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Research Article

Rosmarinic Acid-A New Hope for Liver Diseases Like Cirrhosis, Hepatocellular Carcinoma-Needs Translation to Humans

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Abstract

There is a rising incident of liver diseases that include hepatitis, cirrhosis, fibrosis along with hepatocellular carcinoma (HCC) and managing them remains a massive challenge. The apparent reason for this rise is inadequate nutrition, viral infections alcohol dependency, xenobiotic exposure which help both in development and further progression of liver diseases. Lipid peroxide, production of reactive oxygen species (ROS), peroxy nitrite formation, complement factors and proinflammatory mediators like cytokines and chemokines are some mechanisms of hepatic disease development. Rosmarinic acid (RA) is a polyphenol compound found naturally in the family Lamiaceae, that includes various medicinal plants, herbs and spices. RA possesses various biological properties like antioxidant properties like a ROS scavenger, along with lipid peroxidation inhibitor, inflammatory neuroprotective, antiangiogenic activities besides others. The aim of this review is to highlight how RA may get translated in human liver diseases after studying its mechanism of hepatoprotective effects in animal studies which favour its actions.

Keywords: *Liver Diseases; Rosmarinic Acid (RA); Hepatoprotective Effects; HCC; Cirrhosis*

Introduction

The normal constitution of liver is resident and migratory lymphocytes, macrophages, leukocytes (infiltrated monocytes and neutrophils) which provide surveillance against foreign antigens [1]. But cell types like hepatocytes, parenchymal, the Kupffer, sinusoidal endothelial and stellate (fat storing or Ito) are also present in the liver [2]. These cells of the liver are primary targets for oxidative stress and toxicity that is induced by different agents. The reason being liver is a major site for drug metabolism coupled with the proliferative response of the hepatocytes.

Injury of the liver is one of the main health challenges in the world. Dysregulation of the liver function pathologies which include, hepatitis, cirrhosis, hepatocellular carcinoma etc. The liver diseases progresses from histologic and biochemical changes like hepatocyte death, hepatic stellate cell (HSC) activation, Kupffer cell (KC) activation, peripheral inflammatory cell infiltration and activation, free radical secretion, proinflammatory cytokine production, and extracellular matrix (ECM) protein expression and deposition to irreversible cirrhosis and hepatocellular carcinoma (HCC) [3]. Various factors including inadequate nutrition, viral infection, ethanol and drug abuse, xenobiotic exposure and metabolic diseases have been linked in the development of liver diseases [3,4]. Yet it has been established that

the main etiologic agents for hepatic injury, which triggers onset of liver failure in a special area depends on the prevalent hepatotropic viral infections or patterns of drug use [4].

Worldwide cholestasis has relatively high morbidity and mortality rates, that is caused by impairment of bile formation and /or bile flow. The most chronic cholestatic diseases occur from hepatocellular functional defects or obstructive lesions of the small intrahepatic bile ducts [10]. While hepatitis A, B, C, D, and E are viral infections of the liver with different viruses causing each type of infection. High incidence of hepatitis A is associated with poor hygiene and sanitation [5]. But drug induced injury that is the 2nd major cause of acute liver injury is very common in the developed world. Paracetamol induced hepatotoxicity is the commonest example and other drugs which cause causing liver injury differ by location and current drug use with anti-infectives, anticonvulsants, and anti-inflammatory drugs being the most common, on the other hand herbal or adulterated traditional or complementary medicines have been implicated in East Africa [6].

The normal physiologic repair process i.e. fibrosis following injury or inflammation, can be dysregulated following chronic injury, which accumulation of ECM that fibrosis [6] and ultimate loss of functional cells, thereby impairing liver function. Transforming growth factor beta (TGFβ) is the major pro-fibrotic factor in liver fibrosis that implies inhibiting TGFβ or blocking its downstream signaling pathways will help in preventing the fibrotic process in the liver. But as TGFβ is also an important antiproliferative and anti-inflammatory agent inhibiting it might cause positive/negative effects. Hence Connective tissue growth factor (CTGF), that acts as downstream of TGFβ 1 induced fibrosis remains an alternative target for therapy as its inhibition potent decrease in fibrosis [7].

Alcohol consumption has been liked to liver disease (alcoholic liver disease, ALD) which manifests as acute (alcoholic hepatitis) or chronic steatosis steatohepatitis, fibrosis and cirrhosis form Oxidative stress and changes in lipid metabolism damage to cell membranes and organelles (mainly mitochondria) are implicated in ALD. Individual susceptibility, presence of other liver disease involvement like viral hepatitis, obesity and metabolic syndrome are also contributors. Classical mechanisms of fibrogenesis in ALD are alcohol metabolism, Oxidative stress, methionine metabolism abnormalities, hepatocyte apoptosis and increased lipopolysaccharide (LPS) levels which activates KC [8]. However, in early stage of ALD, lipogenesis has been blamed as a risk factor for the cirrhosis progression. Thus, newer mechanisms involve stimulation of lipogenesis and inhibition of fatty acid oxidation, osteopontin, IL1 signaling and genetic variations [8]. ALD can present in many disease states including asymptomatic fatty liver, steatohepatitis, progressive fibrosis, end stage cirrhosis and HCC [9].

HCC constitutes one of the most lethal and common cancers worldwide, being the 5th leading cause of cancer and has 3rd place in cancer mortality [9]. Inflammation is closely linked to carcinogenesis [26], that is highlighted by HCC, further both chemically or genetically induced HCC depends on inflammatory signaling. Various signaling pathways are involved in injury-Inflammation-regenerational response and human HCC development. Of these signaling pathways inhibitor of β kinase (IKKβ) -dependent classical nuclear factor light chain-enhancer of activated B cells (NFκB)-signaling and signal transducer of activator of transcription (STAT3) were found to be key for compensatory liver regeneration and chemically HCC development [10].

Use of complementary and alternative medicine which includes dietary supplementation with plant derived phytochemicals is on the rise for health promotion along with therapy. Rosmarinic acid (RA, Ig1), an ester of caffeic acid and 3,4 –hydroxy phenyl lactic acids is a major constituent of Chinese and oriental herbal medicines and is commonly found in species of the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae. Presence of RA in medicinal plants, herbs and spices has been associated with the benefits and health promoting effect of plants. RA acts as an accumulated defense compound in humans, having number of biological activities that include antiviral, antibacterial, anti-inflammatory, antimutagenicity and antioxidant. It has very little toxicity, getting rapidly eliminated on intravenous administration from the blood [11].

Methods

Thus, we aimed to review comprehensively how RA has hepatoprotective effects on liver disease for which we conducted a Pubmed, Google Scholar, Web of Science literature search from 1970's to 2019.

Results

We found a total of 120 articles specifically to explain this mechanism in animal models. No meta-analysis was done.

Basic mechanisms of hepatotoxicity

Hepatotoxicity secondary to mitochondrial dysfunction

Inhibition of Mitochondrial-β-oxidation deprivation of cellular energy during fasting [12]. Non-esterified fatty acids (NEFA) and their metabolites also interfere with Mitochondrial energy production [12]. The deficiency of energy liver failure, pancreatitis, coma and even death [12]. Many drugs cause impairment of Mitochondrial-β-oxidation and cause microvascular steatosis via different mechanisms [2]. These are nonsteroidal anti-inflammatory drugs, tetracycline derivatives, glucocorticoids [35], antianginal cationic amphiphilic drugs], along with female sex hormones or pregnancy [13]. β-oxidation and respiration also get inhibited by cationic amphiphilic drugs that concentrate in the mitochondria [13]. The inhibition of respiration production of ROS by mitochondria and causes lipid peroxidation of fat depots. Both lipid peroxidation and ROS cause release of cytokines like TGFβ, Tumour necrosis factor alpha (TNF-α), and interleukin 8 (IL-8), that contribute to the development of non-alcoholic steatohepatitis (NASH). Also, cytolytic hepatitis, a severe liver lesion which can cause liver failure occurs in response to mitochondrial uncoupling, respiratory inhibition and mitochondrial permeability transition caused by opening of permeability transition (PT) pores in the mitochondrial inner membrane [4].

Apoptosis induced by bile acids

Liver produces bile and failure of this bile production is known as cholestasis. With bile constituents getting retained within the hepatocytes during cholestasis. hepatocyte apoptosis and failure to secrete bile liver injury, cirrhosis and death from liver failure. Using rat cultured hepatocytes experimentally, the hydrophilic bile acid glycochenodeoxycholate (GCDC), at appropriate concentrations (20 - 100 nm) can induce Apoptosis. As seen by cell shrinkage, nuclear condensation and caspase activation, DNA fragmentation and phosphatidyl serine externalization]. Either by death receptor pathway or mitochondrial pathway hepatocyte apoptosis occurs. Tumor necrosis factor receptor 1 (TNFR1) and first apoptosis signal (FasR) are the predominant death receptors expressed by the hepatocytes [14]. Ligand independent Fas mediated apoptosis, also is another mechanism of bile acid related injury [15]. Mechanism of bile acid induced Fas activation are alterations in Fas synthesis, Fas compartmentation, and Fas trimerization in the plasma membrane. Bile acid also helps rapid transport of cytoplasmic Vascular Fas to the plasma membrane in a microtubule dependent manner.

CYP2E1 dependent toxicity in HEPG2 cells

The endothelial-inducible form of cytochrome P4502E1 (CYP2E1) activates and metabolizes many toxicologically important substrates like ethanol, carbon tetra chloride, acetaminophen and N-nitroso dimethylamine, in more toxic products [16]. CYP2E1 dependent metabolism of ethanol generates ROS oxidative stress' [17]. Ethanol induced liver pathology correlates with CYP2E1 levels and increased lipid peroxidation that gets blocked by inhibitors of CYP2E1. Hypothesis of the role of CYP2E1 in alcohol induced oxidative stress and hepatotoxicity suggest that various mechanisms might contribute, the linkage between CYP2E1 dependent oxidative stress, mitochondrial injury and increased collagen formation by stellate cells are mechanistic contributors to the toxic action of ethanol on liver [2].

Liver toxicity that involves peroxynitrite

Acetaminophen produces centrilobular hepatic necrosis when taken in overdoses. With initial step in Acetaminophen toxicity involving metabolism to N-acetyl-p-benzoquinone imine (NAPQI), which causes depletion of the reduced form of glutathione (GSH) and

covalent adduct formation. In wild type mice, initiation of nitric oxide (NO) synthesis and subsequently superoxide generation peroxy nitrite formation. However, in inducible NO synthase (iNOS) knockout mice, superoxide increases following acetaminophen, but not NO synthesis. Superoxide anion then causes lipid peroxidation. Hence acetaminophen toxicity might be mediated by nitration in wild type mice and by lipid peroxidation in iNOS knockout mice. Thus. hepatotoxins like acetaminophen, bromobenzene, chloroform and allyl alcohol, which deplete hepatic GSH and encourages peroxynitrite formation will promote hepatic toxicity. Yet with hepatotoxins which cause lipid peroxidation but do not deplete hepatic GSH, such as carbon tetrachloride, NO may scavenge superoxide by forming peroxynitrite, that is then detoxified by GSH [2].

Oxidative stress, inflammation and adhesion molecules in liver diseases

Both systemic and local inflammation, along with macrophage recruitment and neutrophils into the liver vasculature characterizes certain pathologies like sepsis, alcoholic hepatitis, ischemia-reperfusion injury and some drug induced liver toxicities [18]. Function of these macrophages and neutrophils are to destroy invading organisms along with removing dead cells and debris in promoting for preparing for tissue regeneration. Though during course of this some healthy cells may get affected, that can aggravate the original liver injury [2]. Events like drug toxicity, tissue trauma, ischemia-reperfusion, sepsis and other pathophysiological conditions, both neutrophils and KC get activated either directly or via complement activation. Cytotoxic mediators like ROS are then released by KC, along with pro-inflammatory mediators like cytokines and chemokines. Complement factor (like C5a) and cytokines activate neutrophils for promoting their recruitment into the hepatic vasculature. Neutrophils when chemotactically stimulated, they extravasate and adhere to parenchymal cells, that induces necrotic cell death via release of ROS and proteases [2]. Adhesion and subsequent infiltration of leukocytes to extravascular tissues is secondary to induced expression of various critical adhesion molecules on the surface of inflammatory cells (leukocytes, endothelial cells, etc). The overall mechanism of liver disease pathology is shown.

Rosmarinic acid (RA)

RA is a natural phenolic compound having many biological activities. It has 2 phenolic rings, both containing 2 hydroxyl groups in ortho position. Joining the 2 rings are an unsaturated double bond, a carbonyl group and a carboxylic acid group.

RA was 1st isolated from Rosmarinus officinalis in 1958 by Italian chemists, Scarlatti and Oriented hence the name, which is mainly found in species of the family Boraginaceae and subfamily Nepotidae (family Lamicaceae [11]. Other plants that contain RA are *Origanum vulgare* (oregano), *Thymus vulgaris* (thyme), *Mentha spicara* (spearmint), *Perilla frutescens (*perilla), *Ocimum basilicum* (sweet basil) and several other medicinal plants, herbs and spices [19]. Amount of RA production depends on the natural source from which it is obtained [20] which may vary from 1.5 (in Rosmarinus officinalis) to 20.0% w/w in *Perilla frutescens* [21]. RA possesses different biological activities like antitumor [22], anti-angiogenic [23], anti-depressive [24], anti-inflammatory [25], antiallergic [26], antimicrobial [27], neuroprotective [28] and HIV 1 inhibiting properties. Also antioxidant properties as reactive species scavenger along with lipid peroxidation inhibitor have been attributed to it [29]. Various authors have documented antimutagenic activity [26,30].

RA on Liver

RA on LPS and D-galactosamine (D-Galn)-induced liver failure

LPS and D-Galn) induced liver failure is a well established experimental model [31] that uses the ability of D-Galn, a transcriptional inhibitor, to potentiate the toxic effects of LPS in producing typical hepatic necrosis and apoptosis followed by fulminant hepatitis [32]. LPS then activates KC which then secrete different cytokines like interleukin1 (IL1), interleukin 6 (IL-6), and TNFα [31]. TNFαappears to be the major inflammatory cytokine implicated in liver injury caused by LPS and D-Galn [33]. TNFα induced apoptosis, involves the activation of the cysteine protease caspases [68]. Also NO overproduction, that results from upregulation of iNOS expression, gets implicated in the induction of apoptosis of various cell types [34]. All these events can be studied in vivo using the LPS and D-Galn model [33].

RA from Perilla frutescens possesses protective affect against the LPS induced liver injury in D-Galn-sensitized mice [35]. This RA protection's mechanism got confirmed by investigating the effects of anti-TNF antibody, SOD and aminoguanidine in this model. Treatment with RA significantly decreased the increase of plasma aspartate aminotransferase (AST) levels as well as had antiTNF and SOD's effects compared with controls as was confirmed by histological examination. But increase in TNF –mRNA expression in liver and in plasma TNF levels were not significantly decreased by RA. RA did not reduce iNOS mRNA expression or plasma nitrate/nitrite levels as well that suggested that its liver protective effects might be due to scavenging or reducing activities of superoxide or peroxy nitrite rather than to inhibition of RNF [35].

RA on Tert-butyl hydroperoxide (BHP)-induced oxidative liver damage

BHP is commonly used for inducing acute oxidative stress both in vivo and in vitro. It gets metabolized by cytochrome P450 to free radical intermediates like t-Butoxyl and t-methyl radicals [34] that can cause lipid peroxidation [35], GSH depletion [36,37] and DNA damage. t-BHP induced hepatotoxicity is a reaction involving GSH peroxidase that converts GSH to glutathione disulfide (GSSG) with the expense of NADPH oxidation. GSSG is reduced back to GSH and GSH reductase. Decreased GSH and oxidized NADPH contribute to altered Ca2+homeostasis, that is considered a major event in t-BHP induced oxidative liver damage.

Hepatoprotective effects of RA alone and in combination with caffeic acid (CA) was reported in t-BHP induced oxidative liver damage [37]. CA remarkably decreased the oxidative damage in an invitro study more than RA, but in an *in vivo* experiment where 5 days CA or RA alone for 5 days was given before treatment with a single dose of t-BHP (0.5 mmol/kg b.w.,ip) there was a significant decrease of indicators of t-BHP induced hepatotoxicity, like AST; ALT, GSSG, lipid peroxidation. The activities of antioxidant enzymes (catalase, glutathione peroxidase and SOD) were also increased by RA, though the hepatic production appears to be additive with CA [37].

RA on carbon tetrachloride (CCL_4)-induced rat liver fibrosis model

CCL_4 intoxication is the commonest study model of liver injury [36]. CCL_4 gets activated by hepatic microsomal cytochromes to form the trichloro methyl radical, CCL4 which induces oxidative stress by binding to biological molecules. This impairment of several cellular processes which end in necrotic cell damage, inflammation and apoptosis [80]. Nonlethal intoxication triggers liver tissue remodeling and healing via activation of trichloro methyl radical and produces liver fibrosis. CCL4 intoxication hepatic necrosis and increases serum ALT activity [38]. The oxidative/nitrosative stress caused by, CCL4 in the liver usually elevated 3-nitosotyrosine (3NT) and thiobarbituric acid reactive substances (TBARS) formation and a significant decrease in Cu/Zn superoxide dismutase (SOD) activity., CCL4adminstration also triggers inflammatory response in mice livers by activating NF-κB, which coincided with the induction of TNFα and cyclooxygenase2 (COX2) [38]. CCL4 induces typical necrosis but this can be accompanied by increased apoptosis of hepatocytes. Though TGF pushes towards fibrosis, TNFα appear to direct towards activation of caspases and apoptosis [36]. Hence, CCL4 destroys not only plasma membrane but also phospholipid bilayer in mitochondria, which triggers caspase-3 dependent apoptosis.

The *in vitro* and *in vivo* antifibrotic effects of RA on experimental liver fibrosis have also been reported. In the, CCL_4 induced rat liver fibrosis model, liver fibrosis grade and histopathological changes as well as immunohistochemical detected liver TGFβ 1 and connective tissue growth factor (CTGF) expression were noted [7]. In this study, RA inhibited HSC's proliferation, TGF-β1 and α-smooth muscle actin (α-SMA) expression in cultured HSC's. It also decreased fibrosis grade, ameliorated biochemical indicator and histopathological morphology [7].

Similar study also documented that severe acute liver damage caused by, CCL4 administration could be significantly ameliorated by RA [38]. The study reported that though the increase in relative liver weight persisted even with RA treatment, the body weight loss in experimental animals was greatly decreased that suggested a less deleterious effect of, CCL4. RA also dose dependently reduced hepatic histopathological changes of serum ALT levels in, CCL4 intoxicated mice. Also the necrotic area was markedly reduced with RA, along

with expression of active caspase 3 and suppressed oxidative/nitrosative liver damage [82]. Moreover liver inflammation caused by CCL4 intoxication was significantly ameliorated by RA therapy. This study also showed suppression of inflammation by RA was mediated via NF-κB pathway and downregulation of TNF-α and COX2 and submitted that RA has antifibrotic effects that were achieved via inhibition of TGF-β 1.

RA on Extrahepatic cholestasis rat model of bile duct ligation

Both for intestinal nutrient absorption along with hepatic metabolism, bile acids are needed. They get balanced dynamically by intrahepatic biliary tree via secretory and reabsorptive processes under normal condition. Any obstruction of the hepatobiliary system increase in biliary pressure, disruption of the intrahepatic bile duct integrity and leakage of bile into the liver thus predisposing the liver to cholestasis. [3,19]. This stimulatory effect on biliary cholangiocytes, hepatocytes, HSC and KC, that causes many consequences like hepatocyte death and hepatic inflammation, oxidative stress and fibrosis [19,20]. Surgical ligation of bile ducts In rodents can cause extrahepatic cholestasis and has been used as a model for pathophysiological and therapeutic studies of cholestatic liver injury [21-23].

Dietary RA supplementation has been reported to have hepatoprotective benefits using a BDL model of cholestasis in rats [1]. The cholestatic hepatoprotective effect of RA was due to improvement of serum biochemicals and hepatic histopathological changes, fibroses, inflammation and oxidative stress. RA inhibited the development of hepatic fibrosis and suppressed the activation of matrix producing cells and fibrogenic changes. On cultured HSC, it also inhibited TGFβ1 induced stellar cell mitogenic and fibrogenic activation by suppressing post receptor TGFβ1 signaling, fibrogenic molecular expression and cell cycle regulator expression. This study also showed that RA improved BDL-induced oxidative stress along with induction of hepatic toll like receptor4 (TLR4) signaling in BDL rats and its reversal by RA [3].

RA on precision cut liver slices

To study liver damage, an ex-vivo experimental method for liver damage, use of precision cut liver slices from fatty livers (fPCLS) is done to study antifibrotic drugs [39]. Biggest advantage of PCLS cell culture systems is the presence and viability of all cell types and also this model uses the original cell-cell and cell-matrix contacts get retained. In contrast to the in vivo experiments, significant decrease in the number of animals required as about 250 slices of 5 mm diameter and 250 µm thickness can be prepared from a normal rat liver and upto 40 - 45 from a normal mouse liver. As different culturing systems for PCLS are available [39], it makes it easy to use this model. The dynamic organ culture system and the 6 - 12 wells plate incubation systems have been used successfully for the studies of liver fibrosis. Further slices of healthy liver have been used to study the underlying mechanism of early onset of fibrosis and the efficacy of antifibrotic drugs, while slices from fibrotic liver tissue have been used to study the effect of antifibrotic drugs in fully developed fibrotic liver tissue.

The effect of RA was also tested in an *ex-vivo* study using fPCLS [23]. RA had no effect on ATP values of fPCLS but had a concentration dependent reduction in gene expression on the fibrosis markers. It also reduced the total collagen content as found by the hydroxyproline content [18].

Separate mechanisms of RA effect on the liver

The effects of RA and water soluble extract (WSE) of rosemary containing 1.2% RA) following dietary administration of xenobiotic metabolizing enzymes (XME) were studied in rat liver. This was done by examining the modulation pf phase I enzymes like cytochrome P450 (CYP) 1AK, 2E1, 3A and phase II enzymes like glutathione S-transferase (GST), quinine reductase (QR) and UDP-glucoronyl transferase (UGT) by measuring enzyme activities with specific substrates. Protein levels of CYP's and RGST A1/A2, A3/A5, M1, M2 and P1 were also measured using antibodies in Western blots. The extracts containing RA, flavones and monoterpenes increased CYP1A1, 1B1/2, 2E1 and UGT. But RA and caffeic acid did not modify XME (except for a slight increase of UGT activity following CA therapy) thus the induction of

XME by WSE could be explained by flavones, monoterpenes or an additive effect of all components. On the whole, WSE of rosemary markedly increased detoxification enzymes in rat liver.

The liver regeneration potential of RA gas been documented by Lou., *et al* [40]. RA stimulated hepatocyte proliferation during liver regeneration processes. Specifically, RA activated the mechanistic target of rapamycin (mTOR) signaling pathway during liver regeneration and rescued partial hepatectomy (PH), impaired liver function [41]. Treatment with RA increase in body weight and stimulation of proliferating cell nuclear antigen (PCNA) protein expression from days 2 to 4 in mice due to activation of the mTOR/S6K pathway during the early period of hepatic regeneration. Also, in the treated group or significantly increased the level of binuclear hepatocytes in the treated group. This study demonstrated that RA stimulated hepatocyte proliferation during liver regeneration through the mTOR signaling pathway.

The effect of RA on liver ischemia /reperfusion (I/R) injury has also been reported. RA decreases hepatocellular injury following I/R. I/R caused extensive areas of coagulation necrosis with disintegration of hepatocyte cords and inflammatory cell infiltration. These histologic and serum parameters of hepatocellular injury were significantly attenuated in animals that got treated with RA [41]. RA also protects the hepatic parenchyma from I/R induced oxidative stress. RA therapy considerably decreased the lipid hydroperoxide levels in the liver parenchyma. The total hepatic antioxidant capacity and the hepatocellular reserve s of GSH were also partially preserved by RA administration [41]. The study also found that RA reduces hepatic NO content and nitrosative stress after I/R. RA treated animals showed a marked reduction in nitrotyrosine staining exhibited reduced NO levels and decreased levels of both eNOS and iNOS in the liver. RA also attenuated I/R induced inflammatory response in liver, it downregulated polymorphonuclear leukocytes recruitment into the livers of rats subjected to I/R and markedly decreased the accumulation of phagocytes in the liver. It also markedly downregulated I/R induced p65 nuclear translocation and decreased hepatic mRNA levels significantly of proinflammatory cytokines [41].

The suppressive effects of RA on cell proliferation has also been demonstrated [30]. HSC-T6 cells treated with RA grew at a significantly slower rate in a dose and time dependent manner compared to non treated cells with the most effective dose being 8 mg/ml. The effect of RA on the growth of CCC-HEL [30]. The Researchers also investigated the ability of RA to induce apoptosis in HSC-T6 over a period of 48h and observed that treated cells underwent serial changes such as autophagosomes, enlarged rough endoplasmic reticulum (RER), porphyritic chromatin and lower density of mitochondrial matrix which is characteristic of apoptosis in cells [30]. To find whether the effect of RA on HSC –T6 was partly due to the janus kinase/STAT3 signal pathway, they saw that compared with glyceraldehyde-3-phosphodehydrogenase (GADPH) protein level, the expression of phosphorylated STAT3 had a high degree of correlation in the expression of pattern of genes encoding apoptosis inhibitor (Bcl2) and cell cycle regulators (Cyclin D1) protein in HSC-T6 cells treated with RA while the level of STAT3 protein showed no significant changes [30]. A report on the suppressing effect of RA acid on hepatic B biogenesis via suppression of HSC activation, proliferation and induction of apoptosis was given [42]. In the study RA treatment mitigated the proliferation of HSC-T6 cells in a time and concentration dependent manner with no impact on hepatocytes. It also had a concentration dependent reduction in HSC activation, elevation in caspase 3 expression and concentration dependent inhibition of TGFβ 1 production. Giving RA restored AST depletion and decreased lipid peroxidation in the treated group as well as normalized hepatic levels of TGFβ 1 and restored hepatic architecture of treated rat liver.

Discussion

In Europe practically 29 million people suffer from a chronic liver condition still even though progress made in the knowledge and management of liver disease in the past some years with cirrhosis and primary liver cancer cases being the highest. Around 14 - 26 new cases of cirrhosis, per 100,000 inhabitants per year and an estimated 170,000 deaths/year being suggested. In the US, Prevalence of chronic liver diseases ranged from 3.9% in African American and Native Hawaiians to 4.1% in whites, 6.7% in Latinos and 6. 9% in Japanese and > 1 million deaths occur due to liver cirrhosis annually. Various management strategies varying from diet/lifestyle modifications

to surgery including different medications have been used for combating liver problems. Here role of RA in liver diseases via different mechanisms is discussed.

Antioxidant and anti-inflammatory

Oxidative stress is believed to be a critical mechanism of hepatocellular injury and disease progression. It partly explains the pathologic findings of the compromised liver as well as serve as a prognostic indicator. Tissue antioxidant reserves get consumed due to Oxidative stress and it induces massive lipid peroxidation in cellular membranes. Over the years, both RIS and reactive nitrogen species (RNS, have been blamed for causing loss of liver function. Harmful substances production and reduction of bile and redox reaction along with oxidative damage can diseases like subclinical hepatitis, inflammatory necrotic hepatitis, liver cirrhosis and cancer.

This link between oxidation and different liver disease has been reported without any doubt. For e. g in alcoholic liver disease, CYP2E1-dependent Oxidative stress, mitochondrial injury and GSH homeostasis contributes to the toxic actions of cirrhosis on the liver [42]. SOD and glutathione peroxidase (GSH-PX) are significantly decreased in chronic hepatitis, which correlated negatively with serum ALT levels. In chronic hepatitis concentration of MDA are higher than normal values and oxidative damage is closely linked to the pathological damage of hepatic fibrosis. Besides that increase in intracellular oxygen free radicals production in HSC promotes the activation of HSC liver fibrosis. High levels of 8-hydroxydeoxy guanosine (8OHDG) expression in chronic hepatitis can also cause DNA mutations and induce liver cancer. There is also higher serum levels of oxidation protein production like NO and the activities of myeloperoxidase (MPO), arylesterase (AE), and paraoxinase (PON1), in chronic hepatitis. Different authors have shown strong antioxidant effects of RA in various experimental models. Specifically RA was shown to markedly decrease plasma ALT, AST, SOD, GSH long with other enzymes related to antioxidants with reference to particular liver disease. RA also protects hepatic parenchyma from I/R-induced oxidative stress decreased lipid hydroperoxide levels in liver parenchyma and preserved the total hepatic antioxidant capacity along with hepatocellular reserves of GSH specifically [41].

Inflammation is closely related to oxidative stress in view of facts that free radicals can affect intracellular signal transduction and gene regulation cytokine production, important for inflammatory process. In alcoholic liver disease lipid peroxidation causes inflammation and organ fibrosis and in chronic hepatitis the concentration of TNFα and TGFβ are higher than normal values. NF-κB also plays major role in inflammatory process. NF-κB activation triggered by ROS modulates liver injury by producing cytotoxic cytokines like NFκB, p-65 and TNF-α and inducing several other inflammatory properties like COX2 and iNOS. RA has been implicated in a number of inflammatory responses [25,44]. It significantly ameliorated CCL4 induced liver inflammation [6] via the inhibition of COX2 [44], and inhibition of production of inflammatory cytokine [44]. It has also shown the suppression of inflammation by RA can be mediated through the inhibition of NF-κB pathway as well as downregulation of TNFα and COX2 [6], which agrees with other studies. Thus, various studies have shown the therapeutic potential of RA against acute liver toxicity through amelioration of hepatic oxidative stress and suppression of inflammation.

As antifibrotic

This effect of RA was reported in CCL4 induced liver fibrosis in which biochemical parameters, histopathologic changes and immunohistochemical factors were assessed. In the study that RA inhibited HSC's proliferation, TGFβ1, CTGF and α-SMA expression in cultured HSC's were studied. Moreover it ameliorated biochemical indicator, histopathological morphology and decreased the total collagen content in a concentration dependent manner. In an ex-vivo study using fPCLS [45], RA decreased the gene expression of the fibrosis markers and reduced the total collagen content as detected by the hydroxyproline content in a concentration dependent manner. Further reports from an extrahepatic cholestasis rat model [3] showed that RA inhibited BDL-induced biliary fibrosis through mitogenic and fibrogenic inhibition by targeting matrix producing hepatic stellate cells [3]. In this model an observed improvement in hepatic function, parenchymal structure, ductular reaction and fibrosis was seen, which correlated with the suppression of NFκB and API activities, leukocyte

infiltration /activation, and cytokine overproduction [3]. Earlier reports have also shown the involvement of NFκB inhibition in the anti-inflammatory action of RA, thus making NFκB an important signal in the pharmacological effects of RA [3].

Cholestasis

In an ex vivo experiment in rats, RA was shown to have cholestatic, hepatoprotective effect seen as an improvement in serum biochemical and, histopathologic parameters [3]. Antioxidant, anti-inflammatory and antifibrotic effect of RA was corroborated in the same study. The authors found that the antifibrotic effect was mediated by suppression of TGFβ1 provoked mitogenic and fibrogenic activation while the cholestatic hepatoprotective effect was due to the decrease in total bile acid level in BDL rats, which showed that a reduction in bile acid level is associated with RA mediated hepatoprotection. This study also gave a suggestion that RA target of action might be hepatic primary bile acid synthesis, intestinal secondary metabolism and biologic activities [3]. Moreover the study showed that RA improved BDL-induced oxidative stress and oxidative stress has been implicated on playing a pathogenic role in cholestatic liver injury by modulating mitogenic and fibrogenic gene expression along with cell activities involving NFκB and AP-1 mechanisms [45]. Thus, the authors suggested that resolution of Oxidative burden is a mechanism via which RA suppresses redox sensitive NFκB and AP-1 action thus hepatoprotection [3]. Also from this study, an induction of hepatic TLR4 signaling in BDL rats and its reversal by RA was demonstrated pointing to the role of HMGB1/TLR4 signaling pathway in cholestasis associated NFκB and AP-1 activities and suggested that HMGB1/TLR4 derived signals could be another mechanism of action of RA in cholestatic, injury [3]. Thus in toto it is seen that steps of bile acid synthesis/metabolism, hepatic stellate cells, mitogenic and fibrogenic activation, the axis of HMGB1/TLR4 and free radical generation are targets for intervention by RA.

Liver damage

A protective effect on general liver damage has been shown for RA. E. g, in a CCL4 induced liver damage, a less harmful effect was reported with RA administration in mice [38]. RA dose dependently reduce d hepatic histopathological changes and serum ALT levels. It significantly decreased the necrotic area expression of active caspase 3 and suppressed oxidative/nitrosative liver damage [37] along with inhibiting lipid peroxidation which agreed with previous findings. Lipid peroxidation destruction of plasma membrane and intracellular organelles causing a massive destruction of hepatocytes and tissue necrosis, that is central to the toxicity of CCL4 [37]. RA also reduces eNOS and iNOS and NO content. Greater production of NO and superoxide anion in injured liver could the formation of peroxynitrite followed by nitration of protein tyrosine residues that plays an important role in the pathogenesis of hepatic necrosis. The effect of RA on liver damage due to ischaemia reperfusion is also documented [41]. Liver damage secondary to I/R is common in many surgical procedures that involve the liver. In such I/R injuries, cellular damage due to hypoxia is increased after the restoration of blood supply and oxygen delivery. Consequently acute inflammatory response within liver parenchyma immediately after blood flow restoration and it is the major mechanism of injury during the reperfusion phase. The activation of KC and sinusoidal endothelial cells (SEC). CD4+T lymphocyte and neutrophil recruitment to the hepatic interstitium and the subsequent generation of ROS, RNS and cytokines have been found in the most important events in the pathogenesis of liver damage due to I/R. ROS affects the pathophysiology of the liver indirectly by supporting protease activity via the inactivation of antiproteases and by acting as second messengers in signal transduction pathways involved in the regulation of genes encoding adhesion molecules and proinflammatory mediators [2]. NFκB, a key transcription factor, which is activated during reperfusion following an ischaemic event in various organs is made up mainly by a p50 and p65 heterodimer associated with regulatory proteins known as inhibitors of κB (IκB) and has been implicated in neuronal inflammation [46]. Effects of RA on both ROS and NFκB [41] have been reported. Further Li., *et al.* gave the mechanism of how RA protects mice from LPS/d-HGaln-induced acute liver injury by inhibiting MAPK'S/ NFκB and activating Nrf-2/HO-1 signaling pathways.

Xenobiotic metabolizing enzymes

RA along with other flavonoids has been shown to increase detoxification enzymes in rat liver [47]. It was done by examining the modulation phase I enzymes like cytochrome P450 (CYP) 1A, 2B, 2E1, 3A and phase II enzymes like GST, QR and UDP glucuronyl transferase (UGT). Activity of these enzymes were measured using specific substrates and thus used to find the effect of RA on them. Extract that contained CYP) 1A, 2B, 2E1 and GST (especially) RGST A3/A5, M1 and M2), QR and UGT. However, it has no effect on drug metabolizing enzymes probably because of the fact following oral administration, RA gets metabolized before reaching the liver and the resulting metabolites have no effect on the drug metabolizing enzymes. However in a recent study conducted by Kim., *et al.* [48], based on the comparison of the IC_{50} and Ki values, it wa suggested that RA might significantly inhibit the activities of the tested UGT'S (UGT1A1, UGT1A6, UGT B7, rather than CYP's, in clinical settings. This study could give a basis for future studies on clinical significant interactions between UGT substrate, drugs and gerbil medicines containing RA.

Cell proliferation

The effects of RA on Cell Proliferation was investigated both in rat activated hepatic stellate cells (HSC-T6) and normal CCC-HEL cells [30]. RA markedly decreased HSC-T6 cell growth rates in a dose and time dependent manner, but did not have any effect on CCC-HEL cells, demonstrating that it had selective growth inhibition of abnormal cells. It further suggested that the R A inhibitory effect on HSC-T6 proliferation might be associated with G1/S cycle arrest [30], concluding that RA inhibited proliferation of HSC-T6 without any significant toxicity and evident side effects on normal cells [30]. Inhibition of HSC's activation and proliferation along with induction of apoptosis is important in the prevention and treatment of hepatic fibrosis.

Liver regeneration

Removal of liver is a common treatment for liver fibrosis or tumor pts and the ability of the liver to regenerate is important for liver homeostasis. However Liver Regeneration is a very complicated process that involves multiple pathways that operate simultaneously and/or sequentially [50]. The potential of RA on liver Regeneration was studied by Lou., *et al* [40]. He observed that RA stimulated hepatocyte proliferation. Specifically activated the mTOR signaling pathway during liver regeneration and rescued ph impaired liver functions [40]. Hence concluding that RA stimulates hepatocyte proliferation via the mTOR signaling pathway and hence supported the potential usage of RA in liver Regeneration [40].

Conclusions

There has been an increased attention paid on natural products both in general and dietary supplements especially. This might be secondary to the fact that multiple studies imply that diets rich in phytochemicals have benefits in human health. RA is a diphenolic compound, found in many herbs and spices, which is regarded to be having potential pharmaceutical natural product. Hepatoprotective effect of RA via multiple mechanisms have been documented by multiple studies. The mechanisms include scavenging or decreasing activities like superoxide or peroxy nitrite, reduction of indicators of hepatotoxicity like AST, ALT, oxidized glutathione, lipid peroxidation along with enzyme activities that are related to antioxidant like catalase, glutathione peroxidase and superoxide dismutase. RA also inhibits hepatic stellate cell proliferation, TGFβ1, CTGF, and αSMA expression in cultured HSC's. Fibrosis grade is reduced, along with amelioration of biochemical indicator and histopathological morphology by RA. Further it has profound effects on inflammation and different inflammatory mediators. With the animal studies showing how RA has a hepatoprotective role. Further studies possibly will use this knowledge so that RA gets an established agent in liver pathologies after initial human studies substantiating these effects.

Bibliography

1. Calm PF., *et al.* "Recruitment of lymphocytes in the human liver". *Immunology and Cell Biology* 80.1(2002): 52-64.

2. Jaeschke H., *et al.* "Mechanism of hepatotoxicity". *Toxicological Sciences* 65.2 (2002) :166-176.

3. Lin SY., *et al.* "Hepatoprotective activities of rosmarinic acid against extrahepatic cholestasis in rats". *Food and Chemical Toxicology* 108 (2017): 214-223.

4. Taijri K and Shimuzu Y. "Recent advances in the management of pruritus in chronic liver diseases". *World Journal of Gastroenterology* 23.19 (2017): 3418.

5. Wasley A., *et al.* "Hepatitis A in the era of vaccination". *Epidemiologic Reviews* 28.11 (2006):101-111.

6. Guarino M., *et al.* "Direct contribution of epithelium to organ fibrosis:epithelial-mesenchymal transition". *Human Pathology* 40.10 (2009) :1365-1376.

7. Lie Y., *et al.* "Inhibiting connective tissue growth factor by si RNA prevents liver fibrosis in rats". *The Journal of Gene Medicine* 8.7 (2006): 889-900.

8. Fuji H and Kawada N. "Fibrogenesis in alcoholic liver disease". *World Journal of Gastroenterology WJG* 20.25 (2014): 8048-8054.

9. Rantala M and Van de Laar MJ. "Surveillance and epidemiology of hepatitis B and C in Europe: a review". *Eurosurveillance* 13.21 (2008): 18880.

10. He G and Katin M. "NFκB and STAT3-key players in liver inflammation and cancer stem cells". *Cell Research* 21.1 (2011): 159-168.

11. Petersen SM. "Rosmarinic acid". *Phytochemistry* 62.2 (2003): 121-125.

12. Fromenty B and Pessayre D. "Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity". *Pharmacology and Therapeutics* 67.1 (1995): 101-154.

13. Grimbert S., *et al.* "Decreased mitochondrial oxidation of fatty acids in pregnant mice: possibly relevance in development of acute fatty liver in pregnancy". *Hepatology* 17.4 (1993): 628-637.

14. Faubion WA and Gores GJ. "Death receptors to liver, biology and pathophysiology". *Hepatology* 21.1 (1992):1-4.

15. Miyoshi H., *et al.* "Hepatitis after bile duct ligario in the mouse involves Fas". *Gastroenterology* 17.3 (1999): 669-677.

16. Koop DK. "Oxidative and reductive metabolism by cytochrome P450 E1". *The FASEB Journal* 6.1 (1992):724-730.

17. Bonds SC. "Ethanol toxicity and oxidative stress". *Toxicology Letters* 63.1 (1992): 31-241.

18. Van de Bokemp M., *et al.* "Precision cut Liverslices, as a new model to study toxicity induced hepatic stellate cells activation in a physiological milieu". *Toxicological Sciences* 85.1 (2005): 632-638.

19. Shetty K., *et al.* "Food Biotechnology, second ed, CRC Press". Boca Raton (2005): 825-845.

20. Mitchelle Andrade Furtado EAF., *et al.* "Antimutagenicity of Rosmarinic acid in Swiss mice, evaluated by the micronucleus assay". *Mutation Research* 657.2 (2008):150-154.

21. Terao J., *et al.* "Absorption, metabolism, degradation, and urinary excretion of Rosmarinic acid intake of Perila Frutescens extract in humans". *European Journal of Nutrition* 44.1 (2005): 1-9.

22. McKay TBDL. "A review of the bioactivity and potential health of peppermint tea (Mentha Pipperita L)". *Phytotherapy Research* 20.8 (2006): 619-633.

23. Huang BKSS. "Rosmarinic acid inhibits angiogenesis and its mechanism of action n vitro". *Cancer Letters* 239.2 (2006): 271-280.

24. Takeda TMH., *et al.* "Rosmarinic acid and caffeic acid produce antidepressive effects in the forced swimming test in mice". *European Journal of Pharmacology* 49.3 (2002): 261-267.

25. Osakabe TYN., *et al.* "Rosmarinic acid inhibits epidermal inflammatory responses: anticarcinogenic effect of Perila Frutescens in the murine two stage skin model". *Carcinogenesis* 25.4 (2004): 549-557.

26. Takano H., *et al.* "Extract of Perila Frutescens, enriched for Rosmarinic acid, a polyphenolic, phytochemical, inhibit seasonal allergic rginoconjunctivitis in humans". *Experimental Biology and Medicine* 229.3 (2004): 247-254.

27. Morreau S., *et al.* "Antioxidant and antimucribial activities of rosemary extracts linked to their polyphenol composition". *Free Radical Research* 40.2 (2006): 223-231.

28. Ono K., *et al.* "Phenolic compounds prevent amyloid B protein oligomerization and synaptic dysfunctuins by site specific binding". *Journal of Biological Chemistry* 287.18 (2012): 15641-15643.

29. CB K., *et al.* "In vitro antioxidant properties of and phenolic composition of Salvia virgata Jacq from Turkey". *Journal of Agricultural and Food Chemistry* 56.7 (2008): 2369-2374.

30. Zhang JJ., *et al.* "Rosmarinic acid, inhibits apoptosis and induces proliferation of hepatic stellate cells". *Biological and Pharmaceutical Bulletin* 34.3 (2011): 343-348.

31. Blanco M., *et al.* "Chondrocyte apoptosis induced by nitric oxide". *The American Journal of Pathology* 146.1 (1995): 75-85.

32. Watanabe H., *et al.* "Hepatocellular oxidative DNA injury induced by macrophage derived nitric oxide". *Free Radical Biology and Medicine* 30.9 (2001):1019-1028.

33. Wang JG., *et al.* "Boucher Haynes. Nitric oxide mediates hepatocytic injury". *The American Journal of Pathology* 275 (1998): G1117-G1126.

34. Hodgeberg PJ., *et al.* "Further studies on lipid peroxide formation in isolate hepatocytes". *European Journal of Biochemistry* 59.2 (1975): 449-455.

35. Osakabe TYN., *et al.* "Rosmarinic acid, a major compound of Perila Frutescens reduces lipopolysaccharide (LPS) induced liver injury in D-galactosamine (D-Galn) sensitized mice". *Free Radical Biology and Medicine* 33.6 (2002):798-806.

36. Weber A., *et al.* "Hepatotoxicity and mechanism of action of haloalkanes: carbon tetra chloride as a toxicological model". *Critical Reviews in Toxicology* 33.2 (2003):105-136.

37. Chance A., *et al.* "Hydroperoxide metabolism in mammalian organs". *Physiological Reviews* 59.3 (1979): 527-605.

38. Domitrovic R., *et al.* "Rosmarinic acid ameliorates acute liver damage and fibrogenesis in carbon tetra chloride intoxicated mice". *Food and Chemical Toxicology* 51.1 (2013): 370-378.

39. Van de Bovenkamp M., *et al.* "Liver fibrosis in vitro cell culture models and precision cut liver slices". *Toxicology in Vitro* 21.4 (2007): 545-557.

40. Lou K., *et al.* "Rosmarinic acid stimulates liver regeneration through the mTOR pathway". *Phytomedicine* 23.131 (2016): 1574-1582.

41. Ramalho LNZ., *et al.* "Rosmarinic acid attenuates hepatic ischaemia and reperfusion injury in rats". *Food and Chemical Toxicology* 74 (2014): 270-278.

42. Caro AA and Cederbaum AI. "Oxidative stress, toxicology and pharmacology of CYP2E1". *Annual Review of Pharmacology and Toxicology* 44 (2004): 27-42.

43. Kunnukumara AB., *et al.* "Chronic diseases, inflammation, and spices, hpw are they linked". *Journal of Translational Medicine* 16.1 (2018):14.

44. Westra LM., *et al.* "The effect of antifibrotic drugs in rat precision cut fibrotic liver slices". *PLoS One* 9.4 (2014): e95462.

45. Svegliati Baroni G., *et al.* "Fibrogenic effect of oxidative stress on rat hepatic stellate cells". *Hepatology* 27.3 (1998): 720-726.

46. Lu C., *et al.* "Rosmarinic acid counteracts activities of hepatic stellate cells via inhibiting the ROS dependent MMP2 activity: Involvement of N rf2 antioxidant system". *Toxicology and Applied Pharmacology* 318 (2017): 69-79.

47. Fan C., *et al.* "Therapeutic approaches for ischemia-reperfision injury in the liver". *Journal of Molecular Medicine* 79.8 (1999): 577-592.

48. Kim SB., *et al.* "Metabolic interactions of rosmarinic acid with human cytochrome P450 monoxygenases and uridine diphosphate glucuronyl transferases". *Biomedicine and Pharmacotherapy* 110 (2019): 111-117.

49. Michalopoulis GK. "Liver regeneration". *Journal of Cellular Physiology* 213.2 (2007): 296-300.

50. Elufioye TO and Habtemariam H. "Hepatoprotective effects of rosmarinic acid: insight into its echanism of action". *Biomedicine and Pharmacology* 12 (2019): 108600.

Volume 4 Issue 6 September 2019