Antifibrotic Effect of Curcumin on Thioacetamide Induced Liver Fibrosis


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ABSTRACT:

The objective of the present study was to elucidate whether serum ATX activity might be a target for regulation of liver fibrosis and to evaluate the hepatoprotective and antifibrotic effect of curcumin in TAA induced liver fibrosis in rats. Therefore 40 healthy adult albino rats, divided into 4 groups (10 rats in each). Rats in the 2nd group received curcumin (500 mg/kg b. wt /orally every day), the 3rd group injected by thioacetamide (TAA) intraperioteneal (250 mg/kg b. wt) three times a week, the 4th group injected by TAA intraperioteneal (250 mg/kg b. wt) three times a weeks and received curcumin orally (500 mg/kg b. wt every day). The changes in body weight index and histopathological examination. In addition, selected biochemical parameters were also determined. The present study revealed that, oral supplementation of curcumin causing increase of liver weight index, autotaxin (ATX), HDL-c level and decrease of total protein, urea, creatinine and ammonia, total cholesterol, LDL-c and triacylglycerols. Treatment with TAA induced increase in the liver weight index, ATX, ALT, triacylglycerols, ammonia levels and decrease in serum proteins, urea, total cholesterol, HDL-c and LDL-c levels. Histopathological examination revealed severs necrosis, inflammatory cellular infiltration and nodules in TAA group. While the supplementation of rats with TAA and curcumin orally together resulted in increase in liver weight index, ATX, ALT, triacylglycerols levels and decrease in serum total protein, urea, total cholesterol, HDL-c, LDL-c concentration moreover, revealed mild inflammation and necrosis by histopathological examination. Conclusively, the use of curcumin ameliorated the effect of TAA induced liver fibrosis but cannot reach the normal levels.

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1. INTRODUCTION

The liver is a multifunctional vital organ with the primary role in maintenance of body homeostasis. Among the various liver functions are plasma protein synthesis (Tacke et al., 2009), production of hormones, processing dead red blood cells, detoxification (Yu et al., 2011), glucose and lipid metabolism (Liu et al., 2012).

Hepatic fibrosis is the wound response to chronic hepatic injury, including alcohol abuse, viral infection and cholestasis. It is characterized by excessive production and deposition of extracellular matrix (ECM) molecules. It has been established that hepatic stellate cells (HSC) are the primary ECM-producing cell type during hepatic fibrogenesis. So, that its activation, characterized by enhanced cell growth and over production of ECM, That is triggered by the release of mitogenic platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) from this activated cells and fibrogenic transforming growth factor (TGF-β1), mostly from Kupffer cells (Bataller and Brenner, 2005).

For the majority of organs and tissue the development of fibrosis involves a multitude of events and factors including proteins or peptides (profibrotic cytokine, chemokines, metalloproteinase, ect) (Wynn, 2007). But more recent data show significant involvement of phospholipid in the development of fibrosis, the phospholipids including platelet activating factor (PAF), phosphatidylcholine (PC) and lysophosphatidic acid (LPA) (Watterson et al., 2007). Lysophosphatidic acid (LPA), which formed from lysophosphatidylcholine by autotaxin (ATX), a secreted glycoprotein possessing both phosphodiesterase and lysophospholipase D activity (Tokumura et al., 2002), activates hepatic stellate cells, stimulates their contraction and inhibits their apoptosis (Iekeda et al., 2003). LPA has been implicated in certain human diseases such as arteriosclerosis (Siess and Tigyi, 2004) and cancer.
cell invasion (Mills and Moolenaar, 2003). It is likely that most of the LPA actions are explained by G-protein coupled receptors (GPCR) specific to LPA, although LPA was reported to activate the nuclear-type receptor PPARγ (peroxisome proliferative activating receptor gamma) (Mcintyre et al., 2003).

Autotaxin (ATX) was originally identified as a tumour cell autocrine motility factor towards malignant cancer cells and then purified from the conditioned medium of A2058 melanoma cells, where it elicits chemotactic and chemokinetic cellular responses at picomolar to nanomolar concentrations in a pertussis toxin sensitive manner (Stracke et al., 1992), and is widely expressed in tissues such as brain, placenta or high endothelial venules (Nakasaki et al., 2008). In mice with heterozygous loss of the ATX gene the LPA plasma concentration was half of that in wild type mice, whereas complete knock-out of ATX is embryonic lethal due to blood vessel abnormalities (Tanaka et al., 2006).

Recently, a connection between liver fibrosis and serum or plasma LPA and ATX emerged in patients with chronic HCV infection (Nakagawa et al., 2011). ATX activity or protein levels are also elevated in patients with malignant diseases including pancreatic cancer, follicular lymphoma and HCC (Wu et al., 2010).

Liver cancer and hepatitis are the most prevalent and serious global public health problems. Hepatitis or liver inflammation is caused by the hepatitis viruses (Sun and Karin, 2008). The World Health Organization (WHO) estimates that about one-third of the world’s population is infected with hepatitis B virus (HBV) during a lifetime, while about 17.5% remain chronically infected. According to WHO statistics, an estimated 5% of all humans in the world are HBV carriers and a quarter of those would develop serious liver diseases such as chronic hepatitis, cirrhosis, and primary HCC. It is now known that HBV infection accounts for more than 1 million deaths every year (Sun and Karin, 2008), while in Egypt HCV has a very high prevalence and of a high morbidity and mortality with averaging 15%-25% in rural communities, (Khairy et al., 2013).

Approximately 20% of Egyptian blood donors are anti-HCV positive. Egypt has higher rates of HCV than neighboring countries as well as other countries in the world with comparable socioeconomic conditions and hygienic standards for invasive medical, dental, or paramedical procedures. And the most important cause in the high prevalence of HCV was in the past the parenteral therapy for schistosomiasis. Co-infection with viral hepatitis, either HBV or HCV is very common since the regions with a high prevalence of schistosomiasis usually have a high endemicity of chronic viral hepatitis as well. An important cause of the high exposure to HCV was the establishment of a large reservoir of infection as a result of extensive schistosomiasis control programs that used intravenously administered tartar emetic 20–50 years ago (Frank et al., 2000).

Numerous medical studies have demonstrated the important role of nuclear factor-kappa B (NF-kB) signaling pathways (Lee et al., 2010) and oxidative stress (Tanaka et al., 2013) in the pathogenesis of liver diseases and have proved the ameliorative role of dietary antioxidants (Nabavi et al., 2012).

Antioxidants are chemicals that interact with/and neutralize free radicals, thus preventing them from causing damage and producing the diseases. The body produces some of the antioxidants (endogenous). However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs such as fruits, vegetables, and grains are rich sources of dietary antioxidants. The dietary antioxidants include betacarotene, lycopene, and vitamins A, C, and E (Davis et al., 2012).

Curcumin is a biphenyl compound possesses antioxidant, anti-inflammatory, wound healing and antimicrobial activities (Maheshwari et al., 2006) and chemopreventive potential for several cancers by blocking steps in the carcinogenesis (Chen et al., 2006). It is a bright yellow-colored phenolic compound that was initially isolated from Curcuma longa L. (turmeric) rhizomes in 1815 (Gupta et al., 2013). The genus Curcuma is a member of Zingiberaceae family, growing in India, Southeast Asia, and other tropical areas (Martin et al., 2012). Also, it acts on multiple targets and inhibits activation of key cell signaling mediators including NFκB, AP-1, Cox-2, MMP9 and EGFR (Shishodia et al., 2007), the aim of study was to elucidate whether serum ATX activity might be a target for regulation of liver fibrosis and to evaluate the hepatoprotective and antifibrotic effect of curcumin in TAA induced liver fibrosis in rats.

2. MATERIAL AND METHODS

The present study was approved ethical committee of faculty of veterinary medicine, faculty of veterinary medicine, Alexandria Universty

2.1. Preparation with Curcumin: Curcumin was dissolved in 10 % tween 20 (Salama et al., 2013).

2. 2. Experimental animal:
A total of 40 male albino rats were purchased from animal health institute- Doki (3 months old) weighted 120 -160 gm were kept in clean and disinfected metal cages (10 rats/cage) commercial diet and water provided ad libitum. Rats were subjected to the natural photoperiod of 12 hrs light: dark cycle throughout the experiment period (8 weeks). All animals received a commercial diet for 2 weeks before the start of the experiment for adaptation and ensure normal growth and behavior. Rats were maintained in their receptive groups for 8 weeks, monitored closely every day and weight every week.

2. 3. Induction of liver fibrosis: Liver fibrosis was induced in rats by intraperitoneal administration of TAA in a dose of 250 mg /kg body weight. It was dissolved in distilled water (Aydin et al., 2010). The dose was determined according to the animal weight on the day of injection.

2. 4. Experimental design and sampling
Rats were divided into 4 groups.
1st Group control group
2nd Group curcurmin group treated with curcumin which was given orally by stomach tube in a dose of 500 mg/kg body weight every day for 8 weeks.
3rd Group TAA group: received intraperitoneal injection of TAA in a dose of 250 mg/kg body weight three time a week for 8 weeks
4th Group TAA and curcurmin group received simultaneously intraperitoneal injection of TAA in a dose of 250 mg/kg body weight three times a week together with curcumin in a dose of 500 mg/kg body weight given orally by stomach tube every day for 8 weeks.

At the end of the experiment, the animals weighed then the blood was collected from the orbital plexuses of the eye into clean tubes then left to be clotted, the animals were sacrificed by decapitation; all blood samples centrifuged at 3000 rpm for 20 min at 4 °C to obtain serum then samples were transferred to epindorff tubes and stored at -20 °C until analysis. Tissue sample was collected by decapitation, liver was removed, washed with ice cold saline then plotted with filter paper; then weighted for determination of liver weight index (Salama et al., 2013), the liver of each animal was fixed in10 % formalin solution for histopathological examination (Drury and Wallington, 1980). Serum samples for the determination the activites of Autotaxin (ATX) (Sajdok et al., 1995), alanine aminotransferase (ALT) (Young, 1995). In addition, total protein (Burtis, 1999), albumin (Doumas, 1971), globulin (Coles, 1974), ammonia (Kontizer and Vogit, 1963), urea (Rock et al., 1987), creatinine (Henry, 1984), total cholesterol (TC) (Allain et al., 1974), Triacylglycerol (TAG) (Stein, 1987), serum High density Lipoprotein cholesterol (HDL-c) (Burstein et al., 1980) and serum low density lipoprotein cholesterol (LDL-c) (Bauer, 1982).

2. 5. Statistical analysis
Statistical analysis was done by one way analysis of variance (ANOVA). Spss programme was used to perform all calculation and analysis. Data were expressed as means ± standard error (Means ± SE). P<0.01 was set as statistical significance (SAS, 2004).

3. RESULTS
Table (1) revealed that there is significant increase in serum activity of ATX, ALT and ammonia concentration, while a decrease in serum urea concentration and in 3rd G, while in 4th G there were a slight increase in ATX, ALT activity and significant decrease in urea concentration as compared to the control group.

Table (2) revealed that there are significant decrease in serum total protein, albumin and globulin concentration and significant increase in liver weight index in 3rd G, while in 4th G show significant decrease in total protein concentration with a significant increase in the liver weight index as compared to control group.

Table (3) there are a significant increase in serum triacylglycerol concentration and significant decrease in serum total cholesterol, HDL-c and LDL-c concentration in 3rd G, while in 4th G there are a significant decrease in serum total cholesterol, HDL-c and LDL-c concentration as compared to control group.

The liver of 1st G and 2nd G showed normal histological appearance of blood vessels and hepatocytes (fig.1 &2). The microscopical findings of the liver of 3rd G exhibited congestion of blood vessel with mild edema which characterized by faint eosinophilic albuminous fluid and severe hydropic degeneration where the cells are swollen, cytoplasm is replaced by clear fluids and the nucleus not affected either in shape or location (fig. 3) beside necrotic hepatocytes with inflammatory cells infiltrations. Moreover, inflammatory cells infiltrations in the portal areas (fig. 4), were noticed the noticeable lesion 4th G congestion of blood vessel (fig. 5) and mild inflammatory cells infiltrations in the portal areas (fig. 6).
Table 1: Effect of curcumin and/or TAA on ATX activity (µmol/ml/min), ALT (U/L), Ammonia (µg/dl), Urea and Creatinine (mg/dl).

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Mean±S.E</th>
<th>ATX (µmol/ml/min)</th>
<th>ALT (U/L)</th>
<th>Urea (mg/dl)</th>
<th>Ammonia (µg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st G</td>
<td>Autotaxin</td>
<td>4.60±0.12</td>
<td>11.83±0.7d</td>
<td>38.20±3.5a</td>
<td>44.33±3.1b</td>
<td>0.86±0.05a</td>
<td></td>
</tr>
<tr>
<td>2nd G</td>
<td></td>
<td>5.07±0.06b</td>
<td>12.00±1.5d</td>
<td>33.80±2.0b</td>
<td>37.00±4.7c</td>
<td>0.74±0.05c</td>
<td></td>
</tr>
<tr>
<td>3rd G</td>
<td></td>
<td>7.20±0.17a</td>
<td>27.83±2.3a</td>
<td>27.60±3.1c</td>
<td>54.33±4.6a</td>
<td>0.82±0.09b</td>
<td></td>
</tr>
<tr>
<td>4th G</td>
<td></td>
<td>5.24±0.02b</td>
<td>15.50±1.4b</td>
<td>30.80±2.4b</td>
<td>44.33±5.6b</td>
<td>0.86±0.07a</td>
<td></td>
</tr>
</tbody>
</table>

1st (control), 2nd (curcumin), 3rd (TAA), 4th (TAA + curcumin). Value are mean ± S.E

Means within the same column of different litters are significantly different at (P < 0.01).

Table 2: Effect of curcumin and/or TAA on serum total protein, Albumin, Globulin concentration (g/dl) and liver weight index:

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Mean±S.E</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>Liver weight index</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td></td>
<td>5.18±0.41a</td>
<td>3.52±0.24a</td>
<td>1.66±0.27a</td>
<td>2.98±0.11a</td>
<td></td>
</tr>
<tr>
<td>GII</td>
<td></td>
<td>4.82±0.23b</td>
<td>3.40±0.14a</td>
<td>1.43±0.23ab</td>
<td>3.16±0.17a</td>
<td></td>
</tr>
<tr>
<td>GIII</td>
<td></td>
<td>2.67±0.32d</td>
<td>1.74±0.26b</td>
<td>0.94±0.19b</td>
<td>5.42±0.2</td>
<td></td>
</tr>
<tr>
<td>GVI</td>
<td></td>
<td>4.72±0.34c</td>
<td>3.51±0.18a</td>
<td>1.21±0.20b</td>
<td>3.98±0.48b</td>
<td></td>
</tr>
</tbody>
</table>

1st (control), 2nd (curcumin), 3rd (TAA), 4th (TAA + curcumin). Value are mean ± S.E

Means within the same column of different litters are significantly different at (P < 0.01).

Table 3: Effect of curcumin and/or TAA on cholesterol, triacylglycerol, HDL-c, LDL-c (mg/dl) level

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Mean±S.E</th>
<th>Cholesterol (mg/dl)</th>
<th>Triacylglycerol (mg/dl)</th>
<th>HDL-c (mg/dl)</th>
<th>LDL-c (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td></td>
<td>91.20±2.46a</td>
<td>99.60±5.81b</td>
<td>47.80±2.82b</td>
<td>23.48±1.65a</td>
<td></td>
</tr>
<tr>
<td>GII</td>
<td></td>
<td>81.40±3.88b</td>
<td>95.20±5.95c</td>
<td>56.60±4.60a</td>
<td>5.76±1.19d</td>
<td></td>
</tr>
<tr>
<td>GIII</td>
<td></td>
<td>56.80±3.10d</td>
<td>117.60±13.23c</td>
<td>60.00±1.82d</td>
<td>9.22±3.64c</td>
<td></td>
</tr>
<tr>
<td>GVI</td>
<td></td>
<td>73.80±5.80c</td>
<td>101.00±6.78b</td>
<td>35.80±3.37c</td>
<td>15.80±4.97b</td>
<td></td>
</tr>
</tbody>
</table>

1st (control), 2nd (curcumin), 3rd (TAA), 4th (TAA + curcumin). Value are mean ± S.E

Means within the same column of different litters are significantly different at (P < 0.01).

Fig. 1, 2. The microscopical findings of the liver of 3rd G exhibited congestion of blood vessel with mild edema which characterized by faint eosinophilic albuminous fluid and severe hydropic degeneration where the cells are swollen, cytoplasm is replaced by clear fluids and the nucleus not affected either in shape or location.
DISCUSSION

Hepatic fibrosis is traditionally defined as a progressive pathological process involving multiple cellular and molecular events that lead to deposition of excess matrix proteins in the extracellular space including collagen (Iredale, 2008).

In our study thioacetamide (TAA) was chosen for this experiment because it consistently produces liver cirrhosis in rats with histological appearance that is more like to human cirrhosis (Li et al., 2002). Besides, oral and intraperitoneal administrations of TAA are both established methods in the generation of fibrosis and cirrhosis models in rats (Zhao et al., 2002). TAA is a sulfur containing compound that is necrogenic (Landon et al., 1986) and carcinogenic (Kizer et al., 1985). It is commonly used for inducing fulminant hepatic failure (Bruck et al., 1999) and liver cirrhosis in animal models (Li et al., 2002). During the biotransformation of TAA, both flavin-containing monooxygenase (FMO) (Malvaldi et al., 1984) and cytochrome P450 (Lee et al., 2003) reduce di-oxygen to superoxide anion, which is then catalyzed (Ekström et al., 1989) to form hydrogen peroxide (H₂O₂). Therefore, biotransformation of TAA precedes oxidative damage associated liver injury.

In our current study, hepatocyte necrosis and inflammation induced by TAA result in significant increase in serum ALT activity (which are commonly used as biomarker for liver injury) as ALT present in cytoplasm of the cells with the occurrence of necrosis and inflammation it leak in the blood stream causing their elevation (Abul et al., 2010), also, show histopathological changes that manifested by portal inflammation, sever necrosis, septal cirrhosis and cirrhosis, minimally distorted architecture. Liver damage including nodular cirrhosis, liver cell proliferation, resulted in pseudolobules and pranchymal cell necrosis (Sadasivan et al., 2006), also the increase of ATX activity due to increased production or decrease its clearance, also it associated with the stage of fibrosis (Peli et al., 2014). The extent of portal hypertension in patient suffering from esophageal varices or portal hypertensive gastropathy showed significant higher ATX serum activity, higher intrahepatic resistance and portal hypertension lead to increase ammonia hyper ammonimia and thereby contributing the development of hepatic encephalopathy (Ciec´ko-Michalska et al., 2012),

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also the urea concentration decrease due to inhibition of urea cycle by inhibition of 2-oxoglutarate formation, that resulted in increase of ammonia and decrease urea concentration (Cascales et al., 1979). The necrosed cell become unable to metabolized neither lipid nor protein, lead to decrease the total proteins. Increase triacylglycerol (Ismail et al., 2009), decrease in total cholesterol and HDL-c and LDL-c (Tripathi et al., 2003).

Curcumin has hepatoprotective and antifibrotic effect as its administration protect the liver from damage by reducing ALT activity (Fu et al., 2008), that confirmed by histopathological studies (Elhaggagy et al., 2014), so the liver become able to make clearance of ATX from circulation and inhibit mRNA expression (Singh and Misra, 2009), decrease the blood ammonia by stimulating their detoxification and urea cycle as it occur in normal hepatocyte (Huang et al., 2015), and maintain normal metabolism of protein and lipids (Kheder and Kheder, 2014), it decrease triacylglycerol and phospholipid due to decrease free fatty acid synthesis by action of curcumin (Rukkumani et al., 2002) resulted in decreasing ATX.

Also, curcumin ameliorate the fibrotic effect of TAA, as it maintain the normal liver weight index that agree with (Zhang et al., 2014).

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