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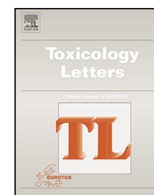

1 # Leishmanial lipid affords protection against oxidative stress induced
2 hepatic injury by regulating inflammatory mediators and confining
3 apoptosis progress

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HIGHLIGHTS

- Leishmanial lipid (pLLD) improves CCl4 induced hepatic insult.
- pLLD reduces oxidative stress and inflammatory injury.
- It also down-regulates apoptotic protein during hepatic intoxication.

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ABSTRACT

Persistence of liver injury alters the internal milieu, promotes deregulation of inflammatory factors, and leads to dysplastic lesions like fibrosis, cirrhosis to hepatocellular carcinoma. Our previous study revealed that leishmanial lipid (pLLD) exerts potential anti-inflammatory activity in sepsis associated hepatic injury. We now show that pLLD gives protection against chemical induced hepatotoxicity in murine system. The beneficial effect of treatment with pLLD on such hepatic injury in mice was analyzed using different assays including ELISA, FACS, western blot and immunohistochemical analysis. pLLD significantly suppressed serum enzymes and rectified the histopathological alteration to induce the antioxidant level in CCl_4 intoxicated liver. Levels of several growth factors including TGF-α, HGF, and EGF were significantly improved in serum and hepatic tissue with consequent reduction of caspase activities and expressions of Bad–Bax p53, and NF-κBp65. Moreover, pLLD modulated inflammatory responses by decreasing the production of several cytokines and chemokines, thus preventing the infiltration of immune cells to the damaged area. It accelerated the repair process in liver damage with modulation of signalling cascade via alteration of apoptotic factors. Our experimental approaches suggest that pLLD effectively prevents liver injury mainly through down regulation of oxidative stress and inflammatory response towards anti-apoptotic changes.

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1. Introduction

8 9 Liver acts as a central regulatory functional unit for detoxification and excretion of destructive agents generated from hazardous chemicals and also maintains metabolic homeostasis including synthesis and xenobiotic metabolism (Bissell et al., 2001). Commonly, toxins are converted into intermediate reactive radicals to exhibit their hepatotoxic impacts. Liver damage is a widespread pathology which can influence these physiochemical functions caused by toxic chemicals (CCl_4) (Jaeschke et al., 2002). 10 11 12 13 14 15 16

Abbreviations: ALP, Alkaline phosphatase; ALT, Alanine transaminase; AST, Aspartate transaminase; CAT, Catalase; CCl_4, Carbon tetrachloride; Cyt C, cytochrome C; DAPI, 4',6-diamidino-2-phenylindole; H2DCFDA, 5-(and 6-)-chloromethyl-2′,7′-dichlorodihydro- fluorescein diacetate acetyl ester; pLLD, pathogenic leishmanial lipid; IL, Interleukin; ROS, reactive oxygen species.

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The hepatotoxicity induced by carbon tetrachloride (CCl_4) is ascribed to its biotransformation to trichloromethyl free radical (CCl_3) or trichloroperoxyl radical ($CCl_3O_2^{\bullet}$) produced with the mixed-function cytochrome P450 oxygenase system of the endoplasmic reticulum; this leads to oxidative stress and membrane damage (Goeptar et al., 1995). These free radicals cause lipid peroxidation which results in hepatocellular damage including centrilobular necrosis, and enhanced formation of inflamed tissues mainly through generation of reactive oxygen species (ROS). ROS are also suggested to play an important role in cytochrome C release from mitochondria followed by apoptotic response (Sipes et al., 1991). Consequently, CCl_4 induced toxic effects on hepatocytes, which is mediated by a direct solvent injury to the cell membranes, and on non-parenchymal cells such as Kupffer cells are mediated by the release of cytokines. This may contribute to a pathophysiological process culminating in hepatocyte apoptosis after toxic injury to liver. Interruption of function of Kupffer cells, hepatic stellates, and sinusoid endothelial cells results in the production of pro-inflammatory cytokines like IL-6, TNF-α, TGF-β, and iNOS. These are intimately involved in chemical induced hepatotoxic process (Bataller and Brenner, 2005; Domitrovic et al., 2011; Friedman, 1999; Taniguchi et al., 2004) and cause infiltration of neutrophils and monocytes into the damaged organ (McGregor and Lang, 1996; Zhang et al., 2004). Thus, higher percentages of induced cell damage are mostly involved in several human pathologies, including liver cirrhosis and fibrosis.

Among several bioactive components, lipids and their derivatives including sphingolipid(s) and ceramide(s) possess potent biological activity, e.g., causing hyper lipidemia or hepatic fibrosis and regulating immune function (Kurek et al., 2013; Moles et al., 2010). They also protect hepatic injury via alteration of different bioactive lipid metabolites like acid sphingomyelinase in pathological liver tissue. Moreover, different classes of lipids isolated from various sources, particularly soybean, safflower, and egg have potent role in hepatoprotection (Imaizumi et al., 1983; Iwata et al., 1992; Kabir and Ide, 1995). Interestingly, leishmanial lipids augmented various inflammatory factors in stimulated macrophages and mammalian lymphocytes (Giorgio et al., 2003). Previously we reported that the lipid from an attenuated strain of *Leishmania donovani* promastigote (MHO/IN/1978/UR6) suppresses several inflammatory mediators in adherent synovial fluid mononuclear cells (SFMCs) isolated from rheumatoid arthritis patients (Majumdar et al., 2008). We also reported that lipid from pathogenic *Leishmania donovani* (pLLD) acts as anti-inflammatory agent and protects from endotoxin induced sepsis in hepatic impairment (Chatterjee et al., 2014). This finding encouraged us to evaluate the efficacy of pLLD in chemical (CCl_4) induced hepatic damage towards oxidative stress and inflammation response to regulate apoptosis.

2. Materials and methods

2.1. Chemicals

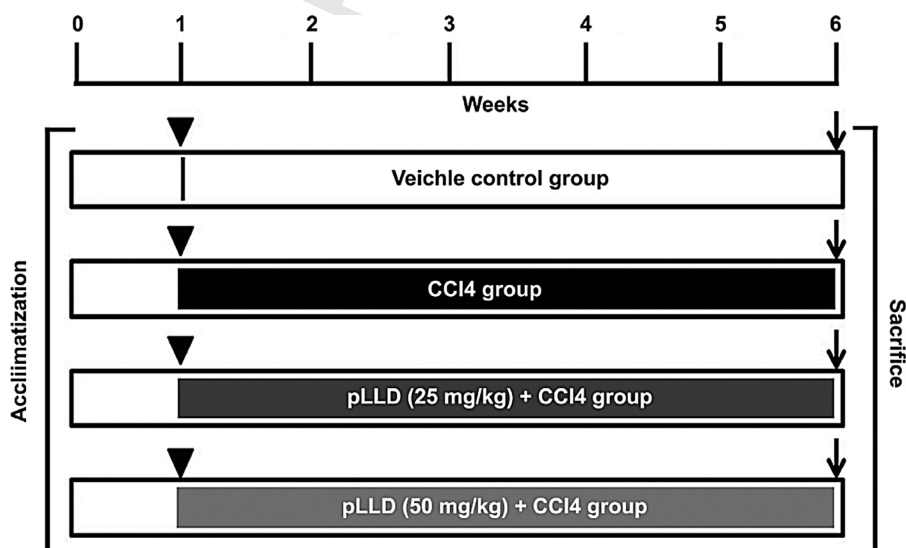
CCl_4 was purchased from Sigma–Aldrich Biotechnology (St Louis, MO, United States). Assays kits for the detection of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were purchased from Cayman (Michigan, USA). Rabbit anti-TNF-α, IL-1β, IL-6, NF-kB/p65, PCNA, TGF-βCOX-2, and iNOS polyclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). IL-1β, IL-6, KC and TNF-α were measured by enzyme-linked immunosorbent assay (ELISA) kits from R&D system (Minneapolis, MN, United States). All other chemicals were of the highest grade commercially available.

2.2. Animals

BALB/c male mice (18–22 g) were obtained from the animal house of CSIR-Indian Institute of Chemical Biology and supplied with certified standard diet and tap water *ad libitum*. Temperature and humidity were regulated at 21–23 °C and 50–60%, respectively. The study protocol was in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals.

2.3. Leishmania donovani *cell culture and isolation of lipid*

L. donovani strain AG83 (MHOM/IN/1983/AG83) was used for the present experiments. AG83 was obtained from Indian kala-azar patients and maintained in golden hamsters (Mukhopadhyay and Madhubala, 1994). Promastigotes obtained after transforming amastigotes from infected hamster spleen were maintained in M199 (Invitrogen) supplemented with antibiotics and 10% fetal calf serum at 22 °C. The Bligh and Dyer method of lipid extraction was used to isolate the total lipid from 1×10^{10} *L. donovani* cells (Bligh



Scheme 1. Experimental regimen.

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