Monitoring redox-homeostasis
Importance in human studies and in animal experiments

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INTRODUCTION

Over the past decade, there has been substansial interest in oxidative stress and its role in the development of different pathophysiological complications as alcoholic or non-alcoholic liver diseases, diabetes, atherosclerosis and associated cardiovascular diseases, cancer, aging, inflammatory bowel diseases and further other conditions such as cataracta or arthrosis. Free radicals and other reactive oxygen species (ROS) are essential for life, because they are involved in cell signaling and are used by phagocytes for their bacteriocidal action. In addition to these well-controlled and necessary functions, ROS are also produced in all respiring organisms as a consequence of mitochondrial respiration, which consumes oxygen in the process of generating ATP by the coupling of electron transport and oxidative phosphorylation. oxidative stress, can also be induced by exogenous factors such as drugs and environmental toxins. Oxidative stress is potentially harmful to cells, and ROS are implicated in the etiology and progression of many disease processes including cancer. However, antioxidant mechanisms that scavenge ROS, by means of low-molecular-weight antioxidants or antioxidant enzyme systems, protect an organism from the damaging effects of oxidative stress. Under normal conditions, these antioxidant defense systems are able to detoxify ROS and prevent damage to cellular macromolecules and organelles. Under conditions of excessive oxidative stress, however, cellular antioxidants are depleted and ROS can damage cellular components and interfere with critical cellular activities.Reactive oxigen species (ROS) produced by oxidative stress can have a profound effect on lipoprotein modification, transciption, cell function and metabolism. During the last five years, oxidant and antioxidant research has taken a new turn. It is now evidence that in living cells ROS and antioxidants as well may selectively regulate certain signal transduction pathways.

Oxidants in cellular signaling

Reactive oxygen species are not only metabolic by-products, they also serve as cellular signaling molecules. Under many environmental stimuli including cytokines, ultraviolet radiation, hyperthermia and even undergrowth factors generate high levels of ROS that can perturb the normal redox balance and shift cells into a state of oxidative stress. Survival or death of the affected cell is dependent on the ability of the cell to adapt to or resist the stress, and to repair or replace the damaged molecules. A number of main stress signaling pathways are activated in response to oxidants injury, such as the nuclear factor (NF?B) signaling system, p53 activation, and the heat shock response, etc. Detoxification of ROS is one of the major problem for the aerobic life forms, and multiple levels of non-enzymatic amd enymatic defenses have evolved to form the oxidant defenses. An imbalance between these defense

mechanisms and pro-oxidative forces has been termed „oxidative stress". One of the most dramatic examples of redox imbalance or oxidative stress is the immflammatory bowel diseases.

Although it isrelatively easy to measure antioxidants activities, it is extremely hard to obtain absolute values for the rate of free radical generation *in vivo*.It is generally accepted that some biomarkers are used to measure the oxidative damage, such as protein carbonylation for protein damage, 8-hydroxydeoxyguanosine (8-OhdG) for DNA damage. Further it was found that increases amount of malondialdehyde (MDA) and 4-hydroxynonenal (HNE), products of lipid peroxidation, are responsible for loss of membrane fluidity with aging in vitro.Metal-catalyzed oxidation of proteins introduces carbonyl groups at lysine, arginine, proline, or threonine in a site-specific manner while oxidative modification converts the side-chain of methionine, histidine, and tyrosine and forms cysteine disulfide bonds. Available data suggest that modification of many proteins is taken place *in vivo* . Oxidation of proteins, specifically the histidine moieties, leads to increased carbonyl values, which are used as an index of protein oxidation to assess the age-related increase in oxidatively altered proteins.

Antioxidant systems

To prevent the accumulation of ROS and possible deleterious effects, antioxidant systems act as ROS scavengeres together. Because of its ubiquitous prevalence, guthathione acts as a major antioxidant puffer within the cell. Moreover, several enzymatic systems detoxify ROS: catalase (CAT) that present in peroxisomes dismutases H_2O_2 , and superoxide sismutase (SOD) eliminates $O2^{\cdot -}$. Gluthathion peroxidase (GPX) present in cytosol and mitochondria catalyses the reduction of peroxides into alcohols using the reducing potential of gluthathione. In addition to these endogenous scavengers, the body can be supplied with exogenous antioxidants such as vitamin E, vitamins A, C, and carotenoids. The liophilic vitamin E (a-tocopherol) is a higly effective antioxidant when incorporated in the lipid core of cell membranes. Major extracellular antioxidant defenses include the metal-binding proteins. It is evidence that the free metal irons and cooper can promote free radical damage, accelerating lipid peroxidation and catalysing hydroxyl radical formation. The body is protected against these effects by binding proteins (transferrin, lactoferrin, coeruloplasmin)which ensure that these metals are remained in a nonreactive state. Similary, haptoglobins, hemopexin and albumin bind hemoglobine and heme, which can also accelerate lipid peroxidation. Various low-molecular-weight molecules such as bilirubin, uric acid, have

antioxidant properities. Furthermore, distinct, extracellular forms of gluthathione peroxidases and SOD enzimes have been described.

Natural antioxidant sources

Epidemiological studies have pointed out clear relations between diet and its protective effect in the different diseases developed on the field of oxidative stress, following the consumption of vegetables and friuts. For a long time antioxidant vitamins (E,C,) and ß-carotene were considered responsible for the positive effects. Owing to the recent results of investigations it became apparent that other compounds of plant origin (mainly polyphenols), especially in dietary vegetables, may contribute to the antiradical and antioxidant effects. A great number of research projects are being carried out to evaluate antioxidant activity of edible vegetables and medicinal plants and the natural compounds isolated from them both in vitro and in the biological systems.

Several studies suggest that dietary supplementation with antioxidants can influence the response to chemotherapy as well as the development of adverse side effects that results from treatment with antineoplastic agents. Administration of antineoplastic agents results in oxidative stress, i.e., the production of free radicals and other reactive oxygen species (ROS).

Flavonoids are the most ubiquitous and structurally evolved class of plant phenolic compounds. Based on a few principal structures multitudinous hydroxylation, methoxylation and glycosilation patterns exist. Investigators examined the hepatoprotective, antioxidant and choleretic activity of flavonoid drugs.

AIMS OF THE STUDIES

In the course of our chemical studies we ivestigated the antioxidant properties of *Raphanus sativus L.var niger, Cichorium intybus* (L.) and the commersially available tea product of *Beiqishen*. We also examined their effect on the carbohydrate and lipid metabolism, futhermore their influence on metal accumulation mainly in the liver tissue in circumstances of experimental hyperlipidaemy. Investigation of dietary antioxidants and fiber compounds in the different medicinal plants became very important in the last decades in order to find the best therapeutic tools and the beneficial nutritional supplements in different chronic diseases especially in dislipidemia or in the chronic inflammatory diseases of the gastrointestinal tract.

Cichorium intybus (L.) is a well-known medicinal plant since the 17 th century. Several studies reported the results of the examinations (most of them were animal experiments) which showed liver protecting or antioxidant property of different kind of chicory herb extracts. Results of the investigations suggest that the observed hepatoprotective

effect of crude plant extract of chicory may be due to it's ability to suppress the oxidative degradation of DNA in tissue homogenate. Roberfroid and his colleagues could confirm the importance of the cichory fructooligosaccharides and it's bifidogenic nature in connection with colon diseases prevention.

In folk medicine the black radish root **(*Raphanus sativus L.var.niger)*** has been used since antiquity as a natural drug against abdominal inflation, insufficient digestion, for the inhibition of gallstone formation and for the stimulation of bile juice production. The drug contains glucosinolates and their derivatives (isothiocyanates, nitriles, cyano-epithioalkanes formed during hydrolysis catalysed by myrosinase), flavonoids and other polyphenolic compounds. In in vitro studies it was detected, that the juice from the choleretic black radish root had hydrogen donating and d-field element-chelating abilities. The juice exhibited strong reducing power property and radical scavenging effect in $H_2O_2/{}^{\cdot}OH$ luminol system.

Antioxidant components of commercially available Beiqishen tea

? *Astragalus mongholicus* is a very old and well-known drug in traditional Chinese medicine. The root of this plant is officially listed in the Chinese Pharmacopoeia. The pharmacologic studies and clinical practice have demonstrated its immunostimulant, cardiotonic anti-perspirant effects. Its isoflavons, including calycosin showed antimicrobila and superoxide aniopn scavenging acitivities.

? The medicinal mushroom *Ganoderma lucidum* has been used for the prevention and treatment of cancerous diseases. The immune enhancing properties of its polysaccharide constituents is well-known, presently the anticancer activity of the triterpenes is in the focus of the investigations.

? *Lycium barbarum* plays multiple roles in pharmacological functions as a well known Chinese traditional medicine. Its bioactive component is a polysaccharide-protein complex, which could upregulate cytokine expression in human peripherial blood mononuclear cells as chinese authors have reported in their studies. The use of cytokines has a long history in immunotherapy. They can regulate the immune response and are capable of mediating tumor regression in some malignancies.

Further aim was to evaluate methods, which appeared to be suitable monitoring the redox-status of the human organism and therefore, may be applied under routine clinical laboratory circumstances. This possibility can lead to a new perspective to set up epidemiological studies in different populations in order to investigate connecting points between the manner of life, alimentary customs (especially consumption of antioxidants), and the risk of chronic or acute illnesses. These methods have already served the work of scientists for many years, helping

them to obtain experience about the pathomechanisms of different diseases. With the help of these measurements it could be possible to monitor the results of medical therapy or surgical treatments, or in the field of chronic illnesses e.g. diabetes, to predict the possible development of late complications. Rutin metabolic parameters were correlated with oxidative stress parameters, and their information value was ivestigated, making differences between the states of pathological conditions in such kind of diseases as chronic liver disease (alcoholic and non-alcoholic), Crohn's disease and diabetes.

Methods of monitoring redox homeostasis and their possible role in the diagnostic practice

Results of the latest investigations confirm that the injury of redox homeostasis of human organism can be the starting point of the development and progress of most of human diseases . The pathogenesis of these diseases, could be cleared up more deeply by the investigation of parameters of oxidative stress and by the observation of their changes in connection with the different organ and tissue damages. It is well known, that individuals with lowered antioxidant defences may be at greater risk of developing diseases induced by free radicals. The goal is to direct attention to the possible role of some redox parameters in detecting of general health status of the human body and in the risk assessment of the different diseases. These measurements can perhaps help the clinician in determining optimal treatment and monitoring its effectiveness. One of the greatest challenges in oxidative stress research today is the identification of specific oxidised proteins in human diseases. Oxidative damage often leads to loss in specific protein function.

Clinical relevance of oxidative stress parameters

Once it has been recognized that oxidative stress plays a significant role in diseases, a major question is to differentiate whether it is a causal factor (etiologic factor) or responsible for promoting tissue injury or a cosequence or an accompanying aspect of the primary disease.

Some examples of different pathologícal conditions associated with evidence of oxidative stress

? *Diabetes*

Diabetes mellitus is a metabolic disease characterized by hyperglycemia. It usually associates with a series of late complications, such as vascular and metabolic abnormalities, during its development. The oxidative stress is greatly increased due to prolonged exposure to hyperglycermia in diabetes. Increased free radical generation, which results from increased non-enzymatic glycosylation, glucose autoxidation, and alterations in polyol pathway activity,

may exert a modulator effect on the level of oxidative stress in diabetes. Endothelial dysfunction, increased oxidation in lipid and lipoprotein and damage in long- lived proteins are some sequences of increased intracellular oxidative stress. Understanding the role of free radicals in the pathogenesis of diabetes will be useful for the treatment and prevention of diabetes and its complications. The oxidative stress is significantly increased in diabetes because prolonged exposure to hyperglycermia increases the generation of free radicals and reduces capacities of antioxidation defense systems

Free radicals also accelerate the formation of advanced glycosylation end-products, which in turn generate more free radicals. It has been demonstrated that reduced scavenging of free radicals by SOD and lake of GSH and ascorbic acid are associated with diabetic vascular pathology. Reduced other antioxidants, such as vitamin E, uric acid, and reduced activity of catalase and GPx are also found in diabetes .The destruction of beta cells results in deficiency and finally total loss of insulin secretion

The activities of SOD, glutathione peroxidase, glutathione reductase and vitamin E levels were significant low in the plasma in NIDDM, with no significant cha nge in catalase activity . The free radical activity in NIDDM patients was increased as measured by the markers of free radicals activity. For diabetic complications, the abnormalities of proteins and lipids are the major causes. In diabetic patients, extracellular long- lived proteins, such as collagen, elastin, laminin, are the targets of free radicals. These proteins are modified to form glycoprotein via glycosylation pathways in diabetes due to hyperglycemia. The modification of these protein and structural changes in tissues rich in these proteins such as lens, vascular wall, and basement membranes are associated with the development of complications in diabetes such as cataracts, microangiopathy, atherosclerosis and nephropathy. Diabetes is almost always related to the changes in plasma lipoproteins. Principally, the modification in diabetic patients includes oxidaton of lipoproteins, mostly LDL. The oxidized lipoproteins are then rapidly internalized by macrophages, which in turn convert to cholesterol- loaded foam cells. The formation of the foam cells is a key mechanism in atherosclerotic lesion. Treatment with antioxidants, vitamin E and probucool decrease the oxidation of lipoproteins without decrease of blood glucose.Vitamin C and Vitamin E are the known free radical scavengers because they inhibit glucose autoxidation and reduces the covalent linking of glucose to serum proteins in vitro. Most studies have found that people with diabetes mellitus have at least 30% lower circulating vitamin C and vitamin E concentrations than that of people without diabetes mellitus. The cellular intake of vitamin C and vitamin E is inhibited by hyperglycemia and promoted by insulin.

Alcoholic liver disease

Although hepatic toxicity of ethanol is likely multifactorial, direct and indirect evidence for the role of ROS in the development of chronic liver disease and hepatic fibrosis has repeatedly been obtained in patients with alcoholic liver disease and in animal models of acute and chronic ethanol injury. A number of experimental studies have demonstrated that either acute or chronic alcohol administration to experimental animals increases the formation of lipid peroxidation products, such as lipoperoxides, conjugated dienes and malondialdehyde (MDA) and decreases tissue levels of antioxidants. The impairment of cellular antioxidant defenses along with the formation of oxygen-derived radicals has been proposed to play a role in causing oxidative damage associated with alcoholic liver disease. However, other free radical intermediates might also contribute to trigger alcohol-dependent oxidative injury.

Non-alcoholic liver diseases

Recently it was demonstrated that acute or chronic fat deposition in the liver, regardless of cause, is associated with lipid peroxidation. In this experimental animal modell the degree of lipid peroxidation increased with the severity of steatosis. One of the end products of peroxidation is malondialdehyde, which activates hepatic stellate cells stimulating collagen production,and hence fibrosis. A further product – 4-OH-nonenal- is strongly chemoattractant for neutrophils. These products may also contribute to inflammation by activating NF-?ß, which regulates the expression of proinflammatory cytokines. It was also demonstrated in animal experiments that a marked increase in cytochrome P450 CYP2E1 is induced not only by ethanol but also by FFAs and ketones, which presumably explains why it is upregulated in diabetes and obesity, where it is capable of generating excess ROS during metabolism of endogenous ketones and dietary costituents as well as ethanol.

? *Inflammatory bowel diseases (IBD)*

A recent conceptual advance in our understanding of the etiology of IBD has arisen from experimental studies suggesting that chronic gut inflammation results from a dysregulated immune response to components of the normal gut flora.The mechanisms of inflammatory intestinal injury, leading to mucosal barrier dysfunction, are not completely clear. Hoewer it is well-known, that chronic gut inflammation is associated with enhanced production of ROS/reactive nitrogen species and protein oxidation, which may be responsible for mucosal barrier dysfunction.

Investigators reported.that mocosal biopsies.taken from either animals or humans with active gut inflammation proposed that the increase in ROS was due to neutrophil or

monocyte-derived oxidants ($O_2^{\cdot -}$, H_2O_2, OH^- , OCL^-) as shown by a decrease in ROS-related chemiluminescence after addition of radical scavengers (oxypurinol).

Chemical methods as tools of examination of antioxidant status

Enzimatic-colorimetriy detected by spectrophotometry

Superoxide Dismutase (SOD)

This enzyme is found in both the cell cytosol and the mitochondria. It can give information about a possible increased degree of oxidative stress in the living organ. SOD seems to be found at high levels in conditions such as systemic sclerosis, myositis, and malignant melanoma. SOD is decreasedin patients with juvenile rheumatoid arthritis, affirming correlations of SOD and inflammation.

Glutathione Peroxidase (GPX)

Glutathione peroxidase is a selenium dependent enzyme found primarily in the cytoplasm (70%) but also in the mitochondria (30%). GPX scavenges lipid peroxides throughout the membrane surfaces and quenches H2O2 converting it to water. High levels of GPX have been associated with certain conditions such as Alzheimer's dementia and Beta-Thalassemia minor.

Reduced Glutathion (GSH)/ Glutathion Reductase (GR)

The measurement of whole blood Reduced Glutathione reflects the body's reserves of this critical molecule. It functions both as an antioxidant (in form of GPX) and as an detoxifying agent for a vast array of xenobiotics.

GR has been used in the detection of hepatic and malignant disease, nutrition assessment of riboflavin status) and detection of genetically determined deficiency states.

Total antioxidant capacity (TAS)

This parameter can give "global" information about the redox-hoemostasis of the body in different healthy or even in pathological conditions(diabetes, chronic liver diseases, inflammatory bowel diseases IBD). It is possible to monitor the efficacy of ways of treatment of diseases, the possible beneficial effects of antioxidants of plant origin e.g. during chemotherapy.The commercially available kit is used for automatic chemical analysers as well so high number of biological samples can be analized in the same time.It also can give the possibility of epidemiological studies on measuring the general health status of any populations.

Methods based on fluorescency or luminometry

Measuring the changes in total scavenger capacity of any biological samples, it is possible to get information about the whole antioxidant status of the body, or even about the antioxidant property of different kind of extracts from medicinal plants. The results of studies show this method more sensitive, so it is possibble to detect even slight changes in the redox-homeostatis.

Measurements of oxidative damage of tissues

Lipidperoxides, lipid-hydroperoxides (F_2 –isoprostanes, 8-epi-Prostaglandin F_{2a})

Recently, the measurement of thiobarbituric acid reactive substances (TBARS), or malondialdehyde (MDA), has been ferquently criticised as too unspecific and prone to artifacts during sample workup. The determination of F_2 –isoprostanes (F_2 –iP) oxidation products of arachidonic acid, has been proposed as a more reliable index of oxidative stress. High levels has been reported in inflammatory diseases e.g. Crohn' disease as compared to healthy control group.

8-hydroxy-deoxyguanosine (8-OH-dG), a biomaker of DNA damage

Levels of oxidized nucleotide , have been shown to accumulate with aging. Perhaps because of ts proximity to the main source of oxidant generation, or because of a limited DNA repair system, mitochondrial is generally considered to be even more sensitive than nuclear DNA to oxidative damage. MtDNA encodes most of the electron transport chain proteins. Because of mtDNA damage, these proteins may not be repaired. Consequently, these defects result in an increased generation of ROS and respiring deficits leading to death. In several tissues, including the central nerve system and muscle, levels of 8-OHdG in mtDNA exceed nuclear DNA (nDNA) 16-fold. Therefore increased level of 8-OH-dG is correlated with increased genome instability, such as mutation and deletion, which is part of the reason for aging process and age-related diseases.

Protein carbonyl groups (CO)

High levels of CO groups have been observed in diseases, such as Alzheimer's disease, rheumatoid arthritis, chronic renal failure, sepsis, and respiratory distress syndrome and inflammatory bowel diseases. It is proposed, that immunblot analysis appear to provide better information than the spectrophotometric assay or ELISA about the oxidative status of proteins in a plasma, tissue homogenates, or cellular extracts especially when they are followed by mass spectrometry for the identification of carbonylated proteins.

MATERIALS AND METHODS

Chemical methods

Methods of measurement of reactive oxigen species

? *Total scavenger capacity by luminometry*

The total scavenger capacity (TSC) of plasma and of erythrocytes was determinated by a chemiluminescence assay adapted to Berthold Lumat 9501 instrument to assay antioxidant activity in rats with hyperlipidemia with and without juice or extract treatment. The sensitivity of the instrument allows detection limits of < 0.1 pg of material. During measurement, the program gives the integrated value of the light reaction. Light emission was initiated by the addition of 0.050 ml of 70 ?mol l^{1} alkaline luminol solution (pH 9.8), 0.30 ml H_2O_2 (1:10 000 dilution) and 0.30 ml of 1 mmol l^{1} micro-peroxidase solution as catalyst. Then 0.05 ml erythrocyte sample, (1 g% haemoglobin content) or 0.1 ml heparinized plasma was added for the measurement.

? *Reducing power*

The reducing power of the liver samples was determined according to the method of Oyaizu (1986) based on the chemical reaction of Fe (III) => Fe (II). The absorbance of the reaction mixture was read at 700 nm. Increased absorbance indicated increased reducing power.

? *Diene-conjugate content*

Diene-conjugate content of the liver was determined at 233 nm by spectrophotometry using the AOAC method (1994).

? Concentration of free SH-groups

Free SH-group concentrations were measured by the Sedlak and Lindsay (1968) method.

? *Total antioxidant status*

ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline-sulphonate]) is incubated with a peroxidase (metmyioglobin) and H_2O_2 to produce the radical cation $ABTS^{+}$ .This has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree which is proportional to their concentration.

Metabolits and enzimes as markers of metabolism

The liver enzyme activities (aspartate and alanin aminotransferases, alkaline phosphatase) and the main metabolite concentrations (cholesterol, triacylglicerides, glucose) in serum samples were measured by spectrophotometric methods by HITACHI 717 automatic chemical analyser.

Other methods

? *Protein content of biological samples*

The protein content of liver homogenates was measured by Lowry et al. (1951).The protein concentration of all homogenates was adjusted to 10 mg/ml using bovine albumin as standard for the measurements.

? *Histological examinations*

In the histopathologic examination the liver tissue were fixed in 4% neutral buffered formalin, embedded in paraffin, and 5? m sections were cut and stained with hematoxylin–eosin.

Phytochemical measurements

? *Total poliphenol content*

It was determined to the rules of Hungarian Pharmacopeae (Ph.Hg. VII), the results were expressed as pirogallol concentrations.

? *Flavonoid content*

The flavonid content of plant extracts were determinated to the rulse of the German Pharmacopeae (DAB 10). With the help of spectrophotometry folowed by an acid hidolysis, teh measurement was carried out using 420 nm wavelength. Results were expressed as hyperoside.

? *Caffeic acid derivatives*

The derivatives of caffeic acid of the plant extracts was determinated by the help of complexometry with spectrophotometry, using 325 nm wavelength.

? *Measuremets by high performance liquid chomatography (HPLC)*

Qualitative composition of the decoct used in the experiments was characterised by HPLC fingerprint. HPLC chromatographic parameters were: mobile phase : A-acetonitrile, B-2% acetic acid in water, column : Hypersil ODS 5 um, flow rate: 1 ml/ min, detection : 325 nm, injection volume: 20 ul, temperature: 25 ^{o}C

Determination of the concentrations of trace elements

Element concentration in the chicory extract and in tissue wet weight liver homogenates was determined by ICP-OES (inductively coupled plasma optical emission spectrometry). Type of instrument: Atom scan 25 (Thermo Jarrel Ash) a sequential plasma emission spectrophotometer. After digestion of the samples (1g) with solution mixture of nitric acid (5 ml) and hydrogen peroxide (2 ml) and dilution to 10 ml with deionised water, the concentration of elements (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Ti, V and Zn) was determined.

Determination of Inulin content

Inuline content of the Cichorium intybus extract was decomposed by inulinase NOVOZYM 230, than for the determination Sucrose/D-Glucose/D-Fructose UV–method test was used (Cath. No.706260.Boehringer Mannheim Test-Combination). Glucose, fructose and saccharose content was determined by enzymatic colorimetry.

For the statistical analysis we used the STATISTICA 6.0 software package. The values are expressed as mean ? SD.

Animal experiments

Extracts of medicinal plants

Wild growing ***Cichorium intybus*** **L. (Asteraceae)** were collected during flowering, identified in the Department of Pharmacognosy, Semmelweis University, where vouchers have been deposited. Diluted alcoholic (40 v %) extract (1:5) was prepared from the whole plant, which was then concentrated and lyophilised.Plant extract applied in this study was determined for the potential bioactive constituents as reported before.

Standardized squeezed juice ***of black radish root (Raphanus sativus (L) var niger*** was prepared by PHARMA Company (Budapest) under the control of the Institute of Pharmacognosy, Semmelweis University.

Beiqishen tea (Beiqishen Healthy Food Co, Daxing'anling, China, permission no.022, 1998) obtained from the commercial network. Ten packets of the tea taken from different boxes were mixed and 2 g of the drug were infused with 250 ml of boiling, doubly distilled water and allowed to stand for 30 min.

Treatments in the dietary groups of animals

Male Wistar albino rats (150-200g) were used in the ten-day experiment. Four groups of rats have been examined with 10-10 animals in each. The first two groups were fed with normal chow (CRLT-N, Biopharm Prompt Kft. Hungary). The next two groups were given a fat rich diet, with 2% cholesterol, 0.5% cholic acid and 20% sunflower oil added to the normal chow. One group with normal diet and one group with lipid rich diet were treated with chicory extract (daily 2 g/body weight kg) using a gastric tube. The animals were divided into four groups: normolipidemic, hyperlipidemic and juice-treated in both dietary groups.

The black radish juice and the infused product of Beiqishen tea was diluted to tenfold with tap water and this solution at a dose (150 ml / body weight kg) also ad libitum had been used instead of drinking water parallel with diet treatments for 9 days.

After treatments the rats were anaesthetised with Nembutal (35 mg/bwkg) and in deep narcosis they were exanquinated via abdominal vein .Plasma and erythrocytes were separated from heparinized blood for chemiluminescent studies, sera for biochemical measurements.

Human studies

42 diabetic patients were randomly (regardless of insulin dependence) selected. The patients were treated by different protocols, but all had relatively good glycemic control (HBA1c < 8%). They were divided into two groups on the basis og the clnical duration of diabetes: in 25 patients the diagnosis was established wihtin the last 10 years, and in 17 patients it was established more than 10 years. Healthy volunteers served as controls (n=15).

27 patients were involved in the examination of non-alcoholic liver disease. There were 12 men (mean age:43.8±11.1 years), and 15 women (mean age : 53.9±8.4 years). By laboratory measurements factors which could couse other cronic liver diseases were excluded (ethanol consumption, toxical damage). Helthy volunteers served as controls(n=16).

Patients suffering from inflammatory bowel diseases were divided into two groups, 30 patients with Crohn's disease (age: 37,6±10.0 years), 11 patients suffered from colitis ulcerosa (age:37.5±7.5 years). 16 healthy volunteers (age: 35.8±7.2 years) served as a control group.

Chemical Materials

1,1-diphenil-2-picrylhidrazyl-dithiobis-nitrobenzoic acid, luminol, microperoxidase were obtained from Sigma (St.Luis, USA). Haemisol reagentwas bought from Human Oltóanyag (Gödöllo, Hungary). All other reagents were purchased from Reanal (Budapest, Hungary).

This study was adopted by the Regional Committee of Science and Research Ethics Semmelweis University, License Number TUKEB 186/1998.

RESULTS

Results of Chicory treatment in experimental hyperlipidemy

On the basis of literature data and our results, it may be assumed that the main active components of chicory extract (polyphenols, flavonoids, and caffeic acid) have a favourable effect on lipid metabolism in hyperlipidemic rats. In the groups on lipid rich diet, chicory supplementation resulted in significant decrease in serum glucose concentration. This change can be explained either by the effect of fructooligosaccharides, modulating glucose metabolism which can realised through increased insulin levels after inulin consumption , or by the potential hypolipidemic effect of oligosaccharides. The authors found decreased hepatic activity of glycerol-3-phosphate acyltransferase, suggesting a decrease in acylglycerol synthesis, and lower activity of fatty acid synthase, which is a key lipogenic enzyme. These findings have also been supported by our results which show a decrease in triacylglycerol-concentration of rat sera in the chicory treated hyperlipidemic animal group. In feeding experiments after 10 days on a fat rich diet the hepatic lipid metabolism was changed significantly. Lipid droplets were accumulated and necrotic lesions were observed in the liver tissue without lymphocyte infiltration. The morphological picture was more favourable after chicory extract treatment, although the reducing activity did not change significantly compared to that of the control animals. Polyphenols and inulin contributed to maintaining the tissue structure during fat rich diet treatment.

In this “short term” experiment a surprisingly high increase in element concentration elevation was found in the treated groups. The absorption and accumulation of trace elements may be influenced by the indigestible carbohydrate components of the *Cichorium intybus* (L.) plant extract which can keep acidic pH in the colon as they are fermented by the bacteria. This condition is favourable especially to the absorption of the Ca ions.

Our results indicate that the compunds of *Chicorium Intybus* L. identificated in herb

decoct which was used for treatment of rats fed normal and lipid rich diet, influenced beneficially the lipid metabolism and were possessed of antioxidant property as the chemiluminescence intensity of pancreas tissue homogenate which was higher to the effect of lipid rich diet decreased significantly through the luminometric measurements. Beneficial changes could be observed in the intensity of liver tissue homogenate as well to the effect of decoct treatment,but only in the normal dietary group. The solution of the lyophilizated chicory decoct could not prevent liver status from the injury of lipid rich diet in such concentration as the results of the biochemical measurements of the enzyme activities show in this short term animal experiment.

The presence of inulin, owing to its acidic fermentation in the intestines, elevates Ca, Mg, Fe and Cu enteral reabsorption and similarly the chicory extract containing inulin may enhance metal ion absorption or reabsorption. The improvment of metal ion absorption is critical in the case of Fe and K. These data were confirmed by literature as well. On the basis of these findings and our results there must be a strong relationship between the element or oligosaccharide content and the relatively poor antioxidant property of Chicory extract.

Results of black radish juice treatement in experimental hyperlipidemy

With the help of short term animal experiment we examined the in vivo effect of *Raphanus sativus* root juice on hyperlipidemia in rats. In our study, using experimental hyperlipidemic condition, it has been detected, that black radish root juice could moderate the injurious effect of lipid rich diet in rats in vivo.

Supplementation of the lipid rich diet with squeezed juice from black radish root in hyperlipidemic rats, resulted remarkable changes in total scavenger capacity of plasma and erythrocytes, as well as in values of cholesterol and triacylglycerols in the sera of the animals. These favourable changes can draw attention to the antioxidant and liver protecting properties of the bioactive compounds (polyphenols) of black radish root extract with beneficial effect as they can play important role in the prevention of the lipid peroxidation processes. It needs further investigations to find the most important points of the effect on the lipid metabolism , or to identify the main types of enzymes, which could be influenced by the compounds of black radish root. Considering the changes of liver enzyme-values, we could control the possible liver protecting effect of the root squeezed juice in alimentary induced hyperlipidemia.

Results of studies with Beiqishen tea

Meanwhile the 14 day consumption of the tea the concentrations of the As, Mo, and the Ni elements icreased significantly in the liver homogenate samples of the treated animals. The Cd and the Pb also could be detected . These metal ions may change the redox balance of the liver, induce the production of reactive oxigen species causing damage of the tissue.

The increased concentrations of glucose, uric acid and cholesterol as the main metabolic markers also could be detected in the plasma samples of the animals. By the measurements of total scavenger capacity and the reducing power it could be verified that the antioxidant balance of the liver tissue weakened to the consumption of the tea.

Results of human studies

Evaluating the different antioxidant parameters, in case of inflammatory bowel diseases, albumin and the total antioxidant status or the parameter of total scavenger capacity could well complemented and confirmed each other in their diagnostical information values.

Strong correlation was found between total antioxidant status (TAS) and albumin, or TAS and uric acid parameters in colitis ulcerosa.

The difference between the redox homeostasis of patients with colitis ulcerosa and Crohns' disease was well detected by the method of luminometry of total scavenger capacity in accordance with the degree of illness, but there were not any difference between the genders' antioxidant capacity.

In non-alcoholic steatohepatitis positive correlation was found between increasing uric acid values and the improving of the reduction ability parameters.

The parameter of total scavenger capacity was suitable for estimation the difference between the two types of cell injury in alcoholic and non-alcoholic liver diseases in accordance of the inflammatory response reaction.

With the help of the measurement of free SH-groups it was succeeded to make difference between the status of patients suffering from alcoholic and non-alcoholic steatohepatitis (NASH). Controlling the efficacy of the applied therapy of NASH, evaluating the concentration values of free-SH-groups, the change of the patients' condition could be monitored.

Examinating the effect of clinical duration of diabetes mellitus along with that of redox homeostasis and glycemic control, it could be established, the longer the duration of diabetes the antioxidant status of patients worthens.

DISCUSSION

It can be verified in our experiments, that black radish juice and chicory extract significantly influenced carbohydrate and lipid-metabolism in rats, in experimental hyperlipidemy. In the cases of cholesterol and triacylglycerols we detected beneficial changes.

The effect of chicory on the redox balance of pancreas was determinated in hyperlipidemic rats. It was verified, that in chicory extract treated animals the total antioxidant capacity of pancreas tissue was significantly higher than in the controls.

The metal ion content was determinated in the chicory extract and also in the liver tissue of the animals by inductively coupled plasma emission spectrometry (ICP-OES). Significantly higher concentrations of iron, cooper, sodium and potassium could be detected in the liver tissue of chicory treated animals either in the normal or in the hyperlipidemic dietary groups.

In the hyperlipidemic dietary animal group, to the effect of chicory treatment significantly higher calcium, cooper, iron, phosphorous and zinc could be determinated than in the non-treated animal group.

It can be verified, that the metal ion content of plant extracts can modify the radical scavenger activity of poliphenols and flavonoids, especially in the case of toxic metal ion contamination. As it could be detected in our examination of Beiqishen tea, metal ions could influenced significantly the production of free radicals and the total scavenger capacity of tissues.

We must be carefull about these commercial products, becasuse of their possible harmfull effect on the redox-balance of tissues of the organ, causing damage of the biochemical processes through out the living organism.

The change of redox-homeostasis of the organ can be well detected by the help of the diagnostical parameter of Total antioxidant status (commercially available) or by the chemiluminescent method of measuring the total scavenger capacity. Compearing the two analitical methods, it was evaluated that luminometry is more sensitive, even slight changes could be welldetected.

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