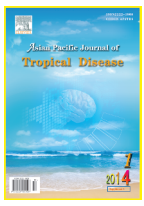
Asian Pac J Trop Dis 2014; 4(Suppl 1): S64-S70

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

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



Document heading doi: 10.1016/S2222-1808(14)60416-3

Ameliorative effects of phyllanthin on carbon tetrachloride-induced hepatic oxidative damage in mice

Rajesh Krithika, Ramtej Jayaram Verma*

Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad-380 009, India

PEER REVIEW

Peer reviewer

Dr. Komal Shah, Research Associate, Indian Council of Medical Research (ICMR), Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad-380009, India.
Tel: +91-79-2630 2362
Fax: +91-79-26308545
E-mail: komal_12885@yahoo.com

Comments

This is a good paper describing the hepatoprotective property of phyllanthin. The authors have performed appreciable work. The authors can further extend their study to analyze the results on molecular level which will shed more light on the exact nature of protection conferred by this isolated molecule.
Details on Page S69

ABSTRACT

Objective: To evaluate the liver protecting efficacy of phyllanthin, a lignin, isolated from the leaves of *Phyllanthus amarus* using mice model.
Methods: Phyllanthin was orally administered with or without CCl_4 for 30 d. Serum levels of hepatic marker enzymes namely alanine transaminase and aspartate transaminase were evaluated. Oxidative stress was ascertained by measuring hepatic lipid peroxidation levels and by estimating non-enzymatic antioxidants such as glutathione, total ascorbic acid, enzymatic antioxidants namely catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione transferase. Histopathological and ultramicroscopic analyses were also carried out.
Results: Oral administration of CCl_4 caused significant increase in lipid peroxidation. The hepatic levels of both non-enzymatic antioxidants and enzymatic antioxidants were significantly lowered in CCl_4-treated mice as compared to control. Treatment with phyllanthin significantly mitigated these changes in the CCl_4-treated mice. Histopathological and ultramicroscopic studies correlated well with the biochemical findings, as phyllanthin treatment reversed the alterations induced by the toxin and the subcellular features of phyllanthin treated mice were similar to those present in the normal mouse liver.
Conclusions: This study reports the *in vivo* anti-hepatotoxic potential of this isolated molecule phyllanthin, which may be responsible for the liver protecting property of *Phyllanthus amarus*.

KEYWORDS

Phyllanthus amarus, Phyllanthin, Hepatoprotection, Oxidative stress, Glutathione

1. Introduction

The use of carbon tetrachloride (CCl_4) to induce experimental oxidative stress and liver damage is quite common[1]. It is also well known that CCl_4 rapidly metabolizes to free radical products in the hepatic tissue with subsequent initiation of lipid peroxidation (LPO)[2]. Repeated exposure with this hepatotoxin is known to exhaust the endogenous antioxidant defense pool of biological systems. To correct this oxidative imbalance, antioxidative principles derived from varied sources are utilized. Some naturally occurring antioxidative principles originating from plant resources have exhibited enormous potential in resolving this oxidative stress which is also considered as the root cause for many pathogenic disorders affecting humans.

The Indian sub-continent through ancient ages had followed a rich tradition of employing medicinal plants for treatment of various ailments. Various experiments have been carried out using plants and their products as liver protecting alternatives. The genus *Phyllanthus* is represented by nearly 1 200 plant species which are distributed in tropical and subtropical countries and used in traditional medicine to treat chronic liver

*Corresponding author: Prof. Ramtej J. Verma, Department of Zoology, School of Sciences, Gujarat University, Navrangpura, Ahmedabad-380 009, India.
Tel: +91-79-2630 2362
Fax: +91-79-26308545
E-mail: ramtejverma@gmail.com

Article history:
Received 15 Dec 2013
Received in revised form 29 Dec, 2nd revised form 2 Jan, 3rd revised form 9 Jan 2014
Accepted 23 Jan 2014
Available online 28 Jan 2014

disease[3-5]. The aerial parts of *Phyllanthus amarus* (*P. amarus*) has been widely used in folklore in India and other tropical countries for the treatment of various diseases such as jaundice, diarrhea, kidney ailments, malaria, genitourinary infections[6]. *P. amarus* is one of the extensively studied and reported plants for its hepatoprotective property against various toxins[7]. The crude extract of *P. amarus* has been found to attenuate paracetamol[8], ethanol[9], aflatoxin B1[10] and galactosamine-induced hepatotoxicity[11].

Many high performance thin layer chromatography and high performance liquid chromatography (HPLC) methods have been standardized for the quantification of phyllanthin and hypophyllanthin[12,13], the major lignins from *P. amarus* responsible for its antihepatotoxic property. Some *in vitro* data on the hepatoprotective potential of phyllanthin have been previously published[14-16]. Similarly, the *in vitro* antioxidative potential of phyllanthin containing microcapsules on human fibroblasts and keratinocytes and its growth inhibitory activity towards *Staphylococcus aureus* has also been demonstrated by Lam *et al*[17].

In this study, we have assessed the hepatoprotective and antioxidative efficacy of phyllanthin against the standard and well established hepatotoxin CCl_4 in mice model. The *in vivo* liver protecting effect of phyllanthin, one of the major lignin and lead molecule of *P. amarus* is determined.

2. Materials and methods

2.1. Chemicals

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). CCl_4 (HPLC grade) and all other solvents used in the study were procured from Merck Specialties Pvt. Ltd., Mumbai, India.

2.2. Phyllanthin isolation

Silica gel column chromatography using gradient elution with hexane-ethyl acetate solvent mixture was used for the isolation of phyllanthin as reported elsewhere[15]. The purity of the isolated compound was confirmed by reversed-phase HPLC analysis. This isolated compound was used in the present study.

2.3. Animals

Swiss strain female mice (*Mus musculus*) weighing between 32-35 g were maintained under controlled conditions of (25± 2) °C temperature and 12 h light/dark cycle in the animal house of the Department of Zoology, Gujarat University, Ahmedabad and used in the study. They were maintained on pelleted rodent (Amrut Feeds) obtained from Pranav Agro Industries Limited, Pune, India. All experimental procedures were assessed and approved by the Committee for the Purpose of Control and Supervision of Experiment on Animals (Reg-167/1999/CPCSEA), New Delhi, India.

2.4. Experimental design

The mice were separated into four different groups consisting of 10 mice in each group. Group I received 0.2 mL of olive oil which was used as the vehicle to dissolve CCl_4 and phyllanthin. Group II was marked as the phyllanthin-treated group and mice were administered 10 mg/kg body weight per day of phyllanthin[18]. The mice in Group III were administered CCl_4 (826 mg /kg body weight)[19] and Group IV mice received CCl_4 along with 10 mg/kg body weight per day of phyllanthin. Treatments in all the groups were continued daily for 30 d. On Day 31, blood was collected by cardiac puncture in clean sterilized tubes and serum was separated by centrifugation at 2800 r/min for 10 min. Liver tissue was dissected out and used for further biochemical, histopathological and ultramicroscopic analysis.

2.5. Biochemical estimations

The LPO was measured by the method of Ohkawa *et al*.[20] which was based on the formation of red chromophore that absorbed light at 532 nm due to the reaction of thiobarbituric acid with products of LPO like malondialdehyde (MDA) and others which were collectively called thiobarbituric acid reactive substances. The tissue glutathione (GSH) was determined by the method of Grunert and Philips in which a saturated alkaline medium was used and the GSH present in the tissues was made to react with sodium nitroprusside to give a red coloured complex which was measured at 520 nm[21]. The method of Roe and Kuether was used to estimate the liver total ascorbic acid (TAA)[22], which was oxidized to dehydroascorbic acid by in the presence of trichloroacetic acid. This couples with 2, 4-dinitrophenyl hydrazine in the presence of thiourea and sulphuric acid yielded a red coloured complex which was read at 540 nm against blank.

Activities of enzymes such as catalase (CAT)[23], superoxide dismutase (SOD)[24], glutathione peroxidase (GPx)[25], glutathione reductase (GR)[26], and glutathione transferase (GST)[27] were measured using standard reported protocols. The protein content was estimated using bovine serum albumin as the standard[28]. Activities of serum transaminases [alanine transaminase (ALT) and aspartate transaminase (AST)] were measured using kits supplied by Agappe Diagnostics, Ernakulam, Kerala, India.

2.6. Histopathological examination

Tissues for histopathological examination were preserved in 10% neutral buffered formalin immediately after autopsy. Standard technique for hematoxylin and eosin (H & E) staining was followed. The tissues were dehydrated by passing through ascending grades of alcohol, cleared in xylene and embedded in paraffin wax (58-60 °C m.p). About 5 μm thick sections were cut on a rotary microtome and stained in H & E,