöcking et al., 1983; Eichhorn et al., 1984; Hils et al., 1986). One of the antivirally most active synthetic polymers is the oxidation product of caffeic acid, KOP, the effect of which on HSV-1 *in vitro* compared with that of naturally occurring peat HA is shown in Figure 1.

Further investigations corroborate the ability of HA-like polymers to inhibit selectively viruses for human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), cytomegalovirus (CMV) and vaccinia virus (Schols et al., 1991; Neyts et al., 1992). No inhibition was found against poliovirus type 1, Semliki forest virus, parainfluenza virus type 3, reovirus type 1 and Sindbis virus. Adenovirus

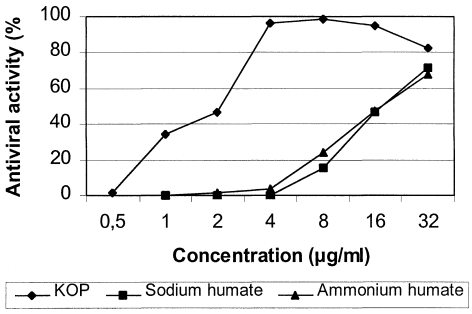

Fig. 1 Antiviral activity to HSV-1 of the synthetic HA-like polymer KOP and naturally occurring peat water HA (as sodium and ammonium salts, respectively). Test substances were added to Vero cells immediately before virus infection. After incubation at 37 °C for 120 h, cell viability was detected using the XTT-based tetrazolium reduction assay EZ4U according to Klöcking et al. (1995).

type 2 and ECHO virus type 6 showed little or no response to natural HA. Half-maximum Anti-HSV-1 inhibition concentrations (IC_{50}) and half-maximum cytotoxic concentrations (CC_{50}) of HA and HA-like polymers are summarized in Table 2, indicating the selective antiviral effect of the polymers tested.

With most viruses, the inhibitory effect of HA and HA-like polymers is directed specifically against an early stage of virus replication, namely virus attachment to cells (Klöcking and Sprößig, 1975; Schols et al., 1991; Neyts et al., 1992). As for CMV, it appears likely that the polyanionic HA occupy positively charged domains of the viral envelope glycoproteins, which are necessary for virus attachment to the cell surface (Neyts et al., 1992).

The effect of HA and HA-like polymers on an early stage of herpesvirus replication has been confirmed by the results of animal experiments. The number of lesions in the cornea of HSV-1-infected rabbits was strongly reduced when a solution of the synthetic HA-like polymer KOP (1%) was applied into the conjunctival sac of the eye along with or immediately after the infectious agent. However, KOP had no effect on the developing lesions when applied 1 and 24 hours later, respectively (Klöcking, 1994). Current interest is directed to the prophylactic effect of HA-type substances on recurrent HSV infection. It is known that topical application of KOP may significantly reduce or even completely suppress experimentally induced herpes in the mouse ear (Dürre and Schindler, 1992), though the mechanistic basis of this effect remains to be elucidated.

A low-molecular weight HA-like polymer (HS 1500, M.W. = 1500 Daltons), synthesized from hydroquinone was found to strongly inhibit HIV-1 *in vitro* (Schneider et al., 1996). Studies on the mechanism of action revealed virus penetration into host cells as the target of the anti-HIV-1 activity. HS 1500 has passed a panel of preclinical tests including eye irritation according to Draize, as well as pregnancy risk in rats. Neither sensitizing nor irritating effects were detectable in concentrations of up to 10% HA (Wiegleb et al., 1993; Lange et al., 1996a,b). SP-303 is another phenolic polymer with antiviral activity, which was isolated from a

Tab. 2 Half-maximal cytotoxic concentrations (CC_{50}) and half-maximal antiviral inhibitory concentrations (IC_{50}) of humic acids and humic acid-like polymers

Test substance	*M.W. (Da)*	*Starting compound*	*CC_{50} (µg/mL)*	*IC_{50} (µg/mL)*
BOP	5300	Catechol	69	26
3,4-DHTOP	3800	3,4-Dihydroxytoluene	>128	42
POP	8000	Protocatechuic acid	70	9.6
3,4-DHPOP	6000	3,4-Dihydroxyphenylacetic acid	>128	9.6
HYKOP	6000	Hydrocaffeic acid	>128	8.0
KOP	6000	Caffeic acid	>64	2.3
CHOP	14000	Chlorogenic acid	>128	6.4
HYDROP	5200	Hydroquinone	>128	3.7
2,5-DHTOP	4700	2,5-Dihydroxytoluene	>128	1.6
GENOP	5500	Gentisinic acid	>128	2.2
2,5-DHPOP	5500	2,5-Dihydroxyphenylacetic acid	>256	0.7
2,5-DHBQOP	3400	2,5-Dihydroxybezoquinone	>512	322
Sodium humate	7500	not known	>128	18.2
Ammonium humate	7900	not known	108	17.8

Euphorbiaceae shrub. The polymer inhibits a panel of respiratory viruses, such as parainfluenza virus type 1, respiratory syncytial virus, influenza A viruses, and influenza B viruses (Gilbert et al., 1993; Wyde et al., 1993). Hemagglutination and other studies suggested that SP-303 at least partially inactivates viruses by direct interaction with virus or host cell lipid membranes. SP-303 at antiviral concentrations did not induce interferon or inhibit virus attachment; however, it abolished RSV penetration into host cells (Barnard et al., 1993). Administered as a small-particle aerosol to influenza A/HK virus-infected mice and RSV-infected cotton rats, SP-303 at 0.5–9.4 mg/kg/day for 3–4 days increased both the percentage and duration of survival of mice. Taken together, results so far show that HS are promising candidates for prophylactic rather than therapeutic use in the treatment of viral diseases.

3.2 Anti-inflammatory Effect and Pro-inflammatory Properties

Various healing effects of peat therapy were attributed to the anti-inflammatory activity of HS. Taugner (1963) showed in the rat paw edema model that sodium humate significantly inhibits the development of various edemas compared with untreated controls. As found by Klöcking et al. (1968) in the same model, ammonium humate isolated from peat water exceeds the anti-inflammatory effect of sodium humate and is twice as effective as acetylsalicylic acid and aminophenazone, respectively. Amosova et al. (1990), in evaluating the biologic activity of HA from Tambukan therapeutic mud in animals, found HA (10 mg/kg) to suppress both phases of the inflammatory process: the exudation (by 44 %) and the proliferation process (by 50–55%).

The anti-inflammatory effect of HS has been supported by a plausible biochemical explanation. As demonstrated by Schewe et al. (1991), naturally occurring HA, and even more synthetic HA-like polymers, inhibit the lipoxygenase pathway of the arachidonic acid (AA) cascade. AA is an integral part of the cell membrane, and the substrate for the synthesis of eicosanoid-based inflammation mediators such as leukotrienes, thromboxane and prostacyclin. Recently, HA (sodium humate) as well as various synthetic HA-like polymers were also found to suppress the heat-induced (42 °C, 6 h) AA release of human promonocytic U937 cells (Dunkelberg et al., 1997; Klöcking et al., 1997). The inhibition of AA release was most pronounced in cells treated with nontoxic concentrations (20 μg/mL) of 3,4-DHPOP (96%) and 3,4-DHTOP (92%), respectively (Table 3). CHOP, sodium humate, KOP and BOP protected cells from membrane damage at 65–90%. These findings may be indicative for membrane-protective activities of HA type substances.

Unlike 5-lipoxygenase, phospholipase A_2 (porcine pancreas), the rate-limiting key enzyme of the AA cascade, is strongly activated at low HA concentrations (0.1–

Tab. 3 Influence of naturally occurring humic acids (sodium humate) and of synthetic humic acid-like polymers on the heat-induced (42 °C, 6 h) [^{3}H]arachidonic acid (AA) release of U937 cells. AA release of untreated control cells = 100%. MEC, Maximum effect concentration; SD, Standard deviation; *Significant, $p \leq 0.05$.

Polymer	*MEC μg/mL*	*[^{3}H]AA release % of controls ± SD*
Sodium humate	80	26.5 ± 8.9*
BOP	160	10.1 ± 14.2*
3,4-DHTOP	20	8.1 ± 10.1*
3,4-DHPOP	20	4.3 ± 2.4*
KOP	40	18.1 ± 7.1*
CHOP	80	34.9 ± 12.6*

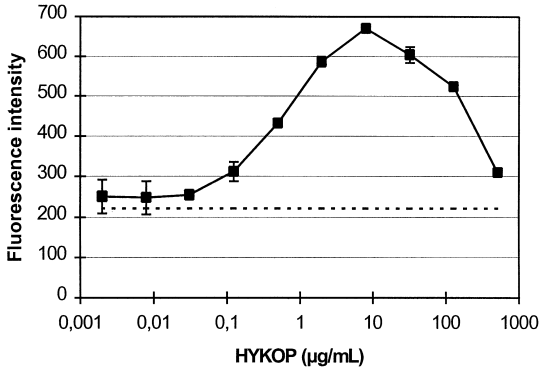

Fig. 2 Influence of the synthetic HA-like polymer HYKOP on the PLA_2-catalyzed hydrolysis of NBD-C_6-HPC using the method of Bennett et al. (1991). Dotted line = Reference value (without HYKOP).

1 µg/mL) and normalized or slightly inhibited at high HA concentrations (Klöcking et al., 1999). Figure 2 shows the typical concentration-dependent course of the dose–response curve for HYKOP, the oxidation product of hydrocaffeic acid. The shape of the dose–response curve suggests HS to have a regulatory function on PLA_2 activity.

Little is known about the influence of different molecular weight fractions of HS on inflammation. Pro-inflammatory activity has found to be associated with the synthetic low-molecular weight HS 1500, which activates human neutrophiles similar to TNF-α (Zeck-Kapp et al., 1991). Liang et al. (1998), while investigating rabbit articular chondrocytes, revealed an inhibitory effect of the ethyl acetate fraction of the commercial Aldrich HA (100–500 µg/mL) on the survival of chondrocytes. Cell injuries were attributed primarily to $O_2^{\bullet-}$ production, which is converted into H_2O_2, thus initiating lipid peroxidation followed by cell necrosis.

In referring to the function of HS as electron donor–acceptor system, Jurcsik (1994) discussed the behavior of HS as the consequence of a 'buffering effect'; this means that HA are able to produce as well as to bind activated oxygen species. This regulatory system is assumed to be important for the favorable influence of HS on wound healing and killing of cancer cells (Jurcsik, 1994).

3.3 Influence on Blood Coagulation and Fibrinolysis

Prophylaxis and therapy of fusions after tubal or ovarian inflammations as well as the postoperative treatment of sterility operations in order to prevent secondary adhesions and repeated occlusions of the ovarian duct are important indications of peat therapy. Fusions are caused by postoperatively reduced degradation of fibrin to soluble fibrin degradation products. As shown by Mesrogli et al. (1988) in laparotomied rats, postoperative baths in peat extract, peat pulp or HA have a clear adhesion-inhibiting effect. A possible explanation of this effect could be the activated fibrin degradation due to the HA-induced release of tissue-type plasminogen activator (t-PA). t-PA is regarded as the regulator of the antithrombotic defense mechanism. It activates plasminogen to plasmin, which splits insoluble fibrin to soluble fibrinogen degradation products (Klöcking, 1991). In addition, HA inhibit the coagulation enzyme thrombin, thereby suppressing the formation of fibrin monomers from fibrinogen (Klöcking, 1994; Klöcking et al., 1999).

Compared with other polyanionic compounds (heparin, pentosanpolysulfate), the anticoagulant effect of HA was found to be less pronounced.

3.4 Estrogenic Activity

Since the first detection of estrogenic substances in the bitumen fraction of peat by Aschheim and Hohlweg (1933), attempts were made to identify the nature of these substances. The assumption that steroid hormones are responsible for the colpotropic effect of peat could hardly be confirmed by chemical analysis. Therefore, the question arose whether, in addition to the lipid-soluble hormones, other peat components might contribute to the estrogenic activity of peat. Studies in castrated ICR (Institute of Cancer Research, USA) mice showed both naturally occurring peat humic acids and synthetic HA-like polymers to be positive in the Allen-Doisy test (Klöcking et al., 1992). The estrogenic activity of sodium humate was found to be 1/3000 of the estriol standard preparation. Referring to the HA content of dry peat, the estrogenic activity of the high-moor peat studied was 5000 times as high as has been supposed to date. Although the components responsible for the estrogenic activity of peat remain under discussion, the results suggest that HA – provided that they can penetrate the skin – may contribute substantially to the estrogenic effect of peat. These findings may have also implications for the use of HS in dermatology and cosmetics.

4 Veterinary-Medical Applications of Humic Substances

In veterinary medicine, HS are successfully applied as drugs for prophylaxis and therapy of gastrointestinal diseases in small animals. Furthermore, HS are utilized as antidotes to prevent intoxication (Kühnert et al., 1989). In order to bind and possibly metabolize as-yet resorbed poisons in the stomach-gastrointestinal tract, HA are given orally as a 20–30% watery solution or suspension in a dosage of 0.5–1.0 g/kg twice daily for 3–5 days (Kühnert, 1996). As observed by Ridwan (1977), a HA concentration of 0.1% is sufficient to reduce significantly the incorporation of lead and cadmium in rats, thus minimizing the risk of heavy metal intoxication. Experiments in mice have shown that orally administered lead humate is less toxic than lead acetate (Klöcking, R., 1980). Opposing results have been obtained following parenteral application of the same compounds. Clearly, the application route is an important factor in deciding whether a metal bound to HS is toxic or detoxified.

5 Humic Substances and Environmental Health

5.1 Mutagenicity

As HS are naturally widespread in the environment and present in surface water, studies on their genotoxic potential are justified, particularly as by-products of chlorination and ozonization in HS containing drinking water are known to be extremely active in bacterial genotoxicity tests (Meier et al., 1987; Meier, 1988). Mutagenesis studies carried out on fractions of drinking water samples have shown that 3-chloro-4-(dichloromethyl)-5-hydroxy-(5H)-furanone (MX) is one of the main chlorination intermediate products, responsible for more than 20% of observed mutagenicity (Holmbom, 1984; Kronberg et al., 1985, 1991). However, in-vivo mutagenicity tests have provided con-

flicting results, possibly due to the great reactivity and instability of the furanones formed (Dayan, 1993). Furanones occur also in foods, where they appear mainly as a result of Maillard reactions between sugars and amino acids during heating. Furthermore, they play an important role in the flavor of fruits and as an essential antioxidant food component (ascorbic acid, vitamin C) for humans (Colin-Slaughter, 1999). Although furanones are mutagenic to bacteria and cause DNA damage in laboratory animals, these compounds are, in practice, very effective anti-carcinogenic agents in the diets of animals which are being treated with known cancer-inducing compounds such as benzo[a]pyrene or azoxymethane. Evidence for the desmutagenic activity of HA has also been reported by Cozzi et al. (1993), De Simone et al. (1993), and Ferrara et al. (2000).

5.2 Protection against Ionizing Irradiation

Oris et al. (1990) were able to show that dissolved humic materials at concentrations in the range of 1 to 7 μg/mL significantly reduced acute photo-induced toxicity in fish (*Pimephales promelas*) and daphnia (*Daphnia magna*). The phenomenon is explained by selective attenuation of the active wavelengths of solar UV radiation by dissolved HS.

A protective effect of HA to injuries caused by an external whole-body ^{60}Co gamma irradiation in female Wistar rats has been reported in a World Patent Application (WO 9858655). HA were extracted from a 3000- to 7000-years-old fen peat standardized by topographic and paleobotanical characterization. The HA preparation (240 mg/animal/day) was applied by gastric intubation to female Wistar rats of 190–220 g bodyweight for 7 days before irradiation (7 Gy), followed by an additional 4-week treatment with the same dose after irradiation. No injury of the hemopoietic system occurred in the HA-treated animals. Lower dosages of HA (90 mg/animal/day) were also effective, albeit to a lesser extent. The HA-containing preparation is intended to improve the regeneration of the hemopoietic system in case of accidental radiation effects, and possibly to mitigate against injuries due to chemotherapy.

A therapeutic effect of sodium humate given as a single dose to experimental mongrel rats 5–10 min following irradiation with lethal doses of ^{60}Co led to 43% survival of animals after 60 days (Pukhova et al., 1987).

In addition to the protection against radiation-induced injuries and the supporting effect on tissue regeneration, HA show indirect detoxifying effects, e.g. by preventing the photoactivation of polycyclic aromatic hydrocarbons. As demonstrated by Nikkila et al. (1999), HA reduced the toxicity of UV-B-irradiated pyrene to *Daphnia magna* in a dose-dependent fashion. The effect was assumed to be due to the decrease in the photomodification of the dissolved pyrene by diminishing the light penetration into the water, and possible interaction with the intact parent compound.

5.3 Blackfoot Disease

Artesian drinking water containing a high concentration of greenish-blue fluorescent HS and/or arsenic has been implicated as one of the etiological factors of Blackfoot disease (BFD), which occurs endemically in the southwest coast of Taiwan (Lu, 1990). Clinically, BFD is a peripheral vascular disorder with symptoms similar to those of arteriosclerosis obliterans and thrombotic vasculopathy. The disease can be induced experimentally in mice receiving fluorescent HS at a daily dose of 5 mg per 20 g body mass for at least 22 days. The pathogenesis of the disease has not yet fully established. In-vitro studies

with purified commercial HA revealed a destruction of human erythrocytes at (relatively high) HA concentrations of 50 – 100 μg/mL, probably due to the generation of oxidative stress (Cheng et al., 1999). Recently, Gau et al. (2000) demonstrated an inhibition of lipopolysaccharide-induced expression of NF-κB in HA-pretreated cultured human umbilical vein endothelial cells.

6 Outlook and Perspectives

The impact of HS on the quality of human health is increasingly recognized as an important subject of future research work. Investigations of HS aimed at the molecular structure and mechanism of action encompass specialized investigations within such diverse fields as physical, analytical, environmental and food chemistry, cell biology, molecular genetics, pharmacology and toxicology.

As outlined in this chapter, some of the naturally occurring or synthetically prepared biopolymers of the HA type have the potential of highly effective drugs. Therefore, in addition to the classic use of peat in balneotherapy and veterinary medicine, the application of isolated HS as well as synthetic HA-like polymers may play a considerable role in future. There are a large number of phenolic compounds which can be transformed into HA-like substances targeted for special functions such as antivirally active agents, heavy metal-chelating compounds, toxic chemical-binding polymers and substances protecting against ionizing radiation. However, the use of HS as therapeutic drugs make high demands on pharmacologically evidenced efficacy, toxicological safety standards and a clearly defined chemical composition of the preparation used.

To elucidate the chemical structure of synthetic HA that originate from comparatively simple individual starting compounds remains an important goal for the near future. The results will definitely stimulate and facilitate the much more complicated exploration of natural HS.

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