The Journal of Basic & Applied Zoology (2016) **76**, 31–41

HOSTED BY

ELSEVIER

The Egyptian German Society for Zoology

The Journal of Basic & Applied Zoology

www.egsz.org
www.sciencedirect.com

Hepatoprotective efficacy of gallic acid during Nitrosodiethylamine-induced liver inflammation in Wistar rats

Uzma Latief [1], Hadiya Husain [1], Devoshree Mukherjee, Riaz Ahmad [*]

Biochemical and Clinical Genetics Lab, Section of Genetics, Department of Zoology, Aligarh Muslim University, Aligarh 202002, India

Received 21 May 2016; revised 17 June 2016; accepted 13 July 2016

KEYWORDS

Gallic acid;
Hepatic fibrosis;
Hepatoprotection;
Inflammation;
Nitrosodiethylamine

Abstract Gallic acid (GA), a popular phenolic acid is found in gallnuts, grapes, pomegranates, tea and oak bark. It possesses anti-cancer, anti-bacterial, anti-depressant, anti-asthmatic and anti-obesity effects. N′-Nitrosodiethylamine (NDEA) is a well-known hepatotoxin, carcinogen and mutagen. In this study, we have examined the hepatoprotective effect of gallic acid against liver inflammation induced by NDEA in Wistar rats. Hepatic damage in the animals was induced by 10 ml kg^{-1} b.wt of 1% NDEA (i.p.) solution in normal saline once in a week. Another group received GA supplement (i.p.) in 100 mg kg^{-1} b.wt wk^{-1}. Animals belonging to control group were administered equal amounts of saline or GA. LPO, SOD and membrane-bound ATPase (Ca^{2+}- and Mg^{2+}-ATPase) activities were determined in liver homogenate of control and treated rats. Alterations in liver architecture were assessed by H&E and Masson's trichrome stainings of 5 μm thick liver sections. Immunohistochemistry (IHC) was performed to localize the inflammatory marker, Cyclooxygenase-2 (COX-2). Our results demonstrate a significant increase in malondialdehyde, and decrease in SOD and ATPases (Ca^{2+}/Mg^{2+}) in NDEA-treated rats. Histopathology data showed inflammation, activated HSCs, deposition of collagen, periportal as well as bridging fibrosis in NDEA-treated liver specimens. Immunohistochemistry of NDEA-treated liver sections exhibited COX-2 positive cells. Gallic acid supplement revert the hepatic functioning in rats injured with NDEA probably by inducing Nrf2-mediated antioxidant enzymes and attenuating the inflammatory mediators COX-2 through NF-κB inhibition pathway. Therefore, gallic acid supplement may be a useful promising bioagent in combating liver injury.

Introduction

Phyto-constituents, especially phenolic compounds, are gaining increasing interest due to their beneficial effects on longevity and disease prevention (Kris-Etherton et al., 2002). Out of

* Corresponding author.
E-mail address: ahmadriaz2013@gmail.com (R. Ahmad).
[1] Authors have equal contribution.
Peer review under responsibility of The Egyptian German Society for Zoology.

http://dx.doi.org/10.1016/j.jobaz.2016.07.002

many, gallic acid (GA) or trihydroxy benzoic acid has been well accepted to possess numerous biological properties including anti-cancerous and anti-inflammatory (Faried et al., 2007), anti-bacterial (Chanwitheesuk et al., 2007) and anti-obesity (Hsu and Yen, 2007). Besides, GA also exhibits hepatoprotective activity against CCl_4 mediated liver fibrosis (Wang et al., 2014). GA is found in gallnuts, grapes, pomegranates, tea, hops and oak bark both as free and as part of hydrolyzable tannins. It is estimated that at least thirty Ayurvedic herbs and their formulations used in Asian sub-continent for treatment of several diseases contained a high percentage of GA (Borde et al., 2011).

The rising burden of chronic liver disease in the world causing majority of deaths every year is a growing concern. Liver fibrosis is one of those chronic liver diseases in which there is excessive accumulation of extracellular matrix (ECM) protein resulting from increased synthesis and decreased degradation of collagen (Friedman, 2003; George and Chandrakasan, 1996). The main ECM producing cells in liver are hepatic stellate cells (HSCs) (Friedman, 2008; Ahmad and Ahmad, 2012). In normal liver, HSCs store vitamin A but upon activation they trans-differentiate into myofibroblast-like configuration acquiring fibrogenic properties and start secreting ECM (Milani et al., 1990; George et al., 2001). Activated HSCs express myogenic markers like α-SMA, c-myb and myocyte enhancer factor. This distorts the architecture of liver and subsequently leads to fibrosis and then cirrhosis. Cirrhosis ultimately leads to increased intra-hepatic resistance to blood flow and hepatocellular dysfunction which result in portal hypertension and ultimate liver failure (Friedman, 2008). Liver fibrosis is also caused by viruses, parasites, drugs and various toxins (Ahmad and Ahmad, 2012).

In the present study, we have taken N′-Nitrosodiethylamine (NDEA), a nitroso compound and a hepatotoxicant (Wills et al., 2006) for inducing liver fibrosis. Nitrosodiethylamine is reportedly present in a wide range of food items such as non-fat dry milk; smoked, dried and salted fish; cheese; cured meat and alcoholic beverages (Scanlan, 1983). Long term exposure to sources rich in NDEA may induce hepatic damage or fibrosis. The treatment of various human diseases, especially hepatic fibrosis, through synthetic medication is perhaps effective but prolong exposure to these medicines may be fatal as the burdened liver is the definitive target for drug metabolism and detoxification. Therefore, researches to explore suitable and friendly alternative medicines are on priority in the present times since they exhibit amazing curative action which is free of any side effect. These genial therapeutic options exert their protective effect by one or a combination of the mechanisms such as activation of detoxifying enzymes, inhibiting formation of reactive carcinogenic metabolites, scavenging ROS, influencing apoptosis and cell proliferation. Owing to their plenteous remedial properties including antioxidant, anti-inflammatory and anti-carcinogenic, naturally occurring biologically active polyphenols derived from common dietary sources are gaining increasing interest as potential therapeutics. Therefore, in this study we investigate the hepatoprotective effect of GA in a mammalian disease model of hepatic fibrosis *in vivo*. Further, we elucidate the probable mechanism of GA action in restoring the hepatic function in the diseased animals. To our knowledge, the scientific literature on the NDEA-induced hepatic fibrosis is sufficient (Pradeep et al., 2007; Jin et al., 2009; Nakazato et al., 2010; Avasilcăi et al., 2011; Zhang et al., 2013a). Reports on the effect of GA in this particular disease model are scanty, however we encountered only a few reports demonstrating effect of GA in a paracetamol-induced liver damage and CCl4 mediated liver fibrosis in mice (Rasool et al., 2010; Wang et al., 2014).

Materials and methods

Chemicals

Adenosine 5′-triphosphate (ATP), Tris buffer, N′-Nitrosodiethylamine, 3,3′-diaminobenzidine and 2-Thiobarbituric acid (TBA) were procured from Sigma–Aldrich chemicals Pvt. Ltd. Gallic acid, Pyrogallol, Hematoxylin and eosin stains were obtained from SRL (India). COX-2 polyclonal mouse antibodies were purchased from Cayman chemicals (Cat. No.: 160106), goat anti-mouse IgG-HRP conjugated antibodies were procured from Trends Bioproduct Pvt. Ltd. India (Cat. No.: M30907), while all other chemicals of AR grade were procured from SRL.

Animal care

Adult, male albino rats (*Rattus norvegicus*) of Wistar strain, 7 ± 1 weeks weighing about 150 ± 10 g were kept in well-aerated polycarbonate cages with proper humane care in the animal house facility of the department with light: dark exposure of almost equal durations. They were acclimatized for about a week and given free access to food and water. All the experiments were performed according to the ethical guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Experimental design

The experimental animals were divided into four groups of five rats each. Before the start of experiment, randomly selected animals were tested for liver function. Animals with normal range of liver function test (LFT) parameters were selected for experimentation and assigned group number, as given below.

Group-1: Control, comprised of rats received normal saline in quantities given in other groups.

Group-2: Rats administered GA in doses of 100 mg kg^{-1} body weight in a single dose intraperitoneally. The selection of dose was based on a few previous studies (Niho et al., 2001; Rasool et al., 2010) as well as based on some pilot experiments carried out earlier. Niho et al. (2001) further demonstrated that intake of GA (119 mg kg^{-1} day^{-1}) for 13 weeks is safe and considered to be no-observed adverse effect level (NOAEL) in male rats.

Group-3: Animals were given 10 ml kg^{-1} body weight of 1% NDEA solution intraperitoneally in a single dose.

Group-4: Rats administered 10 ml kg^{-1} body weight of 1% NDEA solution intraperitoneally in a single dose along with GA in doses of 100 mg kg^{-1} body weight. Based on some initial experiments, GA was given after two hours of NDEA treatment to the animals. After fourteen days of the NDEA + GA administration, the animals were sacrificed.