ORIGINAL ARTICLES

Counteracting Methionine Choline-Deficient Diet-induced Fatty Liver by Administration of Turmeric and Silymarin

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is currently the most common liver disease in the world. Feeding rats a methionine choline-deficient (MCD) diet had served as a nutritional model of nonalcoholic steatohepatitis (NASH). In this study, we aimed to evaluate the probable effect of turmeric and silymarin with high polyphenolic content on experimental nonalcoholic steatohepatitis. Thirty-two male Sprague-Dawley rats were divided into equal four groups: group 1, as control was fed on methionine choline-sufficient (MCS) diet; group 2 was fed on MCD diet; group 3 was fed on MCD diet supplemented with 1% turmeric and group 4 was fed on MCD diet supplemented with 1% silymarin. MCD diet-fed rats developed steatohepatitis at week 6 due to hepatic lipid accumulation. MCD diet induced hepatic damage was manifested by a significant increase in serum activities of the enzymes [alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALK-P)] in addition to products of lipid peroxidation (thiobarbituric acid reactive substances, TBARS). A significant decrease in reduced glutathione (GSH) content was observed. Treatment of rats with turmeric and silymarin reversed these altered parameters towards normal values. Histological evaluation of the liver samples revealed that turmeric and silymarin had abated the severity of steatosis among the MCD-fed rats. These results suggest that turmeric and silymarin protect against NAFLD by limiting hepatic lipid accumulation and liver injury mainly due to their antioxidant and anti-inflammatory activities.

Key words: methionine choline-deficiency, turmeric, silymarin, nonalcoholic steatohepatitis

Introduction

The liver regulates many important metabolic functions, so the hepatic injury is associated with distortion of these metabolic functions (Wolf, 1999). Nonalcoholic fatty liver disease (NAFLD) is a broad spectrum of liver abnormalities ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and in some patients this is followed by progression to fibrosis, cirrhosis and hepatocarcinoma (Zafrani, 2004).

Nonalcoholic fatty liver disease is characterized by liver damage similar to that caused by alcohol, but without a history of ingesting significant quantities of alcohol (Preiss and Sattar, 2008). Methionine choline-deficient (MCD) diets have been used to study liver disease. Rodent fed a MCD diet will develop measurable hepatic steatosis by 2-4 weeks, which progress to inflammation and fibrosis shortly thereafter (Weltman et al., 1996).

Many factors contribute to herbal medicine's appeal, supporters of herbal medicine claim that herbs may both treat and prevent diseases, this adds to a deep belief that these treatments are safe because they are "natural" and fit into the image of a gentle and therefore, harmless alternative to conventional medicine (Stickel and Schuppan, 2007).

Turmeric (Curcuma longa) is a perennial herb, has been used as a spice and coloring agent in foods, current traditional Indian medicine claims to use curcuma longa powder against biliary disorders, cough, diabetic wounds and hepatic disorder. Curcumin is a major ingredient in turmeric (Fig. 1), being responsible for its biological action including antioxidant, anti-inflammatory agent with hepatoprotective, anti-carcinogenic and antimicrobial properties (Limtrakul et al., 2004).

Silymarin, a flavonolignan from milk thistle (Silybum marianum) plant is used almost exclusively to treat liver, spleen and gallbladder disorders (Abenavoli et al., 2010). Silymarin major constituents are the flavonoids silibinin, isosilibinin, silidianin, and silichristin (Fig. 2), of which silibinin is the biologically most active compound and used for standardization of pharmaceutical products (Pradhan and Girish, 2006).

We aimed to investigate the effect of turmeric and silymarin on the biochemical and histological changes of fatty liver caused by methionine choline-deficient diet intake in rats.

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Fig. 1: Chemical structures of curcuminoids of *Curcuma longa* extract.

Fig. 2: Structures of the components of silymarin.

**Materials and Methods**

*Supplements and chemicals:*

Turmeric powder was purchased from local market, Cairo, Egypt. Silymarin was obtained from Chemical Industries Development (CID) Company; Giza, Egypt in the form of capsules, each capsule contains 140 mg. All other chemicals were of analytical quality grade and purchased from commercial sources.

*Animals:*

Thirty-two male Sprague-Dawley rats weighing 90-100g were obtained from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt). Upon arrival, the rats were allowed to acclimatize for one week, during which they were fed a control basal diet prepared in accordance with AIN-93M formulation (Reeves et al., 1993) and water *ad libitum*. The animals were housed in stainless steel cages at a temperature and light-controlled room.

*Diets:*

Two diets were used in this study, control basal, that was methionine choline-sufficient (MCS) diet and methionine choline-deficient (MCD) diet. Ingredient of control diet (g/100g diet): corn starch, 61.94; casein, 14; sucrose, 10; corn oil, 4; cellulose, 5; mineral mixture, 3.5; vitamin mixture, 1; DL methionine, 0.3; choline chloride, 0.25 and tert-butylhydroquinone, 0.008.
In methionine choline-deficient diet, DL methionine and choline chloride were excluded.

Experimental design:

After one week acclimatize period on a control diet rats were divided into four groups (8 rats for each). The first group (MCSD) served as normal control and kept on control diet. The second group (MCDD) was fed on MCD diet to induce liver steatosis (Weltman et al., 1996). Rats of the third group (MCDD + Turmeric) kept on MCD diet supplemented with 1% turmeric (Pulla Reddy and Lokesh, 1994). However rats of the fourth group (MCDD + Silymarin) fed on MCD diet supplemented with 1% silymarin (Sarhan et al., 2007). Rats had free access to food and water for the entire study period (6 weeks) and were weighed every week.

Sample collection:

On completion of the experiment, all rats were fasted overnight before euthanasia. The abdominal cavity was opened and blood was withdrawn from hepatic portal vein into tubes to separate serum by centrifugation and kept at -20°C for biochemical analyses.

Livers were dissected out rinsed in cold isotonic saline solution, dried by blotting between filter papers and weighed. Fatty liver was initially diagnosed by altered coloration (pink color due to lipid accumulation) and later confirmed by histological analysis (criteria described below). A portion of the liver was fixed in 10% buffered formalin, while the remaining liver tissue was stored frozen at -20°C for further analysis.

Biochemical analysis:

Serum alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALK-P) activities were measured spectrophotometrically using kits from Analyticon, Germany.

Tissue homogenate and supernatant were prepared from liver for assessment of the following: Lipid peroxidation levels in liver homogenate were measured as thiobarbituric acid reactive substances (TBARS) following the method described by Uchiyama and Mihara (1978). The supernatant was analyzed for reduced glutathione (GSH) by the method of Beutler et al. (1963).

Serum total protein (TP) and albumin were assayed using colorimetric kits (Analyticon, Germany). Total lipids (TL), triacylglycerols (TAG) and total cholesterol (TC) were measured in serum using total lipid-test, triacylglycerols-E-test and cholesterol-E-test kits (Analyticon, Germany), respectively.

For liver lipid analysis, total hepatic lipids were extracted with a mixture of chloroform: methanol (2:1) and measured according to Folch et al. (1957). Liver TAG and TC were measured enzymatically as described above; phospholipids were measured using commercial kit.

Liver histological examination:

Liver sections were fixed in 10% buffered formalin (pH 7.4), dehydrated in graded ethanol, cleared in xylene and embedded in paraffin. Sections 4-5 µ thick were prepared, stained with Hematoxyline and Eosin (H&E) and examined under light microscopy at 400 X magnification (Bancroft et al., 1996).

Statistical analysis:

Significance of differences was tested by one-way ANOVA. Fisher's Protected Least Significant Difference procedure was used for Post Hoc testing. SPSS statistical package version 11.0 was used to perform all calculations. Differences were considered significant if P <0.05. Data were expressed as mean ± SD.

Results:

Effect of MCD diet on rats biological characteristics:

After 6 wk of methionine choline-deficient diet, rats weighed less than control (Table1). Such a loss of weight is a common feature of the MCDD. Liver tissues were taken and weighed, and to take into account those differences in body mass, tissue mass was expressed per 100g of body mass. MCDD-fed rats showed a 39% elevation in liver relative mass (hepatosomatic index). Whereas turmeric and silymarin treatment of MCD diet protocol decreased the liver index significantly when compared with the MCDD group (P <0.05) and brought this parameter back to values very similar to that observed in the control group.
Effect of turmeric and silymarin on serum protein and lipids as well as hepatic lipids:

Rats fed MCD diet exhibited several metabolic abnormalities. First, serum total protein and albumin levels were significantly lower than in control (MCSD) rats. Second, the rats developed hypolipidemia and hepatic steatosis, indicated by about 40% decrease in serum total lipids, triacylglycerols and total cholesterol, with a 2.5 fold increase in hepatic triacylglycerols and total cholesterol concentrations compared with MCS control rats (Table 2).

Depriving rats of methionine and choline for 6 weeks caused a marked reduction in hepatic phospholipids content. This was accompanied by a significant decrease in serum triacylglycerols concentration. In rats receiving MCDD plus turmeric or silymarin, serum total protein, albumin and lipids were markedly (P<0.05) elevated in comparison with rats receiving MCDD only. On the other hand, turmeric or silymarin resulted in a reduction of hepatic TAG concentration to 24% and 27%, respectively also TC concentration to 56% and 46%, respectively of MCDD fed rats.

Effect of turmeric and silymarin on liver injuries:

The ALT, AST, GGT and ALK-P were determined to assess the liver function. As shown in table 3, MCD diet-fed rats had elevated sera ALT, AST, GGT and ALK-P levels to the extent of 72%, 112%, 117% and 107%, respectively compared to the control rats (P<0.05). However, treatment with turmeric and silymarin markedly reduced these levels to 40%, 9%, 42% and 7% by turmeric and to 39%, 9%, 27% and 16% by silymarin, respectively compared to MCD diet-fed rats, implying that turmeric and silymarin had executed a protective effect against the MCD diet-induced liver injuries.

### Table 1: Biological data of rats fed MCS or MCD diet for 6 weeks.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MCSD</th>
<th>MCDD</th>
<th>MCDD + Turmeric</th>
<th>MCDD + Silymarin</th>
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<tbody>
<tr>
<td>Final body weight (g)</td>
<td>139.50 ± 17.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>118.25 ± 12.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>120.63 ± 13.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>128.75 ± 14.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>4.31 ± 0.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.93 ± 0.43&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.08 ± 0.97&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.31 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hepatosomatic index (g%)</td>
<td>3.02 ± 0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.20 ± 0.53&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.38 ± 0.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.73 ± 0.32&lt;sup&gt;d&lt;/sup&gt;</td>
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</table>

Data are means ± SD for 8 rats.

### Table 2: Biochemical serum and liver parameters of rats fed MCS or MCD diets for 6 weeks.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MCSD</th>
<th>MCDD</th>
<th>MCDD + Turmeric</th>
<th>MCDD + Silymarin</th>
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<tr>
<td>Serum Total protein (g/l)</td>
<td>44.79 ± 3.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.19 ± 2.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>42.24 ± 2.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.95 ± 1.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>23.64 ± 1.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.01 ± 0.50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23.59 ± 1.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.43 ± 1.11&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total lipids (g/l)</td>
<td>4.48 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.69 ± 0.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.42 ± 0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.54 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Triacylglycerols (mmol/L)</td>
<td>1.27 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.75 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.05 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.04 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
<td>2.27 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.29 ± 0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.05 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.23 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
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Data are means ± SD for 8 rats.

### Table 3: Liver enzymes activities and antioxidant status of rats fed MCS or MCD diets for 6 weeks.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MCSD</th>
<th>MCDD</th>
<th>MCDD + Turmeric</th>
<th>MCDD + Silymarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (µkat/L)</td>
<td>0.36 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.62 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.37 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.38 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>AST (µkat/L)</td>
<td>0.51 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.08 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.98 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.98 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>GGT (µkat/L)</td>
<td>0.12 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.26 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.15 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.19 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALK-P (µkat/L)</td>
<td>0.72 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.49 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.38 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.25 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>TBARS (mmol/g)</td>
<td>2.21 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.55 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.53 ± 0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.51 ± 0.12&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH (mg/g)</td>
<td>17.10 ± 0.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.20 ± 0.30&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.30 ± 0.30&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14.1 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
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</table>

Data are means ± SD for 8 rats.

Means in a row without common letter differ, p <0.05.
Effect of turmeric and silymarin on hepatic oxidative status:

In order to explore the effect of turmeric and silymarin on oxidative stress status, liver TBARS and GSH contents were assessed. After 6 weeks of MCD diet feeding, the TBARS level in liver homogenate was greatly increased compared to MCS-fed group (3.55 mmol/g versus 2.21 mmol/g). Lipid peroxidation was efficiently counteracted by the treatment with 1% of turmeric and silymarin as compared with the untreated MCD diet-fed rats. The enhanced TBARS level was associated with a reduction in the GSH content in MCD diet-fed group by 46%, compared to control group. However, as shown in table 3, administration of turmeric and silymarin significantly increased the GSH content by 45% and 53%, respectively compared to untreated MCD diet-fed rats.

Effect of turmeric and silymarin on histopathological examination:

Liver sections of control group exhibited normal global histological features (Fig. 3A). In contrast, feeding MCD diet for 6 weeks caused a marked accumulation of fat in hepatocytes revealed a macro-vesicular steatosis (Fig. 3B). Treatment with turmeric (Fig. 3C) and silymarin (Fig. 3D) resulted in a general improvement in the grade of steatosis associated with the MCD diet, silymarin treatment was more beneficial than turmeric.

![Fig. 3: Histological appearance of the liver samples from rats fed diets (MCS, MCD, MCD + turmeric and/or MCD + silymarin). Liver sections were stained with H & E X 400. (A) Control group had normal hepatic lobule, (B) MCD group showed kupffer cells activation and cytoplasmic vacuolization of hepatocytes, (C) MCD + Turmeric group had small vacuoles in the cytoplasm of some hepatocytes and (D) MCD + Silymarin histologically look normal.](image)

Discussion:

Methionine choline-deficient feeding has become popular in recent years as a model of hepatic steatosis and steatohepatitis (Weltman et al., 1996). With the rising interest in complementary and alternative medicine, notably in the western hemisphere, several natural products have raised interest for their potential beneficial effects in liver disease. Among such attractive novel therapeutic possibilities are milk thistle and its major active compounds (Ligeret et al., 2007). Also, curcumin, a yellow coloring ingredient of the spice turmeric has been used in indigenous herbal medicine as a novel therapeutic strategy for nonalcoholic steatohepatitis (Anand et al., 2008).

These substances have caught our interest because of their various properties namely antioxidant, hepatoprotective and mitochondrial protective properties.

As has been reported previously, MCD feeding provoked significant weight loss attributed to a diet-induced increase in metabolic rate (Leclereq et al., 2000). Importantly, unlike human or other diet-induced rodent models of NAFLD, rodent fed MCD diets lose weight due to a vastly lower caloric intake and do not become insulin resistant, this is in contrast to the typical human with NASH, who is obese and insulin resistant (Rinella and Green, 2004).
Most people with NASH feel well and are not aware they have a liver problem, liver enzymes are usually elevated, especially GGT, ALT and AST (Sakbuja and Malhotra, 2006).

In the present study, we demonstrated that the sequence of pathogenic events during feeding the MCD diet involves early lipid accumulation and lipid peroxidation in hepatocytes, followed by liver cell injury and inflammation (increased liver enzymes activities) and eventually steatohepatitis.

Methionine and choline deficiency for 6 weeks caused a marked reduction in hepatic phospholipids concentration. This was accompanied by a significant decrease in serum TAG concentration, which was, presumably attributable to a phospholipids-related defect in hepatic TAG secretion. A side from its role as a methyl donor, choline is needed for synthesis of the phospholipids in cell membranes, cholinergic neurotransmission, transmembrane signaling and lipid-cholesterol transport and metabolism (Zeisel and Blusztajn, 1994).

Low serum TAG level in MCD-fed rats was accompanied by reciprocally high concentration of hepatic TAG. The decrease in serum TAG found here may be attributed to impaired mobilization from liver, in agreement with observations of triacylglycerols accumulation in liver. Also liver weight-body weight ratios were high in MCD-fed rats, indicative of steatosis.

Elimination of methionine and choline from the diet causes fat to accumulate rapidly within the liver (Leclereq et al., 2000). In this situation, steatosis is the result of impaired hepatic triacylglycerol secretion, which in turn is attributed to defective assembly of VLDL. Methionine and choline are important precursors of phosphatidylcholine, the principal phospholipids comprising the outer coat of VLDL particles. When these nutrients are in short supply, VLDL production is impaired and triacylglycerols accumulate in hepatocytes (Yao and Vance, 1988).

As reported previously, liver cholesterol concentration was also reduced with turmeric intake (table 2). Babu and Srinivasan (1997) have suggested that such a cholesterol-lowering effect could be mediated by the stimulation of hepatic cholesterol - 7α hydroxylase activity.

The finding that curcumin reduces hepatic fat suggests that it lowers the fatty acid synthesis: oxidation ratio. Curcumin activates a key fatty acid oxidizing enzyme, acyl-CoA oxidase (Asai and Miyazawa, 2001), a deficiency of which can lead to hepatic steatosis (Yeon et al., 2004) this might be one way curcumin prevents lipid accumulation.

According to our data, Haddad et al. (2011) found that treatment with silibin was very efficient at reversing the progression of NASH. Indeed, accumulation of fat in the liver was significantly reduced, as indicated by the improved histological grade of steatosis and by the normalization of liver weight, liver index as well as plasma lipid levels.

The previous findings coincided with a significant increase in serum aminotransferases, GGT and ALK-P activities. These observations in MCDD rats were supported by previous studies reporting a relation between liver damage and elevation of serum ALT and AST (Grattagliano et al., 2000; George et al., 2003).

In our opinion, damage to cellular lipids may result in structural alterations, such as membrane fluidity and fragility and in functional alterations, which led to leakage of the enzymes and consequently increase their levels.

Our data were in harmony with the results of Vizzutti et al. (2010) and Aghazadeh et al. (2011) who stated that the increase in serum ALT caused by the MCD diet was significantly reduced by curcumin after 4 weeks and a significant reduction was observed in the sera ALT and AST by the treatment with the *silybum marianum* extract.

In our study, turmeric and silymarin caused apparent improvement in liver functions by restoring total protein and albumin levels that were decreased by MCD diet. The structural similarity of silymarin to steroid hormones is believed to be responsible for its protein synthesis facilitatory actions. Silymarin can enter inside the nucleus and act on RNA polymerase enzymes resulting in increased ribosomal formation. This in turn hastens protein and DNA synthesis. This action has important therapeutic implications in the repair of damaged hepatocytes and restoration of normal functions of liver (Basiglio et al., 2009).

Hypoalbuminemia is known as metabolic syndrome X, this syndrome is also associated with NAFLD (Reaven, 1994), and the hypoalbuminemia was due to the decrease of synthesis by liver due to hepatocellular injury or hepatic inflammation. However, turmeric administration significantly increased serum albumin and total protein suggesting its immunopromoting effect.

Methionine and choline deprivation blocks hepatic lipid secretion, thus, in animals fed on MCD diet, any fatty acid that are either taken up by or produced within the liver, if not required for cellular metabolism, are trapped within hepatocytes. Under MCD fed conditions, accumulation of lipids was associated with liver cell damage and lysis. On the basis of our results, it is conceivable that this event could drive, at least in part, from an alteration of the oxidative balance in fatty liver. The lack of methionine reduces glutathione synthesis and impairs the antioxidant defense against free radicals attacks, also choline deficiency is reported to induce certain level of lipid peroxidation of the polyunsaturated fatty acids contained in the mitochondrial and nuclear membranes (Ghoshal and Farber, 1993). However, all of the most widely used experimental models of liver...
Steatosis (caffeine, lipotrope-deficient diet and alcohol) are associated with increased generation of reactive oxygen species (ROS) (Dianzani et al., 1991; Ghoshal and Farber, 1993; Letteron et al., 1996). Thus, steatosis appears to be associated with oxidative events regardless of the specific cause. Our experimental model is certainly representative of this hepatic condition. George et al. (2003) revealed that livers of MCD diet-fed rats exhibited lowered levels of GSH and elevated TBARS.

Curcumin is a phenolic substance with a potent antioxidant action, available evidence documents that multiple molecular mechanisms may contribute to its protective action. Firstly, curcumin inhibits lipid peroxidation by scavenging free radicals and thus blocking the lipid chain reaction (Venkatesan, 1998). The inhibitory action on lipid peroxidation in the present study was reflected in the decrease in level of TBARS in the turmeric-treated group.

Sreejayan et al. (1997) claimed that the presence of phenolic groups in the structure of curcumin is fundamental to explain its ability to eliminate oxygen free radicals from the medium, and that methoxyl groups increase this activity. Secondly, the observation that curcumin treatment was accompanied by an increase in hepatic glutathione content, suggests that this treatment may augment the action of these naturally occurring sulphhydril groups to maintain membrane integrity and help promote the non-enzymatic detoxification of hydroxyl radicals and lipid peroxides. Thirdly, the capacity of curcumin to stabilize membranes has also been demonstrated.

A number of studies have indicated that antioxidants such as plant-derived flavonoids and isoflavonoids can prevent or attenuate the extent of lipid peroxidation among various experimental NASH model systems (Laurent et al., 2004; Pradhan and Girish, 2006). Our results similarly confirmed that silymarin reduced the hepatic level of TBARS, a byproduct of free radical reactions and it also attenuated the hepatic glutathione depletion.

Recently, Haddad et al. (2011) mentioned that treatment with silibinin improved liver steatosis and inflammation and decreased lipid peroxidation, plasma insulin and TNF-alpha; in addition, silibinin decreased the release of free radicals and restored GSH levels.

There are four overarching hepatoprotective activities of silymarin: (i) its effects against the pro-oxidant products mainly through free radical scavenging and the ability to increase cellular GSH content; (ii) its ability to regulate membrane permeability and increase membrane stability in the presence of xenobiotic damage; (iii) its capacity to regulate nuclear expression through steroid-like effects; and (iv) its inhibition of the transformation of stellate hepatocytes into myofibroblasts, which mediate the deposition of collagen fibers, leading to cirrhosis (Kren and Walterova, 2005; Kiruthiga et al., 2007).

The biochemical findings were supported by microscopic examination of H&E-stained thin sections of the liver, which coincided with the study of Vizzutti et al. (2010) in which MCD diet caused a rapid and marked accumulation of fat in hepatocytes with a predominant macro-vesicular pattern. In addition, treatment with curcumin resulted in a general improvement of inflammation associated with the MCD diet. On the other hand, Haddad et al. (2011) stated that liver biopsy evaluation is presently the gold standard test for the diagnosis of NASH, and the liver sections from rats in the NASH group revealed that more than one-third of hepatocytes contained macrovesicles of fat, while treatment with silibinin promoted significant improvement in histology.

Conclusion:

The prevention of hepatic steatosis or limiting hepatic lipid accumulation and injury using unique dietary approaches as turmeric or silymarin may reduce the incidence and/or progression toward more severe forms of NAFLD.

Acknowledgment

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References


