JOURNAL OF FUNCTIONAL FOODS 9 (2014) 254–263

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jff

The protective efficacy of spirulina against bacterial endotoxin potentiated alcoholic liver disease

Arumugam Sarumathi [a], *Subramaniam Sethupathy* [b], *Nadanam Saravanan* [c,*]

[a] *Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu 608002, India*

[b] *Division of Biochemistry, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar, Tamil Nadu 608002, India*

[c] *Division of Biochemistry, Rani Meyyammai College of Nursing, Annamalai University, Annamalai Nagar 608002, India*

ARTICLE INFO

Article history:
Received 5 November 2013
Received in revised form 19 April 2014
Accepted 24 April 2014
Available online 29 May 2014

Keywords:
Ethanol
Endotoxin
Kupffer cells
Lipid peroxidation
Antioxidant and spirulina

ABSTRACT

One of the most important factors for the development of alcoholic liver disease (ALD) is the activation of immune cells residing in the liver by a substance called endotoxin, which is released by the bacteria living in the intestine. Alcohol consumption can lead to increased endotoxin levels in the blood and liver, which activates Kupffer cell leading to fibrosis. Novel approaches to inhibit these processes might help to prevent ALD. The present study was aimed to find out the protective effect of spirulina on lipopolysaccharide (LPS) potentiated alcoholic liver disease. Male albino Wister rats were randomly divided into five groups. Group 1 received isocaloric glucose. Group 2 received spirulina (500 mg/kg b.w.). Hepatic injury was induced in groups 3–5 by administrating 20% ethanol (5 g/kg b.w for 10 days). Groups 4 and 5 were challenged with LPS on the 11th day of the experimental period. Group 5 was co-administered with spirulina. LPS challenged by alcohol treated rats showed a marked elevation in circulatory lipid peroxidation, DNA damage and hepatic marker enzymes. Liver of LPS challenged alcoholic rats showed marked inflammatory cell infiltrates and fatty changes. Co-administration of spirulina showed reduction in biochemical and histological changes toward near normal.

1. Introduction

Alcoholic liver disease (ALD) is one of the major causes of illness and death worldwide (Liu et al., 2010). The onset of ALD is initiated by different cell types in the liver and a number of different factors, including products derived from the gut, ethanol-induced inflammation, ethanol metabolites, and the indirect reactions from those metabolites (Day & James, 1998). This leads to the formation of fatty liver (steatosis), hepatitis, and alcoholic cirrhosis (Smathers, Galligan, Stewart, & Petersen, 2011). A large number of xenobiotics are reported to be potentially hepatotoxic. Some examples are ethanol, acetaminophen, tetracycline, and carbon tetrachloride. Hepatotoxins may

* *Corresponding author.* Tel.: 914144-237225.
E-mail address: saravanan_74@rediffmail.com (N. Saravanan).
http://dx.doi.org/10.1016/j.jff.2014.04.026
1756-4646/

react with the basic cellular constituents like proteins, lipids, RNA and DNA and induce almost all types of cell damages through oxidative stress (Kang et al., 2014).

The maintenance of the integrity of the intestinal barrier is of crucial importance to preserve a healthy gut–liver axis. In fact, a derangement of the homeostasis between bacteria and host, and a qualitative and quantitative alteration of gut microflora lead to an increased intestinal permeability. This promotes bacterial and endotoxin translocation, triggering the production of pro-inflammatory molecule cytokines and metabolic disorders. In ALD, lipopolysaccharide (LPS) is thought to be derived from a breakdown in the intestinal wall, enabling LPS from resident gut bacterial cell walls to leak into the blood stream (Duerkop, Vaishnava, & Hooper, 2009). LPS has many deleterious effects and plays a significant role in a number of disease processes by increasing the inflammatory cytokine release. LPS is a powerful activator of many immune system cells. It can potentiate the effects of alcohol in activating macrophages, particularly the Kupffer cells. Combined activation, by ethanol and LPS, is to increase liver damage under experimental conditions.

Therapies developed along the principles of modern medicine are often limited in their efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing world. Therefore, treating liver diseases with natural compounds, which are easily available and do not require laborious pharmaceutical synthesis seems highly attractive. Increased use of synthetic drug therapy leads to many side effects and undesirable hazards. Therefore, there is a worldwide trend to return to natural resources, which are culturally acceptable and economically viable. Many of the important and effective drugs used to treat chronic liver diseases are derived from plants and certain species of cyanobacteria. *Spirulina platensis* is blue–green algae (Cyanobacterium, family Oscillatoriaceae). It is gaining more attention because of its nutritional and various medicinal properties (Sharma, Sharma, Kumar, & Kumar, 2007a). Spirulina is the only blue–green alga commercially cultivated for food use. In 1996, the United Nations World Health Organization (UN WHO) declared Spirulina as 'The best for tomorrow', and it has gained popularity in recent years as a food supplement. Its nutritional value derives from its high protein content (Simpore et al., 2006) and natural biochelated vitamins (Kapoor & Mehta, 1993). Several studies have reported that Spirulina can prevent or inhibit cancers in animals (Roy et al., 2007). *In vitro* and animal studies have suggested that spirulina possesses antiviral effects (Shih, Tsai, Li, Chueh, & Chan, 2003). It is a powerful stimulant for the immune system, as shown in animal experiments, by increasing the phagocytic and natural killer cell activities (Qureshi & Ali, 1996). Moreover, hypocholesterolemic effects have been described in some animal studies (Nagaoka et al., 2005). The anti-inflammatory property of spirulina was also demonstrated by Vo, Ryu, and Kim (2013). Various studies have shown that spirulina plays a protective role against many toxicants, including mercury (Sharma, Sharma, Kumar, & Kumar, 2007b), D-galactosamine, acetaminophen (Lu, Ren, Wang, Sanada, & Egashira, 2010) and copper toxicity (James, Sampath, Nagarajan, Vellaisamy, & Manikandan, 2009).

C-phycocyanin is a protein-bound pigment found in spirulina. Phycocyanin monomers are themselves built up of two distinguishable protein subunits designated A and B, which contain at least three covalently attached bilin chromophores and open chain tetrapyrroles with no metal complexes (Duerring, Schmidt, & Huber, 1991). These prosthetic groups account for close to 4% of the algae mass, indicating the presence of about 16 chromophoric groups per unit molecular weight (Eocha, 1962). It occurs in four different structural forms, monomeric, trimeric, hexameric and decameric (MacColl & Guard-Friar, 1987), and is the most abundant pigment in blue–green algae, accounting for more than 20% of algal dry weight (Richmond, 1990). Natural pigments like phycocyanin exhibit various beneficial biological activities such as antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective activities (Pangestuti & Kim, 2011). The chemical structure of the bilin chromophores in C-phycocyanin (open chain tetrapyrroles) is snug to that of bilirubin. Stocker, Yamamoto, McDonagh, Glazer, and Ames (1987) reported that bilirubin is an antioxidant of possible physiological importance because it could scavenge peroxyl radicals by donating a hydrogen atom from the C-10 bridge of the tetrapyrrole molecule to make a carbon radical with resonance stabilization extending over the entire bilirubin molecule. It is well known that reactive oxygen species (ROS) are implied in a variety of important processes in medicine including, among others, inflammation, atherosclerosis, cancer and reperfusion injury (Kehrer, 1993). One means by which a substance can interfere with these cognitive operations is by working as an antioxidant or free radical scavenger. The present study was planned to study the preventive effect of oral supplementation of spirulina, which contains C-phycocyanin, against hepatotoxicity triggered by bacterial endotoxin obtained from the gut microbial flora (LPS) after chronic administration of alcohol which has not yet been considered.

2. Materials and methods

2.1. Materials

LPS was purchased from Sigma Chemicals Co., St. Louis, MO, USA. Spirulina was a generous gift from Maan Spirulina Company Pvt. Vellore, Tamil Nadu, India. Absolute ethanol used in our survey was obtained from Himedia Laboratories, Mumbai, India and all other chemicals and solvents used were of analytical grade.

2.2. Animals

Male albino Wistar rats aged 8 weeks, weighing 120–150 g were obtained from the Central Animal House, Rajah Muthiah Medical College and Hospital, Chidambaram (Reg No: 166/1999/CPCSEA). They were acclimated for 7 days at room temperature (25 ± 3 °C) and relative humidity of 66% in a 12-h light/dark cycle in a room under hygienic condition. The animals had free access to standard pellet diet (Pranav Agro Industries Ltd, Bangalore, India) and water *ad libitum*. The study was approved by the Institutional Animal Ethics Committee, Rajah Muthiah Medical College (Approval number: 909/2012) and was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals.