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The Effect of Curcumin on Experimental Non-alcoholic Fatty Liver Disease in Rat Models: A Biochemical Study

Magda Abdel-Ghany Megahed[1*], Hassan Moustafa Fayed[1], Mohammed Mohammed Shamseya[2], Safia Mohammed Hassan[3], Mahmoud Mahfouz Shaaban[1]

[1] Department of Biochemistry, Medical Research Institute, Alexandria University, Egypt.
[2] Department of Clinical and Experimental Internal Medicine, Medical Research Institute, Alexandria University, Egypt.
[3] Department Histochemistry and Cell Biology, Medical Research Institute, Alexandria University, Egypt.
[*]**Corresponding Author**
Magda Abdel-Ghany Megahed
Department of Biochemistry, Medical Research Institute, Alexandria University
Egypt
Email: magda.megahad@alexu.edu.eg
Tel.: +20 3 4282331/73
Fax: +20 3 4283719

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Abstract

Introduction: Nonalcoholic fatty liver disease [NAFLD] is considered the commonest form of liver disorders around the globe. Curcumin is known to have protective anti-oxidative characteristics. **Objective:** The present work aims to investigate the effect of curcumin on NAFLD. **Materials and Methods:** 60 Wistar male rats divided into four groups: Group I: Control group received rat chew diet for 12 weeks. Group II: Fatty liver group, animals received high-fat diet for 12 weeks. Group III: Fatty liver group injected intraperitoneally [IP] with 1 ml/kg body weight dimethyl sulfoxide [DMSO] every other day for 8 weeks. Group IV: Fatty liver group injected with 50 mg/kg body weight, curcumin dissolved in DMSO, IP every other day for 8 weeks Animals were sacrificed at the end of the experiment. Blood was collected for separation of sera and liver tissues to prepare liver homogenates. **Results:** Fasting blood sugar, insulin,and homeostatic model for insulin resistance [HOMA-IR] were significantly higher in rats of NAFL group than control group ($p<0.05$, $p<0.001$, $p<0.001$ respectively) while insulin and HOMA-IR were lower in curcumin treated group (group IV) when compared to DMSO group (group III) ($p<0.05$, $p<0.001$respectively). Cholesterol concentrations in liver homogenate were siginificantly decreased in curcumin group when compared to DMSO and NAFL group ($p<0.05$).Serum TG of NAFL group was significantly higher than DMSO and curcumin groups ($p<0.05$).Triglycerides in liver homogenate of curcumin group was lower than that of NAFL and DMSO groups ($p<0.001$,$p<0.05$ respectively). Serum LDL-cholesterol was significantly higher in NAFL group when compared to control one ($p<0.05$) but decreased in curcumin group when compared to NAFLand DMSO groups ($p<0.001$). Serum VLDL in NAFL group was significantly higher than that of DMSO and curcumin groups($p<0.05$). MDA concentration was higher in NAFL group compared to control group ($p<0.001$) and was significantly decreased in curcumin group compared to DMSO and

NAFL groups (p<0.001). Total glutathione levels in liver homogenate was significantly decreased in NAFL group compared to control group and increased in curcumin group compared to NAFL and DMSO groups (p<0.001). Triglycerides in liver homogenate had a positive correlation with HOMA-IR (r=0.724, p<0.05) and malondialdhyde [MDA] in curcumin group (r=0.807, p<0.001) There was a positive correlation between insulin level and serum VLDL (r=0.547, p<0.05) in curcumin group. **Conclusions:** Enhanced oxidative stress and reduced antioxidant status of the plasma and liver are greatly involved in the induction and progression of NAFL. Curcumin has antioxidant defense mechanism, improves serum lipid profile, decreases steatosis in the liver and improves insulin sensitivity in nonalcoholic fatty liver disease.

Keywords: Non-alcoholic fatty liver, Curcumin, insulin resistance , liver cholesterol, liver triglycerides, malondialdhyde, liver glutathione, Oxidative stress

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is considered the commonest form of liver disorders around the globe.[1] NAFLD has a wide spectrum ranging from simple steatosis, steatohepatitis, cirrhosis up to liver cancer. Its pathogenesis is due to insulin resistance, oxidative stress, and some inflammatory cascades. According to the "multiple hit" theory [2,3] imbalanced lipid metabolism and insulin resistance (IR) are considered as the first hit. Hyperinsulinemia leads to steatosis. After the development of steatosis, the adipokine/cytokine imbalance, bacterial toxins in the intestine and oxidative stress leads to activation of stellate cells and Kupffer cells which eventually leads to liver injury.

Curcumin, extracted from Curcuma longa herb, is known to have anti-oncogenic protective effects due to its anti-oxidative characteristics. [4-6] It has been revealed to limit the activity of inflammatory transcription factors, reduce oxidative stress, and to suppress pro- fibrogenic cytokines and connective tissue growth factors in hepatic stellate cells [HSCs] [7,8]Moreover, curcumin has been shown to limit multiple signaling pathways and to modify proteins and gene product for cell endurance and proliferation.[9] Being of low cost and of negligible toxicity, it is important to investigate the role of curcumin in experimental NAFLD as a pre-clinical research The present work aims to investigate the effect of curcumin on experimental NAFLD as regard biochemical characterestics.

2. Materials and Methods

All procedures were done according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and after approval of the local Animal Ethics Committee. This study was conducted on 60 Wistar male rats (devided on 4 equal groups) weighing approximately 100-120 grams obtained from Animal House of Medical Research Institute [MRI], Alexandria University. Animals were housed 5 per cage with food and water ad libitum, on 12:12-hours light-dark cycle at 23 ± 1 ° C. Rats were divided equally into four groups:**Group Ⅰ** : "The control group" which received standard rat chew diet [23 % of protein, 49 % of carbohydrate, 4 % of total fat, 5 % of fiber, 7 % of ash, and 6 % of vitamins] for 12 weeks.**Group Ⅱ**:"The fatty liver group" animals received high-fat diet (Fiorese et al, 2008) [20% protein, 20% fat, 48% carbohydrate, and 4% fiber] for 12 weeks. **Group Ⅲ**:Fatty liver group injected intra-pretoneally (IP) with 1 ml/kg body weight dimethyl sulfoxide [DMSO][Sigma,Aldrich] after induction of fatty liver, every other day for 8 weeks.

Group Ⅳ: Fatty liver group injected IP with 50 mg/kg body weight, curcumin [Sigma,Aldrich] dissolved in DMSO after induction of fatty liver, every other day for 8 weeks.

Animals were sacrificed at the end of the experiment. Blood was collected for separation of sera and liver tissues were used to prepare liver homogenates.

The following parameters were investigated on serum and liver homogenate:

1. Liver profile tests: Serum alanine aminotransferase[ALT], serum aspartate

aminotransferase[AST], serum alkaline phosphatase[ALP], serum gama-glutamyltranseferase[GGT] and serum Bilirubin.[10]

2. Lipid profile: Total Cholesterol in serum & liver, triglycerides (TG) in serum & liver, serum high-density lipoprotein (HDL) cholesterol, serum low-density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol.[10]

3. Lipid peroxides as malondialdhyde [MDA] in liver homogenate.[11]

5. Antioxidant parameter in liver homogenate & serum: total glutathione [GSH] content.[12]

6. Insulin resistance parameters: Fasting blood glucose and fasting serum insulin then homeostatic model for insulin resistance (HOMA-IR) index was calculated using the formula: HOMA-IR = [fasting serum insulin (mIU/L) × fasting plasma glucose (mg/dL)]/405.[13]

Results were analysed statistically using ANOVA test and Student t-test. The statistical correlation between parameters were also investigated.

3. Results

The results of serum biochemical parameters including liver function profile tests (ALT, AST, GGT, ALP & total bilirubin) and serum lipid profile among the studied groups with their statistical differences are shown in Table 1.

The results of the present study revealed that the mean serum ALT activity was significantly increased in group II than control group ($p<0.001$). On the other hand, the mean serum ALT activity in group IV was significantly decreased than all other studied groups ($p<0.001$).

Regarding serum AST activity, the mean serum AST activity was significantly increased in group II than control group ($p<0.001$). On the contrary, the mean serum ALT activity in group IV was significantly decreased than all other studied groups, while group III showed a significant decrease compared with group II ($p<0.001$).

Also, the present study revealed a significant increase in the mean value of serum GGT in group II compared with control group ($p<0.001$).

A significant decrease in the mean value of serum GGT activity in group IV compared with group II was observed, but a significant increase was shown compared with control group ($p<0.001$).

Group IV showed a significant decrease in mean serum ALP activity when compared with group III ($p<0.05$).

Results of the serum total bilirubin levels are still within normal limits even after NAFL induction in group II and NAFL induction and curcumin injection in group IV. However, there was a significant decrease in group IV compared with control group and group III ($p<0.05$).

In this study, the mean values of serum triglycerides in groups III and IV were significantly decreased compared to group II ($p<0.05$).

Concerning the results of serum cholesterol, the mean values of serum cholesterol in groups IV were significantly decreased compared to group III ($p<0.05$).

There was no significant difference among studied groups as regard serum HDL-C concentration.

There was significant increase in groups II and III compared with control group ($p<0.001$). While in group IV there was a significant decrease compared with groups II and III ($p<0.001$), and a non significant difference from control group. the mean values of VLDL-C in groups III and IV were significantly decreased compared to group II ($p<0.05$).

The results of insulin resistance parameters, lipid content in liver extract, lipid peroxide (malondialdhyde), and glutathione in liver and serum among the four studied groups are shown in Table 2.

Regarding Fasting serum insulin, the mean values of Fasting serum insulin were significantly higher in group II compared with control group ($p<0.001$). On the other side, group IV showed significant decrease in Fasting serum insulin levels compared to groups II and III ($p<0.001$).

Regarding serum Fasting Glucose , the mean values of Fasting serum Glucose were significantly higher in group II compared with control group ($p<0.05$). On the other side, groups III and IV showed no significant difference in Fasting serum Glucose levels compared to control group. As regard HOMA-IR, there was a significant increase in groups II and III compared with control group

(p<0.001) but there was no significant difference between group IV and control group.

The mean values of cholesterol concentration in liver extract showed significant increase in groups II and III compared with control group (p<0.001). On the other hand, group IV exhibited a significant decrease compared with groups II and III (p<0.001), and a non-significant difference from control group. the mean values of triglycerides concentration in liver extract showed significant increase in groups II and III compared with control group (p<0.001).the mean values of triglycerides concentration in liver extract in group III was significantly lower than group II (p<0.001).

Group IV showed significant decrease in triglycerides concentration in liver extract compared with group III (p<0.001).

The present study revealed that there was a significant increase in the mean values of MDA concentration in liver extract in groups II and III compared with control group (p<0.001). Alternatively, group IV exhibited a significant decrease compared with groups II and III (p<0.001), and a non-significant difference from control group.

There was a significant decrease in the mean values of total glutathione in liver homogenate in groups II and III compared with control group (p<0.001). Otherwise, group IV exhibited a significant increase compared with groups II and III (p<0.001), and a non-significant difference from control group.

Concerning serum glutathione, the mean values of serum total glutathione in groups II and III were significantly lower than control group, but group IV exhibited a significant increase compared with groups II and III (p<0.001).

Statistical correlation between these study parameters showed the followings:

1-There was a significant negative correlation between HOMA-IR and HDL-cholesterol, in NAFL group [r=-0.574, p<0.05], While HOMA-IR had a significant positive correlation with MDA [r=0.971, p<0.001].

2-In curcumin group, TG in liver extract had a significant positive correlation with HOMA-IR [r=0.724, p<0.05] and MDA[r=0.807, p<0.001].

There was a significant positive correlation between insulin level and serum TG and VLDL [r=0.547, p<0.05] in curcumin group.

Table 1: Statistical comparisons (Mean±SD) of serum biochemical parameters among the four studied groups (liver profile tests and lipid profile)

Group / Parameter Mean ± SD.	Group I: Control[n=15]	Group II: NAFL [n=15]	Group III: NAFL treated with intra-pretoneal DMSO 1ml/kg[n = 15]	Group IV: NAFL treated with intra-pretoneal curcumin 50mg/kg [n = 15]	*p*
Fasting Insulin(ng/ml)	2.26 ± 0.78	5.23 ± 1.56 [a]	7.11 ± 2.11 [ab]	3.57 ± 1.40 [bc]	<0.001
Glucose (mg/dl)	94.75 ± 10.29	109.63 ± 10.18 [a]	105.45 ± 12.19	100.18 ± 16.87	0.014
HOMA-IR	11.18 ± 3.97	30.37 ± 10.51 [a]	39.79 ± 15.35 [ab]	19.43 ± 9.94	<0.001
Cholesterol in Liver extract (mg/g liver tissue)	24.13 ± 4.70	33.28 ± 9.09 [a]	33.05 ± 7.69 [a]	24.49 ± 7.66 [bc]	<0.001*
Triglycerides in liver extract(mg/g liver tissue)	16.20 ± 0.92	38.19 ± 9.10 [a]	28.50 ± 7.41 [ab]	21.47 ± 4.30 [bc]	<0.001*
MDA in liver homogenate (nmol/g liver tissue)	7.93 ± 3.01	13.17 ± 3.17 [a]	12.33 ± 2.91 [a]	6.40 ± 3.79 [bc]	<0.001*
GSH in liver homogenate (µmol/g liver tissue)	5.10 ± 0.95	2.71 ± 1.06 [a]	2.99 ± 1.83 [a]	5.87 ± 1.56 [bc]	<0.001*
Serum GSH(µmol/ml)	20.32 ± 3.63	12.09 ± 2.40 [a]	10.63 ± 1.85 [a]	16.29 ± 5.67 [abc]	<0.001*

ALT: alanine transaminase, ALP: alkaline phosphatase, AST: aspartate transaminase,DMSO:dimethylsulfoxide,GGT: gamma glutamyletransferase,HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol,NAFL: non-alcoholic fatty liver, VLDL: very low density lipoproteins.
a = significant difference from control group.
b = significant difference from NAFL group.
c = significant difference from DMSO group.
*: Statistically significant at $p \leq 0.05$

Table 2: Statistical comparisons (Mean ± SD) of insulin resistance parameters, lipid content in liver extract, lipid peroxide (malondialdhyde), and glutathione in liver and serum among the four studied groups

Group / Parameter Mean ± SD.	Group I: Control[n=15]	Group II: NAFL [n=15]	Group III: NAFL treated with intra-pretoneal DMSO 1ml/kg[n = 15]	Group IV: NAFL treated with intra-pretoneal curcumin 50mg/kg [n = 15]	*p*
Fasting Insulin(ng/ml)	2.26 ± 0.78	5.23 ± 1.56 [a]	7.11 ± 2.11 [ab]	3.57 ± 1.40 [bc]	<0.001
Glucose (mg/dl)	94.75 ± 10.29	109.63 ± 10.18 [a]	105.45 ± 12.19	100.18 ± 16.87	0.014
HOMA-IR	11.18 ± 3.97	30.37 ± 10.51 [a]	39.79 ± 15.35 [ab]	19.43 ± 9.94	<0.001
Cholesterol in Liver extract (mg/g liver tissue)	24.13 ± 4.70	33.28 ± 9.09 [a]	33.05 ± 7.69 [a]	24.49 ± 7.66 [bc]	<0.001*
Triglycerides in liver extract(mg/g liver tissue)	16.20 ± 0.92	38.19 ± 9.10 [a]	28.50 ± 7.41 [ab]	21.47 ± 4.30 [bc]	<0.001*
MDA in liver homogenate (nmol/g liver tissue)	7.93 ± 3.01	13.17 ± 3.17 [a]	12.33 ± 2.91 [a]	6.40 ± 3.79 [bc]	<0.001*
GSH in liver homogenate (µmol/g liver tissue)	5.10 ± 0.95	2.71 ± 1.06 [a]	2.99 ± 1.83 [a]	5.87 ± 1.56 [bc]	<0.001*
Serum GSH(µmol/ml)	20.32 ± 3.63	12.09 ± 2.40 [a]	10.63 ± 1.85 [a]	16.29 ± 5.67 [abc]	<0.001*

DMSO:dimethyl sulfoxide, GSH: glutathione, HOMA-IR: homeostatic model for insulin resistance, MDA: malondialdhyde,
NAFL: non-alcoholic fatty liver.
a = significant difference from control group.
b = significant difference from NAFL group.
c = significant difference from DMSO group.
*: Statistically significant at $p \leq 0.05$

4. Discussion

Insulin resistance, oxidative stress, and inflammatory cascades play an important role in the pathogenesis of NAFLD. Curcumin has been shown to restrain multiple pathways by interacting with many proteins and modification of their activity like inflammatory cytokines. enzymes, transcription factors, and gene products responsible for cell survival and proliferation.[9]The present study demonstrates the potential value of curcumin in high-fat diet (HFD) induced NAFLD in rat models. In our study, liver activity of enzymes ALT and AST were highly elevated in NAFL rats, an observation which is in the same line with Adams and Feldstein [14]who reported that elevated ALT and AST levels in the absence of other liver disorders may support NAFLD.

Curcumin treatment significantly reversed the elevation of serum ALT and AST activities in NAFL rats, also it improved results of ALP and GGT serum activities. It has been reported that improvement in aminotransferase levels appears to indicate improvement in steatosis and inflammation but not fibrosis.[15] Our study showed that the levels of fasting glucose in NAFL group were significantly high compared with that of control group, also, insulin levels and HOMA-IR were high in NAFL group. In line with that, Singhal et al, [16] demonstrated that insulin resistance with compensated hyperinsulinemia has a link with steatosis and hyperlipidemia in both humans and animal models. In agreement with these results it was reported that once the liver is fatty, the ability of insulin to inhibit hepatic glucose production is impaired, which leads to an increase in the fasting plasma glucose concentration. IR in turn, stimulates insulin secretion resulting in mild hyperinsulinemia and lowering of glucose to near-normal levels, also the inhibitory action of insulin on VLDL production is impaired whereas VLDL clearance remains unaffected which results in hypertriglyceridemia and decreasing HDL-cholesterol.[17, 18]

Results of our study revealed that curcumin significantly reduced HFD-related high serum glucose, insulin levels and HOMR-IR values, these results were in line with a study done by Seo et al,.[19] Both a turmeric extract and a purified curcumin extract reduced hyperglycaemia in a mouse model of type 2 diabetes mellitus. [20] Curcumin also activated peroxisome proliferator-activated receptor gamma [PPAR-γ] in fat cells in vitro and the authors attributed curcumin's hypoglycaemic effect to this mechanism. HSC and Moser cell PPAR-γ were also activated by curcumin in vitro. [21,22]

Dyslipidemia has been reported in 20% to 80% of NAFLD cases.[23] In our study, HFD had significantly increased TG, TC, LDL-C and reduced HDL-C. After treatment with curcumin, serum levels of cholesterol, TG, and LDL-C were significantly decreased, and HDL-C was increased compared with DMSO group. Curcumin was reported to reduce plasma triacylglycerol concentrations in Wistar rats on high-fat diet.[24] In a previous study of Um et al,[25] curcumin suppressed HFD-induced lipid droplet accumulation in the liver and lowered serum TC and TG levels. Curcumin decreases circulating lipid levels resulting in reduced lipid transport to the liver, leading to the inhibition of lipid accumulation in the liver. They suggested that curcumin can have protective effects against HFD-induced hepatic steatosis via enhanced hepatic lipid metabolism via AMPK activation.

In this study, we estimated the liver oxidative stress, measuring, on one hand, the liver antioxidants [total GSH] and on the other hand, the oxidative stress marker MDA. The results revealed that, the liver glutathione was reduced in NAFL group compared to normal, while liver MDA was increased significantly. Livers of experimental animals and patients with NAFLD have also been observed to express low levels of GSH. [26]

Lipid peroxidation initiates release of MDA and 4-hydroxynonenal [HNE] that can join to hepatic proteins forming neo-antigens and initiating potentially harmful immune response.[27] In another study, it was reported that curcumin can increase antioxidant defense mechanism by increasing activity of catalase enzyme and reduce inflammation through reducing the expression of NF-kβ in the liver. [28]

Our study results showed that, curcumin reduced MDA and raised total GSH in liver cells and serum which suggests decreased lipid peroxidation by curcumin.

In addition, a study by Kempaiah and Srinivasaو [24] reported that curcumin reduced membrane and intracellular lipid peroxide levels and induced antioxidant activity in hepatocytes and erythrocytes of rats on HFD. Curcumin chelates and scavenges reactive oxygen species and induces anti-oxidant enzymes.[29,30]

In our study, lipid content in liver was increased in NAFL group compared to control group and decreased after curcumin treatment. The finding that curcumin reduces both hepatic and non-hepatic fat suggests that it lowers the fatty acid synthesis: oxidation ratio. Curcumin activates a key fatty acid oxidizing enzyme, acyl-CoA oxidase,[31]a deficiency of which leads to hepatic steatosis.

Conclusions: From the findings of our study, it can be concluded that high fat diet had serious health consequences. Insulin resistance and oxidative stress are increased in experimental

NAFLD. Curcumin has antioxidant defense mechanism, improves serum lipid profile, decreases steatosis in the liver and improves insulin sensitivity.

Ethics approval

All procedures were done according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and after approval of the local Animal Ethics Committee.

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