

## **1. INTRODUCTION**

### **1.1. BACKGROUND**

HUMET®-R syrup is an orange-flavoured colloidal solution of humic acids and ten organic minerals: Iron, Magnesium, Zinc, Copper, Cobalt, Manganese, Selenium, Vanadium, Molybdenum and Potassium. This colloidal solution forms an optimal carrying agent with the humic acids providing variable function groups, which contain the trace elements and minerals in chelated biochemical structures similar to the human body's own transport-proteins. These are thus more easily absorbed and have high bioavailability. HUMET®-R is formulated to carry the international Recommended Daily Allowances (RDA) of the ten organic minerals.

Once the organic minerals are released into the body, the freed humic acids naturally bond to any heavy metal molecules (cadmium, mercury, lead, etc) and these are removed in the waste. Humet-R has been developed after many years of study into the inter-relationship of the trace element content of cultivated fields, the trace element content of farm animals maintained in those fields, and the area spread of disease due to trace element deficiency. In spite of the fact that both soil and its vegetation contain lavish quantities of the elements needed, animals developed deficiency symptoms. It is known from the literature that the humic acids contained in peat assist in efficiently delivering the required trace elements into the animal organism. This is impossible for inorganic compounds in the form of the simple metal salts.

The eminent veterinarian Dr. Elek Csucska carried out the majority of the veterinary observations mentioned above as well as the early experiments with a peat-based product. Basic experiments were followed by research work for several years. An extraction method was developed to separate humic acid from calcium huminate. By adding the proper metal ions to the humic acid, the product was found to improve the clinical state of mineral-deficient patients.

### **1.2. DATA FROM THE LITERATURE**

The results of recent research works clearly demonstrate that animals are unable to utilise metal ions when they are introduced in the form of inorganic compounds. References reinforce that the efficiency of inorganic metal salts is very low in the replacement of trace elements demanded by the body.<sup>1</sup> Similarly, literature references demonstrate that peat and peat soil possess the capacity to bind metal ions.<sup>2</sup> It was shown that the humic acid component of peat was responsible for the chelate binding of trace elements.<sup>3</sup> It became evident that the administration of peat and peat extracts might improve the introduction of the vital metal ions.

Additionally, it was observed that animal feed enriched with humic acid could be curative not only in deficiency diseases but the feed also improved the reproductive function of several domestic animals and their resistance against infectious diseases. Furthermore, humic acid-enriched animal feed could exponentially increase the utilisation of nutrients and, consequently, body weight gain increased.<sup>4</sup> Obviously, that which proved to be helpful in the animal organism may possess therapeutic value in humans, too. Many well-documented trace element deficiency diseases are known in medical practice and the research of the medico-biological role of trace elements is continuing.

### **1.3. DEVELOPMENT OF A PARAMEDICINAL PRODUCT**

Research workers of HUMET Limited (formerly HORIZON-MULTIPLAN Ltd.), and the laboratories working with the company, have investigated HUMET®-R since 1992. The final composition was determined during this period. The efficiency of Humet®-R was proved in controlled expertly based experiments, its further therapeutic indications were revealed, and its safety tested in toxicity studies.

Based on this data, the National Institute of Pharmacy (OGYI) of Hungary approved the product of the HUMET Ltd. in 1993 and the production started in 1994.

## **2. COMPOSITION OF THE PRODUCT AND THE ROLE OF ITS COMPONENTS**

In HUMET®-R the vehicle is a humic acid preparation of homogenous origin, extracted from geologically

young (about 3,000-7,000 years old) peat. This vehicle is completed with several micro- and macro-elements. This paramedicinal product is a complex trace element preparation and its humic acid vehicle is a biologically compatible chelate-former, which assures the good absorption and bioavailability of the metal ions included. Each of its constituents possesses an individual physiological action and effect, but the general roboration (or tonic) effect results from their synergistic, joint action and interaction.

## 2.1. STRUCTURE AND CHEMICAL PROPERTIES OF HUMIC ACIDS

During the last 50 years, several research works and theoretical studies have focused on the chemical structure of humins. Their common source is lignin, which constitutes the slowly degradable compact skeleton of plants. Lignin undergoes a slow microbiological transformation, caused primarily by bacteria and fungi, and chemical changes in the soil. The joint effect of these changes leads to the enrichment with humic acids of various soils (primary peat and brown coal). The two most important therapeutic groups of humins are humic acids and fulvic acids, which are determined based on their acid/base solubility.

According to present knowledge, humic acids are chemically multi-substituted polyaromatic heterocyclic macromolecules, which incorporate cyclic structures joined by aliphatic carbon chains. This primary structure can fix other organic components, such as carbohydrates, proteins, and lipids, in physical and chemical bonds.<sup>5</sup>

Related to oxidised state, the aromatic and chinoidal structures of the chemical entity contain the oxo-, hydroxyl-, carboxyl-, amine, - and substituted amine groups which can bind several bivalent metal ions in chelate bonds. This chelate-bonding ability of humic acids has been used for many years for the clearance of toxic heavy metals from waste water and superficial water running downhill from mining areas.<sup>6</sup>

## 2.2. BIOLOGICAL ROLE OF HUMIC ACIDS

Humic acids are difficult to characterise by physical or chemical methods. Their place of origin, age and geological past may be more characteristic than actual chemical and physical analysis. Their biological effects may be very different.

There is a humin preparation derived from the peat (Tolpa Torf preparation, TTP) which exerts immunomodulator effect.<sup>7</sup> TTP preparation increases the production of tumour necrosis factor (TNF) in human leukocytes and stimulates the synthesis of interferon.<sup>8</sup> In mice, it can recover the immune responses which have been suppressed by zinc phosphamide.<sup>9</sup> TTP preparation given prophylactically significantly decreases the damage of the gastric mucosa and duodenal ulcer<sup>10</sup> and its regenerative effect was also demonstrated in the liver.<sup>11</sup> Humic acids have been noted to influence the function of the endocrine system. The effect on the thyroid function was studied in mice and this demonstrated that humic acids antagonise the action of thyroxin; this effect is mediated by blocking the activity of the Na<sup>+</sup>/K<sup>+</sup>-ATP-ase.<sup>12</sup> The antibacterial effects of humic acids antagonise the mutant strain of streptococci-producing glucane which is responsible for the development of caries.<sup>13</sup> Anti-viral effectiveness of humic acids is also known. The sodium salt of humic acids, when given together with cadmium (a toxic heavy metal) in experiments in chickens, showed a marked decrease in the absorption of the heavy metal and prevented its incorporation into the liver.<sup>14</sup> At the same time according to our experiments with humans, HUMET®-R decreases the blood level of the toxic heavy metals, such as lead and cadmium, in exposed workers.<sup>15</sup>

It was found that, after surgery, humic acid treatment successfully prevented adhesion in rats.<sup>16</sup>

In other experiments, human endothelial cells were incubated with humic acids and an increase in the tissue factor (TF) expression was found. In conjunction with this, increasing intracellular Ca<sup>2+</sup> was measured, even in the presence of Verapamil (a strong Ca<sup>2+</sup> channel blocker), which led to the conclusion that the influx was independent of the specific Ca<sup>2+</sup> channels.<sup>17</sup>

Desmutagenic activity was observed *in vitro* on CHO cells using four different humic acid preparations against two known mutagens, mitomycin-C and maleic acid hydrazide.<sup>18</sup>

Of possibly outstanding importance, in cell culture, synthetic humin analogues block the human immunodeficiency virus (HIV)<sup>19</sup>, the paralysis<sup>20</sup>, and herpes virus.<sup>21</sup>

The anti-allergic effect (e.g.: in "hay fever") of salts, formed by humic acids with alkali metals, has been positive.<sup>22</sup>

The dermatological efficiency of humic acids is noteworthy. Salts of humic acid formed with ammonia and alkali metals significantly shortened the time of wound healing.<sup>23</sup>

Humic acids have been shown to inhibit the reproduction of malignant tumour cells. Thus, they can be

useful in anti-cancer therapy.<sup>24</sup>

Humic acids, as a natural absorbent of the ultraviolet light, may protect the human skin.<sup>25</sup>

The protective effect of humic acid against radiation was demonstrated in rats using its sodium salt. The lethal effect of gamma radiant <sup>60</sup>Co was prevented by 50 percent in animal experiments.<sup>26</sup>

The increasing use of humic acids in the antiphlogistic treatment and particularly in arthritis yields new important therapeutic indications.<sup>27</sup>

It has been reported several times that humic acids possess toxic heavy metal binding capacity.<sup>28</sup>

The therapeutic applications and the potential curative indications outlined above justify further basic research activity.

### 2.3. THE HUMIC ACID METAL CHELATE

For therapeutic application, the most interesting medico-biological effects are due to the metal binding capacity of humic acids. The metal binding capacity of humic acids is based, in part, on chelate forming. Development of chelate bonding masks the charge of the metal ion. The chelated metal loses its hydrate cover and receives the hydrophilic/hydrophobic characteristics of the chelate-forming compound. Thus, in principle, the chelate could easily pass the hydrophobic cell membrane.

The metal-humin interactions are selective. Namely, humic acids binding the toxic heavy metals (lead, cadmium, and mercury), mobilise and eliminate them from the organism, but some vital macro- and microelements are transported by humic acids into the body to specific enzymes. Humic acids can affect several biological processes by hitherto unknown mechanisms.

Recently, it became evident that **selenite** is essential to the function of antioxidant enzymes (e.g.: glutathion-peroxidase), which are responsible for the elimination of free radicals. This mechanism is important where increased formation of free radicals is present (radiation effect, tumour, increased degradation of lipid and protein, long-lasting starvation, etc.). In the body, the lack of selenite causes muscular tissue deficiency and the tumourigenic effect of cadmium and lead possibly increases in humans, and certainly in animals, respectively. Sufficient selenine supply can prevent cardiomyopathy and muscular dystrophy.

The **molibdate** content of the diet assures the co-factor of xanthin oxydase, aldehyde oxydase, and sulphite oxydase.<sup>29</sup>

The **vanadate** component inhibits the phosphatases, which control the intracellular signal transduction and, thereby, can prolong the duration of hormonal action. In diabetes, the gene expression of certain enzymes changes.<sup>30</sup> K<sup>+</sup> found in the preparation is the most important intracellular kation, which has central role in stimulus conduction, and in the maintenance of basic life processes. K<sup>+</sup> deficiency may occur following drug treatment (e.g. diuretics) or due to some diseases. Besides these metal ions, HUMET® -R contains a further six bivalent metal ions which are chelated with the humic acids.

### 2.4. METAL IONS AND THEIR PHYSIOLOGICAL ROLE

Several monographs widely discuss the biological role of the bivalent metal ions and these may be summarised as follows.

- Iron (**Fe**) is the basic component of the functional group of haemoglobin and myoglobin, transporting oxygen and electron-transporting cytochromes. There are clinical symptoms in iron deficiency (fatigue, headache, stomatitis, gingivitis, loss of appetite, etc.) In chronic deficient state, hypochrome anaemia with microcytemia and bone marrow hyperplasia develop. The presence or absence of the other microelements influences the administration of iron to the organism. At the same time, iron intake potentiates the elimination of the toxic lead.
- Magnesium (**Mg**) is a natural calcium antagonist and thus influences the metabolism of calcium, phosphorus, and sodium. Magnesium is the activator of the glycolysis and plays a significant role in protein metabolism. It modifies the muscular function, participates in the maintenance of circulatory homeostasis, and decreases blood pressure (relaxation of the vascular smooth muscle). Magnesium has a role in energy metabolism and in the reproductive function. Magnesium deficiency is manifested in spastic responses.
- Zinc (**Zn**) is the component of several enzymes. It has a central role in the formation of the steric

structure of insulin and in the synthesis of DNA and RNA. The presence of Zinc is especially important in lead and cadmium exposition: its administration decreases the toxicity of these metals. Zinc deficiency causes typical symptoms (dermal changes, alopecia, disturbance in testicular development, sexual retardation, hepato- and splenomegaly, growth disturbances, delayed wound healing, and decreased immunological defensive function.) Levels of Zinc may decrease following the adverse effect of corticosteroid or diuretic therapy, in sickle cell anaemia, lung tumours or myocardial infarction and, in consequence, of anticonceptive use.

- Copper (**Cu**) has a significant role on the haemopoiesis, celloxidation, energy metabolism, and in cerebral catecholamine metabolism. It influences the iron and zinc balances and the reproductive functions. Its lack may be one of the causes of infertility and the increase in cadmium toxicity. Consistent Copper deficiency evokes anaemia, bone marrow alterations, growth retardation, cerebral dysfunction, and myocardial destruction. Patients with Wilson disease a.k.a. pseudosclerosis of Westphal-Strümpell - a trias of basal ganglia dgeneration, liver cirrhosis and corneal Keyser-Fleischer ring - which is quite rare should not use Humet-R.
- Manganese (**Mn**) is an essential mineral requirement for the formation of bone, cartilage and ligaments. It plays an essential role in the formation of Superoxide dismutase, the cellular antioxidant enzyme responsible for the removal of Superoxide radicals. It stabilises blood sugar levels, and is essential for reproduction, red blood cell synthesis, production of insulin as well as maintaining healthy DNA & RNA.
- Molybdenum (**Mb**) assists the body's antioxidant function, removes protein breakdown products like urea, hydrocarbon based free radicals and sulphites.
- Potassium (**K**) is vital in the maintenance of the body's hydrostatic balance, assists with insulin secretion and overall energy balance, maintains heart function, stimulates gut movement and encourages elimination. Promotes healthy nerves and muscle condition, key component in the potassium:sodium ratio for maintenance of blood pressure.
- Selenium (**Se**) is known as the anti-cancer mineral is a principal antioxidant vital in the removal of free radicals and is a component of the antioxidant enzyme Glutathione peroxidase. Essential for male reproduction, immune system stimulation and cardiac function.
- Vanadium (**Va**) is considered an essential trace mineral. Its primary role is associated with regeneration of red blood cells, imbalance in iron metabolism, maintenance of blood sugar levels via the insulin –mimic effect of vanadyl ions, reduces blood fat and cholesterol levels and prevents dental caries.
- Cobalt (**Co**) influences iron metabolism. It increases the haemoglobin concentration in red blood cells. Cobalt is the metal component of the prosthetic group of vitamin B<sub>12</sub>. It is one of the components of beta-lysine-isomerase, glycerin dehydrogenase, and methionin aminopeptidase.<sup>31</sup>

### **3. PRECLINICAL INVESTIGATION**

#### **3.1. TOXICOLOGICAL INVESTIGATIONS**

##### **3.1.1. ACUTE TOXICITY STUDIES IN RATS (WITH 14-DAY POST-TREATMENT OBSERVATION PERIOD)**

Acute toxicity studies were performed in the form of "limit tests" in two species (mouse and rat), in compliance with the GLP regulations, for the determination of the acute oral LD<sub>50</sub> values.<sup>32</sup>

These studies were carried out in male and female Wistar rats and in CFLP mice. At the beginning of the studies, the animals were 5-6 weeks old. The animals were treated with the endowed humic acid which is the active ingredient of the Humet®-R, (supplemented humic acid - SHA) in total amount of 40 ml/kg.

Related to the standard humic acid preparation containing 15 mg/ml, this 'total amount' corresponds to 600 mg effective dose of humic acid.

The animals were fasted for 18 hours, the active part of Humet®-R (SHA) was given (p.o.) via gavage in a volume of 10 ml/kg, twice per 24 hours (10 males and 10 females in every group). Control groups (10 for each gender) were given the same volume in physiological saline. Animals were maintained for further 14-day (post-treatment observation period).

The lethality per group and the body weight of the animals during the post-treatment period were studied. The necropsy did not reveal any pathological changes in several organs. The single-dose administration of SHA did not cause altered behavioural effects or any other pathological changes.

Acute oral LD<sub>50</sub> value of this trace element preparation could not be properly determined in Wistar rats and CFLP mice. There was no lethality after the doses applied. LD<sub>50</sub>(male) > 600 mg/kg; LD<sub>50</sub>(female): > 600 mg/kg in both species.<sup>27</sup>

### **3.1.2. SUBACUTE (4-WEEK) DIETARY TREATMENT OF RATS WITH Humet®-R**

In the course of a four-week treatment, the effects of Humet®-R on the haematological parameters and the body weight gain were studied.

At the end of the observation period, the mass of several organs (lung, liver, spleen, and kidney) was registered and the possible macroscopic changes were studied.

Treatment was carried out in five groups (n = 10), the animals were given 5, 15, 50, 150, 500 mg/kg/day doses in the different groups. The amount of the Humet®-R preparation was related to the dried content mixed with the trace element deficient food. Control group was supplied with normal food.

In this rat study, the animals were fed continuously with food containing Humet®-R for four weeks. The results may be summarised as follows.

It was demonstrated that the humic acid-containing preparation Humet®-R did not affect the animals' general physical state (motility, food intake), the weight of the whole body or that of different organs or the haematological and blood chemistry parameters during the four week diet treatment.

For the animals fed with 150 and 500 mg/kg/day doses of Humet®-R, loss of appetite and consequent weight loss developed three weeks after starting the treatment but the haematological and blood chemistry parameters remained unchanged. Humet®-R did not influence the survival of the animals in either dose group; lethality did not occur. There was no significant difference between the control and Humet®-R treated any dose group of animals in the value of haematological or blood chemistry parameters.<sup>33</sup>

### **3.1.3. CUMULATIVE TOXICITY EVALUATION IN RATS**

The aim of the study was to determine the cumulative toxicity of Humet®-R.

Study was performed in control and treated Wistar rats (n = 10). The results of the previous studies declared the product non-toxic. The LD<sub>50</sub>-value was arbitrarily declared 150 mg/kg. The rats were orally treated with 9, 13.5, 20, 30, 45 and 68% of this LD<sub>50</sub> value (13.5, 20.3, 30.0, 45.0, 67.5, 101.3 mg/kg/day). Each was given sequentially for four days in a total 24 day interval with daily volume of 5 ml/kg.

Following treatment, the total body weight and the relative organ weight (thymus, lung, heart, liver, spleen, kidney, and adrenals) were determined. Each of these organs was histologically processed. The haematological parameters were determined and differential blood count was taken. Serum iron value and level of the thyroid hormones (T3 - T4) were also measured.

According to the results of this study, Humet®-R did not evoke any changes due to cumulative toxicity. The decrease of the leukocyte count, the haematocrit, the MCV and serum iron was not a toxic effect. Other biologically detectable changes were not observed.<sup>34</sup>

### **3.1.4. MUTAGENICITY STUDIES**

In the **Ames test**, the lyophilised Humet®-R preparation was investigated. The study was carried out in several strains of *Salmonella typhimurium* (TA 98, TA 100, TA 1537, TA 1538) both in the presence and absence of the liver microsomal fraction, activated with Arochlor 1254, using positive and negative control groups. (Concentrations studied of Humet®-R were: 469, 938, 1875, 3750, 7500 microgram per plate.)<sup>35</sup> Results of the study showed that Humet®-R did not possess any mutagenic activity after the administration of the above concentrations, in this test.

## **3.2. PHARMACOLOGICAL INVESTIGATIONS**

In the course of the animal experiments, primarily the biological effects of Supplemented Humic Acid (SHA), which is the active principle of Humet®-R syrup, were studied in well-controlled animal experiments.

### **3.2.1. STUDY OF THE IMMUNOLOGICAL EFFECTS**

To date, Humet®-R was studied in non-controlled clinical studies involving volunteers, most of whom

suffered with a tumour disease. In these cases, the treatment resulted, in part, in the cessation of growth of the tumour, or its healing. It should be stressed, however, that in these studies the administration of Humet®-R syrup was made in the form of adjuvant therapy.

As is known, tumours are cellular agglomerates with abnormal reproduction capacity occurring in several tissues. They have the ability, by deceiving the immune system of the body, to proliferate abundantly and finally destroy it. In this study the purpose was to determine how the growth and proliferation of the tumour cells can be inhibited by the administration of Humet®-R (supplemented humic acid, SHA).

In this study, C57BL (black) 6 male mice were used, their mean age was 3 months, and mean body weight was 20 g. SHA was mixed into the drinking water in a concentration of 1.5 ml/L.

For studying the effect on the immune system, several cell sets were given subcutaneously into the trunk area above the hip. The growth rate of the tumours, injected subcutaneously, and its possible inhibition by the SHA, were studied.

The experimental results showed that tumours could grow *only* in untreated animals inoculated with the least amount of tumour cell (103 cell/animal). Such difference was not seen after inoculation with hundred fold higher concentrations of the inoculation (105 cell/animal) but the growth of tumours was slower in the treated than in the control animals. Among the groups of animals inoculated with one million tumour cells, half of the treated animals were living 2.5 months after beginning the study. However, after only 7 weeks after inoculation, the control animals had to be over-anaesthetised to spare them from suffering due to their enormous tumours.

Subcutaneous tumours developed also in the treated animals but the animals gnawed their tumours causing great wounds. The majority of gnawed wounds cleanly healed without infection, and the remaining part of the tumour did not grow in most cases.

Such responses of animals are usual in the development of inflammatory processes. The study convincingly demonstrated the positive effect of this preparation (SHA) upon the immune system and this can explain the tumour-inhibitory effect, too.<sup>36</sup>

### **3.2.2. EFFECT ON THE IRON METABOLISM**

The objective of these studies was to obtain information about the veterinarian therapeutic usefulness of Humet®-R and an iron-chelate preparation in sows and piglets and further experimental evidence about the effect upon the iron-deficient anaemia of rats treated with SHA and several granulates of solid physical state (this latter investigation was carried out in GLP-conform study).

#### **3.2.2.1. Study of the effect on the iron intake in piglets and sows**

In conventional animal husbandry, the iron-deficiency anaemia regularly develops in piglets. The following factors are involved:

the very low iron level in the body of the new-born piglet which is the lowest in comparison with the other mammals;

increasing iron-deficiency due to the rapid growth;

the low iron level of the milk of the sows which is the only food for the suckling-pigs.

The pigs, born with body weight of 1.2-1.5 kg and 30-50 mg iron reserve, are provided with a total of 30 mg iron, during the first 5 weeks of life. At the same time, 120-150 mg daily iron intake would normally be needed for the synthesis of haemoglobin, and enzymes containing myoglobin or iron (cytochrome, cytochrome-oxydase, catalase, peroxidase, etc.).

For the prevention of iron-deficiency anaemia in pigs, parenteral iron treatment has become widely used which causes a sudden increase in the iron content of the blood and the saturation of transferrin by nearly 100%. However, the negative actor of parenteral iron therapy is the inability of the gut to absorb iron. The natural way for the prevention of iron deficiency is for the oral administration of the iron.

100 ml of Humet®-R syrup was applied on 30g perlite and this provided 140 mg iron intake for a litter consisting of 10 piglets. Among suitable experimental conditions and nutrition, the following conclusion may be drawn:

The highest total body iron content of new born piglets was found in the offspring of sows supplied with Humet®-R; the second was the group supplied with iron-chelate. The lowest iron level was stored in the

animals of the untreated control group.

The blood haemoglobin content of piglets fed with Humet®-R was significantly higher than that of either control group or iron-chelate consuming group.<sup>37</sup>

### **3.2.2.2. Study of the effect of trace element humic acid preparation (SHA) in iron-deficiency rat model**

Pregnant Sprague-Dawley rats were fed with normal, (control) and iron-deficient (Fe<10 ppm) rodent food during the entire gestation and lactation period. After weaning, the following groups were formed:

control group: feeding with normal food,  
iron-deficiency group: feeding with iron-deficient food  
group fed with SHA: feeding with iron-deficient food  
Reference substance was Aktiferrin syrup.

Doses: SHA 0.66 ml/kg (i.e.: 3.7 mg Fe<sup>2+</sup> /kg)

Aktiferrin 0.54 ml/kg (i.e.: 3.7 mg Fe<sup>2+</sup> /kg)

Following weaning, the haematological parameters of control and iron-deficient (anaemic) offspring were determined before starting the 21 day treatment with either SHA or reference substance (Aktiferrin). Offspring exposed to iron-deficiency pre- and postnatally had body weight 60% less than that of the control group. Red blood cell (RBC) count, mean volume of erythrocytes (MCV), haemoglobin (Hb) haematocrit (Htc), serum iron concentration (Fe), and transferrin saturation (Sat) were significantly lower, ratio of zinc-protoporphyrine/hem (ZP) and total iron binding capacity (TIBC) were significantly higher. Both in the offspring of Aktiferrin- and SHA treated animals, the value of Hb, Htc, MCV overtook the value of RBC, ZP, Fe and TIBC approximated the values of the offspring of the control group. There was no significant difference between the effects of Aktiferrin and SHA. Conclusively, according to the animal experiments, SHA (Humet®-R) is outstandingly suitable for the therapy of the iron-deficiency anaemia and it is equally effective with Aktiferrin for iron content.<sup>38</sup>

### **3.2.3. INVESTIGATION OF THE CARDIOPROTECTIVE EFFECT**

Heart failure and several types of arrhythmia due to ischemic heart disease play a central role in the mortality rate of cardiovascular diseases. The aim of the study was to demonstrate the antifibrillatory effect of the endowed humic acid (SHA) during reperfusion period following 25 minute coronary occlusion in isolated rat cardiac preparation. It was possible to obtain experimental data about the cardio-protective action of Humet®-R syrup given in repeated-dose long-term administration.

SHA was administered in an oral dose of 10 mg/kg for two weeks. At the end of the treatment, the heart was exteriorised, a canule was inserted into the aorta, and perfusion was carried out for 10 minutes maintaining constant perfusion pressure, according to Langendorff. During this period, a canule was introduced into the right atria using Neele's method. The value of 'pre-load' and 'after-load' was kept constant during the entire period of the experiments.

In these experiments, the coronary blood flow, the aortic blood flow, the heart rate, and the left ventricular end diastolic pressure (LVEDP) were measured. The ratio of onset of the ventricular fibrillation (VF) and the first derivative of the upstroke phase of the left ventricular pressure (i.e.: contractility: dp/dt<sub>max</sub>) were calculated.

The results of the experiments are shown in the Table 1.

<b>Treatment</b>	<b>CBF ml/min</b>	<b>AF ml/min</b>	<b>HR min<sup>-1</sup></b>	<b>VF %</b>	<b>+dp/dt<sub>max</sub> kPa/s</b>	<b>LVEDP kPa</b>
<b>before ischaemia (n=8)</b>	22.9 ± 0.9	43.4 ± 1.5	265 ± 6.0	0	1026 ± 45	0.51 ± 0.04
<b>after ischaemia</b>	20.4 ± 0.9	13.3 ± 2.5	260 ± 3.9	87.5	609 ± 53	1.53 ± 0.09
<b>SHA treatment 10mg/kg p.o. for 2 weeks (n=8)</b>	24.5* ± 0.8	24.5* ± 2.8	263 ± 3.0	12.5*	788* ± 36	1.09* ± 0.08

$p < 0.05$      $\bar{X} \pm$  S.E.M. (after ischaemia or SHA)

Two weeks oral administration of 10 mg/kg SHA could improve all parameters, which became pathologic after ischemia. Although the dose-dependent character of the response remains to be studied, the cardio-protective effect of SHA seemed to be proven.<sup>39</sup>

#### **3.2.4. EFFECT OF HUMET®-R ON THE MOBILISATION OF TOXIC HEAVY METAL IN PIGS**

Experiments were performed in pigs (body weight range was 16.2-18.2 kg at the beginning of the study). Humet®-R syrup was given in three different doses 2.5; 7.5; 20 ml/day/pig (i.e.: 1.1, 3.3, 8.8 mg/kg/day humic acid).

The aim of the study was to investigate the effect of the preparation on the elimination of <sup>203</sup>Hg isotope from the organism and vital organs.

The treatment with Humet®-R syrup started 5 days before the administration of the Hg-isotope and lasted 11 days after the administration of the isotope. The changes of the elimination from the total body and the fate of radioactivity of faeces, urine, and several organs were measured.

The results of the study are summarised in the Table 2:

<b>Dose mg/lg/day</b>	<b>Faeces</b>	<b>Urine</b>	<b>Faeces/Urine</b>	<b>Total</b>
<b>Control (n=4)</b>	52.8 ± 2.3	12.1 ± 4.4	4.36	64.9 ± 2.8
<b>1.1 (n=4)</b>	53.8 ± 4.0	13.0 ± 4.4	4.14	66.9 ± 0.9
<b>3.3 (n=3)</b>	60.2 ± 6.1	15.4 ± 7.9	3.91	75.6 ± 1.9
<b>8.8 (n=4)</b>	67.9 ± 9.5	18.1 ± 10.1	3.71	86.6 ± 3.5

mean  $\pm$  S.E.M

From the above results, one can observe that Humet®-R syrup increases the amount of  $^{203}\text{Hg}$  in the faeces, the urine and, total value, in tendency. Apart from the lowest dose (1.1 mg/kg/day), the same trend was observed in the other organs, as well. However, numerical value was not given due to the great standard deviation and the low case number. The effect proved to be dose-dependent. Namely, increasing the doses, the biological effect increases. In spite of the non-significant data, it seems evident that one can obtain positive results by performing the experiment with suitable animal numbers.<sup>40</sup>

### 3.2.5. EFFECT OF THE HUMIC ACID ON THE REGENERATION OF THE HAEMO-POETIC SYSTEM

According to the results of our preliminary experiments, there is a possibility to develop a therapeutic preparation for the prevention or regeneration of the damage of the haemopoetic system by using the humic acid and endowed humic acid.

It is known that humic acids are hetero-polycondensates containing highly variable components. They are allomelanins, which can be found in soils, coals, and peats that develop during the slow decomposition of plant residues by means of their chemical and biological transformation.

They contain polymerised phenolic macromolecules, their composition highly depends on the place and time of their origin.

Their chelate forming with metal ions, and especially their iron-binding activity, is well known. Their practical application has been discussed since the Fifties in the literature.

The humic acid preparation, which is the basic material of Humet®-R, can advantageously affect the regeneration of the haemopoetic system damaged by  $^{60}\text{Co}$ -gamma irradiation.

We could not find any literature data or reference about such biological effectiveness of the humic acid.

Detailed examination was performed in animal experiments using several doses of whole-body gamma irradiation to develop a treatment procedure, which could be applied in the human therapy as well. The biological effectiveness of the humic acid preparation produced was demonstrated in the following experimental arrangement:

#### I. Experimental animals

At the beginning of these experiments, the animals were randomised according to body weight. They were male animals (b.w. 190-220 g) of Wistar strain (HUMAN Vaccine and Pharmaceuticals, Co., Ltd., Gödöllő, Hungary). The animals were kept in rooms with controlled temperature ( $23 \pm 3^\circ\text{C}$ ), relative humidity of  $60 \pm 10\%$ , and alternative lighting (light / dark cycle by 12 hours) and in type II plastic cages (5 animals / cage). Drinking water and normal and humic acid enriched foods were provided *ad libitum*.

Rats were adapted to the experimental conditions for two weeks. During the experiments, the general clinical state of the animals were controlled daily.

#### II. Active substance applied

The preparation, which contained 5-15% humic acid, was given by gavage in several doses to the experimental animals.

#### III. Whole-body irradiation

Whole body irradiation of the rats was carried out in a special plastic restraint cage (40 animals/cage). This dose of irradiation was 7.0 Gray (Gy) (dose intensity: 0.82 Gy/min). The LD<sub>50/30</sub> value: 7.5 Gy, which was characteristic for this rat strain.

#### IV. Haematological examinations

On the day 0, 7, 14, 21, and 28 of the experiment, the abdominal aorta was prepared for blood sampling in ether anaesthesia. Haematological parameters determined were leukocytes (WBC), red blood cells (RBC), haemoglobin (Hgb), haematocrit (Htc), platelet (TRO), reticulocyte (RET). Determination was made with types PHA-1 and PHA-2 automatic device (made by MEDICOR, Hungary). The measurement error of the system was 1-3%.

#### V. Experimental groups

In these experiments, treatment with several doses of humic acid preparation was performed in groups of 30 animals. On the figures, the mean value characteristics to the Wistar strain were always demonstrated.

Group 1: Whole body irradiation with 7 Gy  $^{60}\text{Co}$  gamma radiation (standard food + drinking water)

Group 2: 7-day pre-treatment with humic acid (240 mg/animal/day active ingredient), then whole-

body irradiation with 7 Gy and further four-week treatment with 240 mg/animal/day dose of humic acid

Group 3: Whole-body irradiation with 7 Gy then, single-dose administration of 240 mg/animal/day dose of humic acid

Group 4: Seven-day pre-treatment with humic acid (90 mg/animal/day) then, whole-body irradiation with 7 Gy and further four week treatment with 90 mg/animal/day dose of humic acid

Group 5: Whole-body irradiation with 7 Gy then single-dose treatment with 90 mg/animal/day dose of humic acid

Statistical analysis of the test was used for the evaluation of the experimental data.

## VI. Results

Evaluation of the haematological and chemical parameters demonstrated that, independently of the treatments applied, each value was in the proximity of the reference value corresponding with the regeneration of the haemopoetic system, at the end of the experiment (28 day).

Haematological parameters of the rats, treated in the different ways, the changes of leukocytes (WBC) and thrombocytes of the 240 mg/animal/day treated group *and* those of the 90 mg/animal/day treated group were demonstrated on the Fig 1, Fig 2 *and* on the Fig 3, Fig 4, respectively.

*The results obtained after treatment with 240 mg/animal/day dose:*

It was stated that count of leukocytes and thrombocytes significantly decreased one week after whole body irradiation (Group 1, i.e. control group) and in animals which were treated with humic acid by single administration (Group 3). In case of the single-dose administered humic acid, only the thrombocyte count showed moderate increase of regeneration during the third week.

In the animals of the control group (Group 1), the regeneration counts of both leukocytes and thrombocytes appeared only on the third week after irradiation.

In the animals which were pre-treated and following the 7 Gy whole-body irradiation further treated with 240 mg/animal/day dose of humic acid (Group 2), there was no damage of the haemopoetic system. Thus, the count of leukocytes and thrombocytes remained in the proximity of the reference values which were characteristic to the Wistar strain.

Our experimental results demonstrated that humic acid / humin preparation given in adequate dose (240 mg/animal/day) and using the appropriate treating arrangement (pre-treatment at first, then maintenance treatment after irradiation), could prevent the damage of the haemopoetic system due to high-dose irradiation with ionisation irradiation (Fig 1 and Fig 2).

**1= Group 1 without treatment (control group)**  
**2= Group 2 continuous HS treatment**  
**3= Group3 one HS treatment**  
**Wistar average**

**1= Group 1 without treatment (control group)**  
**2= Group 2 continuous HS treatment**  
**3= Group3 one HS treatment**  
**Wistar average**

*Results obtained after the treatment with 90 mg/animal/day/dose:*

One week after whole-body irradiation, both in single-dose and continuously treated animals, the leukocyte count and the thrombocyte count significantly ( $p < 0.05$ ) decreased.

Low cell count was measured also on the second week after irradiation in the irradiation control (Group 1) and single-dose treated (Group 5) animals.

In the animals of the control group which were only irradiated (Group 1) the regeneration of both leukocyte and thrombocyte counts started only after the third week.

In the animals given 90 mg/animal/day humic acid treatment, if humic acid pre-treatment was used (Group 4), the regeneration of both cell types started intensively already after the first week. Until the end of the second week, the values were similar to those of the control animals (Fig 3 and 4).

1= Group 1 without treatment (control group)  
2= Group 2 continuous HS treatment  
3= Group3 one HS treatment  
Wistar average

1= Group 1 without treatment (control group)  
2= Group 2 continuous HS treatment  
3= Group3 one HS treatment  
Wistar average

Conclusively, one can state that, after a whole-body irradiation with high-dose  $^{60}\text{Co}$  gamma radiation, the normalisation of radiation-caused haemopoetic changes was evoked by several therapeutic doses of humic acid preparation.

The most advantageous effect was obtained in the animals, which were also pre-treated (before irradiation) with the preparation of the patent.

The results of these experiments showed that the humic acid/humin preparation might be properly applied for the prevention of the damage of the haemopoetic system, and for the efficient facilitation of the regeneration of the haemopoetic functions already damaged.

Biological efficacy of the humic acid/humin preparation provided the possible successful application of this therapeutic material in human therapy in-patients who were exposed therapeutically or accidentally to ionisation radiation (reactor accident or accidental ionisation of patients or staff).

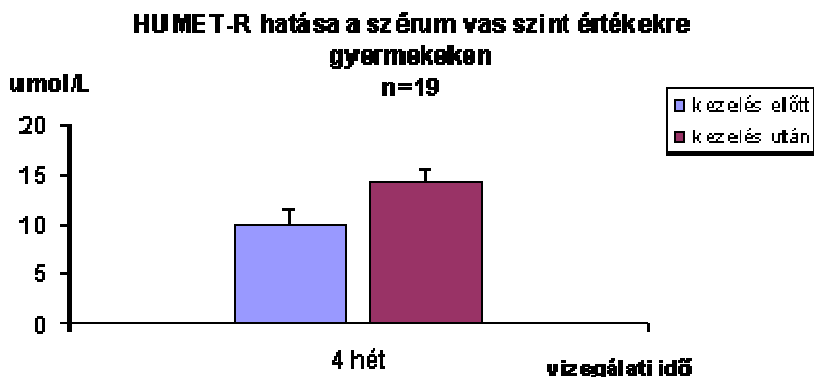
Further experiments show that above preparation is suitable for the acceleration of the regeneration of the haemopoetic damage due to chemotherapeutic treatment.<sup>41</sup>

## **4. CLINICAL OBSERVATIONS**

### **4.1. STUDY OF THE EFFECTS ON THE SERUM IRON LEVEL**

The study was performed in an open clinical trial (Dept. of Paediatrics, Erzsébet Hospital, Hódmezővásárhely, Dr. P. Szűts, Dr. P. Koszó) in anaemic children (age 1-18 years). Anaemia was diagnosed by complete blood count analysis from a blood sample taken before oral administration of Humet®-R syrup. In the case of a small child, 2x1 ml (for age 1-2 years), 2x2.5 - 5.0 ml (for age 2-3 years old) of Humet®-R were given maximum for 3 weeks. Therapeutic efficacy was demonstrated by checking the total body count on the weeks 2 and 4 after treatment. Twenty-two persons were involved in this study.

Results are demonstrated in the following figures 5, 6:



According to the results of the study, three weeks oral treatment with adequate doses of Humet®-R caused significant increase of the serum iron level which was a marked effect on the week 4, too (i.e.: one week after cessation of treatment).<sup>42</sup>

#### 4.2. EFFECT OF HUMET-R SYRUP IN VOLUNTEERS WORKING IN CADMIUM EXPOSITION

The aim of the study was to investigate the influence of the regular intake of Humet®-R syrup, (which contains ten essential elements bound to the mixture of the humic acids and is distributed as a therapeutic product) on the biological parameters (blood and urine cadmium concentration) of subjects working in cadmium exposure conditions; and on their clinical laboratory parameters (haemopoiesis, liver and kidney function) characteristic to their health status.

The workers involved in the study were involved partly in battery production, and partly in over-plating some component with cadmium; the performance of these technical processes needed very simple activity.

The persons studied were exposed to cadmium in the workplace and their blood cadmium level (90 nmol/L) or the urine level (10 nmol/mmol creatinine) remained within the determined biological limit values.

The patients were examined three times: record of the basic state (examination No 1) which was followed by a six-week voluntarily intake of Humet®-R syrup in daily dose of 10 ml (examination No 2), and on the week 14 after cessation of the treatment (examination No 3).

All patients were free of symptoms and complaints and remained capable of work during the entire period of investigation. There was no intermittent disease or illness.

In the course of the study, for characterising the degree of exposure, the biological exposure parameters of cadmium were measured, as follows: level of cadmium concentration in the blood and urine samples (value of quantitative urine determination was corrected to be the creatinine concentration), the effect on the haemopoiesis (total quantitative blood count, serum iron concentration, iron-binding capacity and saturation), the value of the liver function (serum bilirubin concentration, the activity of aspartate amino-transferase (GOT), alanine aminotransferase (GPT), gamma-glutamyl transferase (GGT), and the values of the kidney function (study of general state, and sediment, quantitative protein concentration of urine, N-acetyl-betaD-glucosamidase NAG) activity, the concentration of serum creatinine, carbamide and uric acid (urate). From the results of the study, the three examinations (basic state, after six-week treatment with HUMET-R, and on the week 14 after the treatment) of 16 male patients regarding the cadmium concentration of the blood and urine and the results of the other laboratory parameters are demonstrated in the Tables 3 & 4.

Table 3:  
**Changes of the blood and urine cadmium content in volunteers**

Measurement (n=16 males)	Blood Cd (nmol/L)	Urine Cd (nmol/mmol creatinine)
Basic state	8.5 ± 5.7 (2.3-23.3)	1.0 ± 0.6 (0.3-2.6)
6 weeks after HUMET-R treatment 10mL/day	7.2 ± 4.8* (2.5-18.4)	1.3 ± 0.78* (0.4-2.8)
on the week 14 after treatment	6.3 ± 4.6* (1.1-17.3)	2.0 ± 1.1* (0.5-5.0)

X ± S.D. \* p<0.05

**Table 4:**  
**Effect of Humet®-R treatment on haematological and urine parameters**  
**in cadmium exposition.**

<b>Parameter</b>	<b>examination No1</b>	<b>examination No2</b>	<b>examination No3</b>	<b>Significant difference p&lt;</b>	<b>Significant difference p&lt;</b>
<b>tested</b>	<b>mean ± S.D.</b>	<b>mean ± S.D.</b>	<b>mean ± S.D.</b>	<b>1. vs 2.</b>	<b>2. vs 3.</b>
<b>Red Blood Cell count (T/1)</b>	5.17 0.17	5.06 0.20	5.01 0.28	0.05	NS
<b>Haemoglobin (g/L)</b>	152.8 4.8	149.1 6.3	153.4 6.2	0.01	0.05
<b>Haematocrit (%)</b>	46.1 1.6	44.7 2.3	44.8 1.9	0.01	NS
<b>MCV (fl)</b>	88.8 3.1	87.9 3.4	89.3 3.7	0.01	0.001
<b>Leucocyte count (g/L)</b>	6.6 1.3	6.7 1.8	7.4 1.8	NS	0.01
<b>GPT (U/L)</b>	47.1 36.0	38.6 26.7	41.9 25.1	0.05	NS
<b>Urate (micromol/L)</b>	432.7 110.0	334.6 116.7	340.0 76.6	0.001	NS
<b>Urine protein (mg/mmolcr.)</b>	9.6 17.4	5.9 12.5	6.2 6.8	0.05	NS

Decrease of the blood cadmium concentration (15 and 13%) and the increase of the urine cadmium concentration (25 and 56%) were significant both in response to the six-week Humet®-R treatment and at the end of the treatment-free period (week 14). It was noteworthy that at the end of the six-week Humet®-R treatment the serum GPT activity (from 47.1 to 38.6 U/L), the urate concentration (from 432.7 to 334.6 micromol), the total protein concentration of the urine (from 9.6 to 5.9 mg/mmol creatinine) significantly decreased.

Conclusively it can be stated that the regular daily intake of Humet®-R syrup decreased the absorption of the cadmium and increased its urinary elimination. In respect to the outstandingly cumulative potency of cadmium in the human organism, this clinical finding has extraordinary importance from the point of view of prevention.

#### **4.3. STUDY ON THE EFFECT OF HUMET®-R ON THE LEAD LEVEL OF URINE AND BLOOD IN HEALTHY ADULT POPULATION**

The aim of the experiment was to study the effectiveness of Humet®-R on reducing the lead level of urine and blood in a city-dwelling volunteer population. 51 persons were included in the experiment (25 men and 26 women), aged between 18-60 years.

The laboratory samples of blood and urine were obtained before the administration of Humet®-R syrup and after a 14-day treatment, using the recommended daily dosage.

From the 51 person volunteers for the study, 11 proved to have moderate lead exposure thus this sub-group was evaluated separately. The results are shown below:

**Blood (n=51) and Urine (n=46) Lead Levels upon HUMET-R Administration  
Healthy Adult Population**

**Blood (n=11) and Urine (n=7) Lead Levels upon HUMET-R Administration  
in Lead Exposed Workers**

From these results it can be safely concluded that, for the exposed volunteers (determined as lead level above 1.0 mol/L blood), the lead content of the blood dropped significantly after 2 weeks of treatment. However, the expected elevation of lead level in urine was not significant.

The same tendencies were observed in the non-exposed group, but for them the changes were insignificant.

#### **4.4. STUDY ON THE EFFECT OF HUMET®-R TREATMENT ON LEAD POISONED PATIENTS.<sup>43</sup>**

9 patients were treated at the Department of Internal Medicine of Szent György Hospital between August and October 1994 with lead poisoning due to paprika tainted with minimum ( $Pb_3O_4$ ).

Of the 9 patients 3 required Ca-Na-EDTA acute treatment which eliminated the symptoms of lead poisoning quickly (after the third day of treatment).

The purpose of the study was to evaluate the efficiency of the HUMET®-R treatment (20 ml/day for three weeks) on the remaining 6 patients with less severe poisoning and compare it to a reference compound (Byanodine).

The results clearly revealed that the HUMET®-R preparation would normalize the blood lead level with the same efficiency as the reference compound.

#### **4.5. EFFECT ON THE PHYSICAL PERFORMANCE OF FIRST-CLASS SPORTSMEN**

According to several years' experience, the physical performance of sportsmen, following extreme load, can be characterised by the parameters as follows:

the blood lactate concentration related to the resting haematocrit value,  
the maximal heart rate,  
the maximal  $O_2$  uptake related to body weight kg,  
and the correlation connection between them.

The effect of three week continuous daily intake of HUMET-R preparation (20 ml/day) was studied in complaint free first-class sportsmen separated into groups according to changes related to the resting value of the haematocrit. The blood lactate concentration (LA), the maximal heart rate (HR), the maximal  $O_2$ -uptake related to the kg body weight ( $RVO_2$ ) and their correlation connection were studied.

The Htc and Hb values, related to the initial values, depending on the high or low level of the latter, decreased or increased and the mean values remained in proximity with the mean value of the physiological range. LA, HR and  $RVO_2$ , level of the sportsmen, grouped according to the low (<0.43) and high (>0.47) Htc values, were compared. Examining all sportsmen, there was positive correlation between Htc and  $RVO_2$ , LA and  $RVO_2$  (both Htc and lactate are proportional with the aerobic performance) in low Htc group (value < 0.43), the same correlation was negative in the high Htc group (value > 0.47).

Based on this three week HUMET-R treatment, one can state that the result of the treatment is the optimisation of the value of Htc and Hb and approximation of the physiological reference values.<sup>44</sup>

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