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Original article

Apocynin prevented inflammation and oxidative stress in carbon tetra chloride induced hepatic dysfunction in rats

Md Mizanur Rahman[a], Awale Yousuf Muse[a], D.M. Isha Olive Khan[a], Ismaile Hussein Ahmed[a], Nusrat Subhan[a], Hasan Mahmud Reza[a], Md Ashraful Alam[a,*], Lutfun Nahar[b], Satyajit Dey Sarker[b,*]

[a] Department of Pharmaceutical Sciences, North-South University, Bangladesh
[b] Medicinal Chemistry and Natural Products Research Group, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, United Kingdom

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ABSTRACT

Background: Liver fibrosis is a leading pathway to cirrhosis and a global clinical issue. Oxidative stress mediated tissue damage is one of the prime causes of hepatic dysfunction and fibrosis. Apocynin is one of many strong antioxidants.
Objective: To evaluate the effect of apocynin in the CCl_4 administered hepatic dysfunction in rats.
Methods: Female Long Evans rats were administered with CCl_4 orally (1 mL/kg) twice a week for 2 weeks and were treated with apocynin (100 mg/kg). Both plasma and liver tissues were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase activities. Oxidative stress parameters were also measured by determining malondialdehyde (MDA), nitric oxide (NO), myeloperoxidase (MPO), advanced protein oxidation product (APOP). In addition, antioxidant enzyme activities such as superoxide dismutase (SOD) and catalase activities in plasma and liver tissues were analyzed. Moreover, inflammation and tissue fibrosis were confirmed by histological staining of liver tissue sections.
Results: Apocynin significantly reduced serum AST, ALT, and ALP activities in carbon tetrachloride treated rats. It also exhibited a considerable reduction of the oxidative stress markers (MDA, MPO, NO, and APOP level) which was elevated due to CCl_4 administration in rats. Apocynin treatment also restored the catalase and superoxide dismutase activity in CCl_4 treated rats. Histological analysis of liver sections revealed that apocynin prevented inflammatory cells infiltration and fibrosis in CCl_4 administered rats.
Conclusion: These results suggest that apocynin protects liver damage induced by CCl_4 by inhibiting lipid peroxidation and stimulating the cellular antioxidant system.

1. Introduction

Liver fibrosis is characterized by increased deposition and altered composition of extracellular matrix, such that collagen fiber is an excess to the fibrous site. Deposition of collagen may lead to liver cirrhosis, liver failure, and hepatocellular cancer along with fibrogenic development. Advanced fibrosis and cirrhosis are generally considered to be irreversible conditions even after removal of the injurious agent [14]. Cirrhosis which is the end stage of liver fibrosis is the leading cause of liver disease related morbidity and mortality [36]. Hepatic cirrhosis or fibrosis is known to be an irreversible distortion and alteration of the normal tissue architecture. In this phenomenon, multiple processes are involved in the progression of this lesion including: oxidative stress by free radicals; chronic inflammation mediated in part by the release of pro-inflammatory cytokines from Kupffer cells; and fibrosis induced by the paracrine action of pro-inflammatory and profibrogenic cytokines produced by Kupffer cells and hepatocytes on hepatic stellate cells (HSCs) [21].

Recently, the possible effect of free radical mediated oxidative injury in the pathogenesis of alcohol-induced liver diseases has received increasing attention. Clinical studies have shown that products of lipid peroxidation can be detected in the liver and in the blood of heavy drinkers and that oxidative damage increases proportionately with the amount of ethanol consumed [1]. Harmful oxidative reaction with strong oxidizing compounds is

* Corresponding authors.
E-mail addresses: ashraful.alam@northsouth.edu (M.A. Alam), S.Sarker@ljmu.ac.uk (S.D. Sarker).

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believed to damage cells and tissues, therefore leading to chronic diseases. Free radicals usually contain unpaired electrons in their atoms. These unpaired electrons usually give a considerable degree of reactivity to free radicals. Free radicals, such as superoxide anion radical, hydroxyl radicals, lipid free radicals, nitrogen dioxide and nitric oxide free radicals, together with hydrogen peroxide, singlet oxygen and ozone, are major forms of reactive oxygen species (ROS) in vivo [37]. Many hepatotoxicants including carbon tetrachloride (CCl_4), nitrosamines, and polycyclic aromatic hydrocarbons require metabolic activation, especially by liver cytochrome P450 enzymes to form reactive, toxic metabolites that in turn produce liver injury in experimental animals and humans. CCl_4, a well-known model compound for the production of chemical induced hepatic injury, requires biotransformation by hepatic microsomal P450 to produce hepatotoxic metabolites, namely trichloromethyl free radicals ($^{\bullet}CCl_3$ and/or $^{\bullet}CCl_3OO$). Trichloromethyl ($^{\bullet}CCl_3$) free radicals can react with sulfhydryl groups, such as glutathione (GSH) and protein thiols, and the covalent binding of trichloromethyl free radicals to cell proteins are considered as the initial steps in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell necrosis [15].

Currently, there has been increased interest in using natural antioxidants to prevent the free radical induced hepatic toxicity. Apocynin, also known as acetovanillone, is a natural compound structurally related to vanillin [11]. Apocynin was discovered from *Picrorhiza kurroa* plant during an attempt on activity-guided isolation of immunomodulatory constituents from plant extract. It has been used as an efficient inhibitor of the complex NADPH-oxidase in many experimental models involving phagocytic and nonphagocytic cells [28], and to increase the synthesis of glutathione in alveolar epithelial cells by increasing gamma-glutamylcysteine synthesis through activation of the transcription factor AP-1 [3]. Apocynin administration in HFD-fed animals showed improved insulin sensitivity, reduced the diverse plasma inflammatory cytokines, suppressed gene expression of inflammation-related molecules in both liver and adipose tissue, and decreased the activity of transcription factor NF-κB in liver tissue [25]. Apocynin treatment also remarkably reduced systemic oxidative stress and suppressed hepatic lipid peroxidation and increased antioxidant capacity [24]. However, beneficial role of apocynin in liver fibrosis was not investigated. Thus, the current study was undertaken to evaluate the role of apocynin in CCl_4 induced liver damage in rats.

2. Materials and methods

2.1. Experimental animals and treatment

Ten- to twelve-weeks-old, 24 Long-Evans female rats (150–180 g) were obtained from Animal Production Unit of Animal House at the Department of Pharmaceutical Sciences, North South University, and were kept in individual cages at room temperature of 25 ± 3 °C with a 12 h dark/light cycles. They have free access to standard laboratory feed (pellet food crushed to coarse powder) and water, according to the study protocol approved by Ethical Committee of Department of Pharmaceutical Sciences, North South University, for animal care and experimentation.

To study the hepatoprotective effects of apocynin (APC), experimental rats were equally divided into four groups (six rats in each group): Group I (Control), Group II (CCl_4), Group III (Control + APC) and Group IV (CCl_4 + APC). Rats of group I were treated with 1 mL/kg of saline (0.85%) and olive oil (1 mL/kg) intragastrically twice a week for two weeks. Rats of groups II and IV were treated with CCl_4 (1:3 in olive oil) at a dose of 1 mL/kg intragastrically twice a week for two weeks. However, animals of groups III and IV were treated with apocynin 100 mg/kg orally every day for two weeks. This dose was selected based on literature search about apocynin effect on liver diseases [12]. The weighed quantity of apocynin was dissolved in olive oil. Olive oil was chosen as the vehicle because apocynin is soluble in olive oil. Animals were checked for the body weight and water intake on a daily basis. After two weeks, all animals were anesthetized using ketamine, and then all the animals were weighted, sacrificed, collected the blood and organs like heart, kidney, spleen and liver. Immediately after collection of the organs, they are weighted and stored at −20 °C for further studies. Blood was drawn via syringe and centrifuged at 8000 rpm for 15 min at 4 °C. Then serum was transferred using a micropipette into micro centrifuge tubes and stored at −4 °C until analyzed.

2.2. Chemicals

CCl_4 was obtained from Merck (Germany) and apocynin was purchased from the Kuri & Company, office: 78, Motijheel C/A Dhaka-1000, Bangladesh. Thiobarbituric acid (TBA) was purchased from Sigma Chemical Company (USA) and trichloroacetic acid (TCA) from J.I. Baker (USA). Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase assay kits were obtained from DCI Diatec diagnostics (Budapest, Hungary), 50, 50-dithiobis-2 −nitrobenzoate (Ellman's reagent) from Sigma (USA) and sodium hydroxide from Merck (Germany). All other chemicals and reagents used were of analytical grade.

2.3. Assessment of hepatotoxicity

Liver marker enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were estimated in plasma by using Diatec diagnostic kits (Hungary) according to the manufacturer's protocol.

2.4. Preparation of tissue sample for the assessment of oxidative stress markers

For determination of oxidative stress markers, liver tissue was homogenized in 10 vols of phosphate buffer containing pH 7.4 and centrifuged at 8000 rpm for 15 min at 4 °C. The supernatant was collected and used for the determination of protein and enzymatic studies as described below.

2.5. Estimation of lipid peroxidation as malondialdehyde (MDA)

Plasma concentrations of thiobarbituric acid reactive substances (TBARS) are the index of lipid peroxidation and oxidative stress. Lipid peroxidation in liver was estimated calorimetrically measuring MDA using thiobarbituric acid reactive substances (TBARS) followed by previously described method [27]. The absorbance of clear supernatant was measured in Elisa reader against reference blank at 535 nm.

2.6. Estimation of nitric oxide (NO)

NO was determined according to the method described previously as nitrate and nitrite [31,32]. In this study, Griess-Illosvoy reagent was modified by using naphthyl ethylene diaminedihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). NO level was measured by using standard curve and expressed as nmol/gm of tissue.