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Effect of sweet grass (*Hierochloe odorata*) on the physico-chemical properties of liver cell membranes from rats intoxicated with ethanol

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ABSTRACT

Changes in the composition and physicochemical properties of liver cell membranes due to ethanol intoxication are due mainly to reactive oxygen species (ROS). The destructive action of free radicals can be neutralized by administration of antioxidants. The purpose of this study was to investigate the efficacy of sweet grass on the physicochemical and biochemical properties of the rat liver membrane altered by chronic ethanol intoxication. Qualitative and quantitative composition of phospholipids and proteins in the membrane were determined by HPLC. Ethanol increased phospholipid levels and altered the level of integral proteins as determined by decreased phenylalanine, cysteine and lysine. Ethanol significantly enhanced changes in the surface charge density of the liver cell membranes as determined by electrophoresis. Administration of sweet grass to rats intoxicated with ethanol significantly protects lipids and proteins against oxidative modifications. Therefore, sweet grass protects against some of the deleterious membrane changes associated with ethanol exposure.

1. Introduction

Animal and human organisms are constantly being subjected to various types of oxidative stress, such as ingested chemicals including ethanol. Alcohol metabolism to acetaldehyde and acetate is accompanied by reactive oxygen species (ROS) formation. Both ethanol and its metabolites can react with cell components including biological membrane components. Ethanol reduces the cell membrane surface hydration and affects the membrane protein–lipid structure (Klemm and Yuritas, 1992). Acetaldehyde and ROS react with proteins and modify their composition and functions (Grimsrud et al., 2008). ROS are also responsible for lipid peroxidation (Ponappa

Abbreviations: BHT, tert-butylhydroxytoluene; C_{TA}, acidic functional group concentration; C_{TB}, basic functional group concentration; Cys, cysteine; HPLC, high performance liquid chromatography; K_{AH}, average association constants with hydrogen ions; K_{BOH}, average association constants with hydroxyl ions; NP-HPLC, normal phase; PC, phosphatidylcholine; Phe, phenylalanine; PI, phosphatidylinositol; PMSF, phenyl–methyl–sulfonyl fluoride; PE, phosphatidylethanolamine; PS, phosphatidylserine; Lys, lysine; ROS, reactive oxygen species; TFA, trifluoroacetic acid; Tyr, tyrosine.

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and Rubin, 2000). Polyunsaturated fatty acids in membrane phospholipids are particularly vulnerable to peroxidation. The oxidative stress-induced alterations in cell membrane properties manifests as changes in membrane asymmetry and phospholipid, protein and fatty acid composition (Freikman et al., 2011; Edelfors et al., 2002). The changes in phospholipid and protein content influence in a significant way both the electrical properties of the membrane and the equilibria between cell membrane components and environmental components.

The deleterious consequences of membrane peroxidation have stimulated studies on the efficacies and mechanisms of action of biologically relevant antioxidants. One of the most important natural antioxidants sources is sweet grass (Hierochloë odorata), which belongs to the *Graminacae family* (Zainuddin et al., 2002). Sweet grass is a strongly aromatic perennial grass normally found growing in the rich, moist soil of North America, Asia and Europe. Sweet grass extracts promote the retardation of the lipid peroxidation (Pukalskas et al., 2002). Its chemical composition and biological properties have not been investigated extensively. However, this herb is known to contain coumarin and its derivatives 5,8-dihydroxycoumarin and 5-hydroxy-8-O-β-D-glucopyranosycoumarin (Bandonienie et al., 2000).

The aim of this work is to determine the biochemical and electrical properties of rat liver cell membranes after chronic ethanol intoxication. In addition, the efficacy of sweet grass as a protective agent was investigated.

2. Materials and methods

The sweet grass extract used in the experiment contained coumarin (312 mg/l), 5,8-dihydroxycoumarin (4.2 mg/l) and 5-hydroxy-8-O-β-D-glucopyranosyl-benzopyranone (3.1 mg/l) was used. The level of these compounds was analyzed using a gas chromatograph (GC; Agilent Technologies) equipped with a mass spectrometry (MS)/MS detector in the electron-impact ionization mode (GC System 7890A with GC/MS Triple Quad 7000) and HP-5MS capillary column (30 mm × 0.25 mm i.d., 0.25 μm film). The carrier gas was helium, at a constant flow of 1 ml/min. The initial oven temperature was 50 °C, with the temperature increased at 10 °C/min until the final temperature reached 280 °C, which was maintained for 10 min. The injector was maintained at 280 °C in split mode (split 1:2). The sample size was 1 μL. The MS unit was operated in scan mode (50–600 *m/z*). The coumarin peak was identified by comparison of the retention time with the standard and its mass spectrum from the National Institute of Standards and Technology Virtual Library (NIST).

2.1. Animals

Twelve month old male Wistar rats were used for the experiment. They were housed in groups with free access to a granular standard diet and water and maintained under a normal light–dark cycle. The rats were weighed every week of the experiment changes in the weight of animals from the different groups were not statistically significant. All experiments were approved by the Local Ethics Committee in Białystok (Poland) in accordance with the Polish Act Protecting Animals of 1997.

The animals were divided into the following groups:

- *Control group*: Rats were treated intragastrically with 1.8 ml of physiological saline every day for 4 weeks ($n = 6$).
- *Sweet grass group*: Rats received sweet grass water beverage (coumarin content 10 mg/l of water) ad libitum instead of water for one week. Next they were treated intragastrically with 1.8 ml of physiological saline and received sweet grass water beverage ad libitum instead of water every day for 4 weeks ($n = 6$).
- *Ethanol group*: Rats were treated intragastrically with 1.8 ml of ethanol in doses from 2.0 to 6.0 g/kg b.w. every day for 4 weeks. The dose of ethanol was gradually increased by 0.5 g/kg b.w. every three days ($n = 6$).
- *Sweet grass and ethanol group*: Rats received sweet grass water beverage ad libitum instead of water for one week. Next they were treated intragastrically with 1.8 ml of ethanol in doses from 2.0 to 6.0 g/kg b.w. and received sweet grass water beverage ad libitum instead of water every day for 4 weeks.

After the above procedure, the rats were sacrificed under ether anaesthesia (six animals in each group). Livers were removed quickly and placed in iced 0.15 M NaCl solution.

2.2. Phospholipid isolation and analysis by high-performance liquid chromatography (HPLC)

Liver cell membranes were prepared by differential centrifugation method as described by Ipata (1967), Evans (1970). Phospholipids were extracted in chloroform–methanol as desribed by Folch et al. (1957). Normal phase (NP)-HPLC separations were done using a Merck HPLC system equipped with a pump, an ultraviolet (UV) detector, an analog interface module (D-6000 A) and System Manager software. Phospholipids were separated using a silica gel column, with an acetonitryle–methanol–phosphoric acid (85%) mixture (130:5:1.5 volume ratio) by isocratic elution at 1 ml/s flow rate and 214 nm wavelength (Dobrzyńska et al., 2005).

2.3. Extraction of membrane proteins

The cell membranes were rinsed with NaOH prior to solubilization in 30 ml buffer containing 20 mM Tris/HCl (pH 7.4) and 1% Triton X-100 at 4 °C. The suspension was centrifuged at 1000xg for 10 min. The supernatant was incubated at 32 °C for 2 h and was then dialyzed against distilled water and evaporated until dry (Tani et al., 1997).

2.3.1. Trypsin hydrolysis of proteins

The membrane protein extract was hydrolysed by trypsin, at an enzyme:substrate ratio of 1:25 (Persaud et al., 2000). Following hydrolysis, the peptides were separated by HPLC on a LichroCART RP-18 column 100A (5 μm, 250 mm × 4.0 mm) equilibrated with solvent A (0.1% trifluoroacetic acid (TFA) in H_2O) and eluted with a linear gradient of 20–100% solvent B (0.1% TFA in acetonitrile) using a flow rate of 1 ml/min (Szachowicz-Petelska et al., 2012). A typical separation of the