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Curcumin Effects on Hepatic Steatosis and Histopathology in an Obese Mouse Mode

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Authors' contributions

This work was carried out in collaboration between all authors. Author AN designed the study, assisted with the statistical analysis and wrote the first draft of the manuscript with literature searches, authors BH and AM managed the chemical analyses for the study, had input on writing the manuscript. Authors HZ and AM performed histopathological analysis, author LA secured grant funding and wrote, with authors JN and DB, the initial study design. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Curcumin is a popular spice and part of the ancient medicinal system *Ayurveda*. It is known to have anti-inflammatory, antimicrobial and antioxidant properties; research has shown curcumin to have beneficial effects on induced liver damage in animals.
Study Design: Based on our own observations demonstrating hepatic improvement with curcumin in both obese and wild-type animal models, as well as the work of others, we determined that an n of at least 6 in each group at the selected time point would allow for greater than 80% probability of finding significant differences at a level of P< 0.05 between curcumin treated versus control fed OB-OB mice in the proposed experiment. Realizing that histomorphometric data are more variable and to ensure statistically valid data, the n per group was raised to 10, which allowed us to significantly detect a 10% difference between groups using a power analysis program and statistical evaluation of the data (Statistica, Statsoft).

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Place and Duration of Study: With a protocol approved by the University Animal Care Committee, we tested the hypothesis that the natural fatty livers in leptin k/o mice (OB/OB) would be improved by adding curcumin to food pellets at doses equal to human use (mg/ kg body weight).
Methodology: Ten curcumin-treated and ten untreated control OB/OB mice were fed 2 months then studied for effects. Food pellets (Harlan-Teklad) were prepared with and without 180 mg curcumin in the daily dietary intake (9 gm). Calories for both diets: protein 15.2%, carbs 62.5%, fat 22.3%.
Results: Histopathology on organs and cytokine analysis on serum parameters were measured on the two groups. Blood glucose was elevated in 8/10 curcumin-treated mice (p=0.07 a trend). Cytokines adiponectin and TNFα were strongly decreased by the curcumin diet and tissue superoxide was reduced. Liver % fat was significantly reduced by the curcumin additive, P=.01.
Conclusion: Dietary curcumin in OB/OB mice produces significant improvement in liver health by reduction of % hepatic fat as well as by improved cytokine and inflammatory values. An increase in blood glucose by this treatment remains unexplained.

Keywords: Hepatic steatosis; curcumin; animal model; histopathology.

1. INTRODUCTION

Nonalcoholic fatty liver disease is the most common liver disease today [1]. Many studies are in process in order to explain the mechanisms of liver injury and in particular the inflammation at the molecular level. Molecular mechanisms of liver inflammation and injury continue to be a subject for intense study. Many of these studies look at site-specific phosphorylation as critical modulators of liver gene expression, cell-cycle progression, as well as tissue inflammation and repair. A recent review discusses the many inflammatory pathways leading to NASH [2]. While much is known about the metabolic features of liver steatosis such as over-nutrition, insulin resistance, or hyperglycemia, far less is understood about the nature of hepatic inflammation and recruitment of the inflammatory cells and cytokines. The key pro-inflammatory signaling pathways in NASH involve many cytokines such as TNFα and IL -1 but the upstream, initiating components of liver inflammation are unknown and may even originate outside of the organ (gut microbes?) This inflammatory cascade creates a state of chronic inflammation, damaging to the liver parenchyma, the biliary ducts, and the perfusion system [3]. With the premise that the origin of NASH involves a chronic state of hepatic inflammation [4-6], the present study describes the effect on the liver and its related adipocytokines of a natural product that has been used for centuries in *Ayurvedic* medicine for anti-inflammatory effects. Curcumin, a plant polyphenol compound [7], is derived from the herb *Curcuma longa*, and is the major ingredient in the common spice turmeric [8]. Physiological effects attributed to curcumin have been widely reported. For example, curcumin acts as an anti-inflammatory [9,10], an antimicrobial [11,12] and as an antioxidant [13,14]. Curcumin has also been used as an antiproliferative compound [15,16]. Studies have shown its effectiveness in preventing IL-6-induced liver damage as well as blocking inflammation mediated through the NFkB pathway [17,18]. When used in combination with other drugs, curcumin improves their effect by inhibiting the efflux transporters that play a role in multidrug resistance [19,20]. These findings, added to the long-term use of curcumin by humans without noxious side effects or toxicity led us to choose curcumin in order to evaluate its efficacy in an animal model of a common inflammatory pathology similar to human NASH. Since leptin is an adipocyte hormone interacting with areas of the brain that control hunger and appetite [7] the OB/OB leptin knockout mouse is a recognized model of obesity and nonalcoholic steatosis. We hypothesized that a curcumin supplemented diet would reduce both hepatic steatosis and inflammation (measured by blood and tissue cytokine levels and histopathological analysis) in the OB/OB mouse model as compared to animals fed the same diet without curcumin.

2. MATERIALS AND METHODS

The study was approved by the University Animal Use Committee for 20 OB/OB leptin knock-out mice. The 10 treated mice were fed pellets (Harlan-Teklad) containing 180 mg of curcumin per 9 gm (average daily intake), a curcumin amount equivalent to a dose given in human clinical trials (mg/kg body weight). The 10 controls received pellets containing the same

percent of carbohydrates, fat, and protein without curcumin, supplied by the same manufacturer. Feeding experiments lasted 60 days. Animal weights were taken weekly and at sacrifice time.

At necropsy, tissues and blood were collected for analysis. Portions of the tissues were fixed in 10% formaldehyde, embedded and stained by H&E. The histological damage was analyzed /quantified by two pathologists unaware of the slide identification, according to a technique previously described and applied to lungs, kidneys and pancreas of experimental animals [21]. Liver damage was assessed by evaluating changes in the fat droplet presence, especially around bile ducts and portal veins as well as vasculitis and ductal fibrosis. A subjective rating for each slide ranging from 5 (minimal) to 50 (severe and extensive damage) was assigned to each component of the organ. The damage severity was then evaluated by statistical methods.

Serum, blood and tissue homogenate analyses were performed, including non-fasting blood glucose, serum cytokine levels (IL-6, adiponectin, TNFα, insulin), and liver enzyme alanine aminotransferase (ALT). Superoxide dismutase (SOD) was measured by ELISA on liver homogenate supernatants. The degree of inflammation in liver was measured histologically; lung and respiratory airway inflammation were compared between curcumin and control groups by cell counts of broncho alveolar lavage and counts of inflammatory cells in lung tissue.

Score and ratio data were analyzed by analysis of variance (Statistica, Statsoft, Tulsa, OK). Where appropriate, comparisons were made using Fisher least significant difference, and Weekly weight changes were analyzed by the multiple comparisons test. The level of statistical significance for comparisons was set at P=.05.

3. RESULTS

3.1 Histopathology

There were significant differences between the average histopathology scores for livers of the two groups. Inflammation, liver congestion, bile duct hypertrophy, and vasculitis were significantly decreased for the curcumin treated group compared to the controls. All histopathology differences had a statistical significance of P=.01. There was also a significant decrease (P=.01) in percent liver fat deposition as measured on slides at 400x. This finding suggests that liver fat deposition at different points during treatment should be measured, preferably as a dose-response to the anti-inflammatory intervention (curcumin in this study). The differences in the various parameters of liver damage are shown in Fig. 1. Representative sections of the livers of the mice control group and of the curcumin treated group are shown in Figs. 2, a-d.

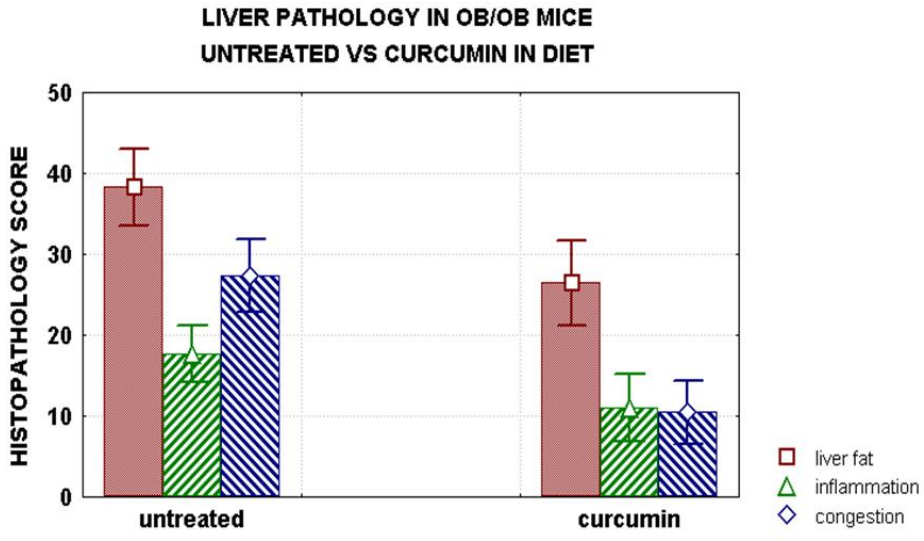

Fig. 1. Liver pathology in OB/OB mice as a function of curcumin vs control

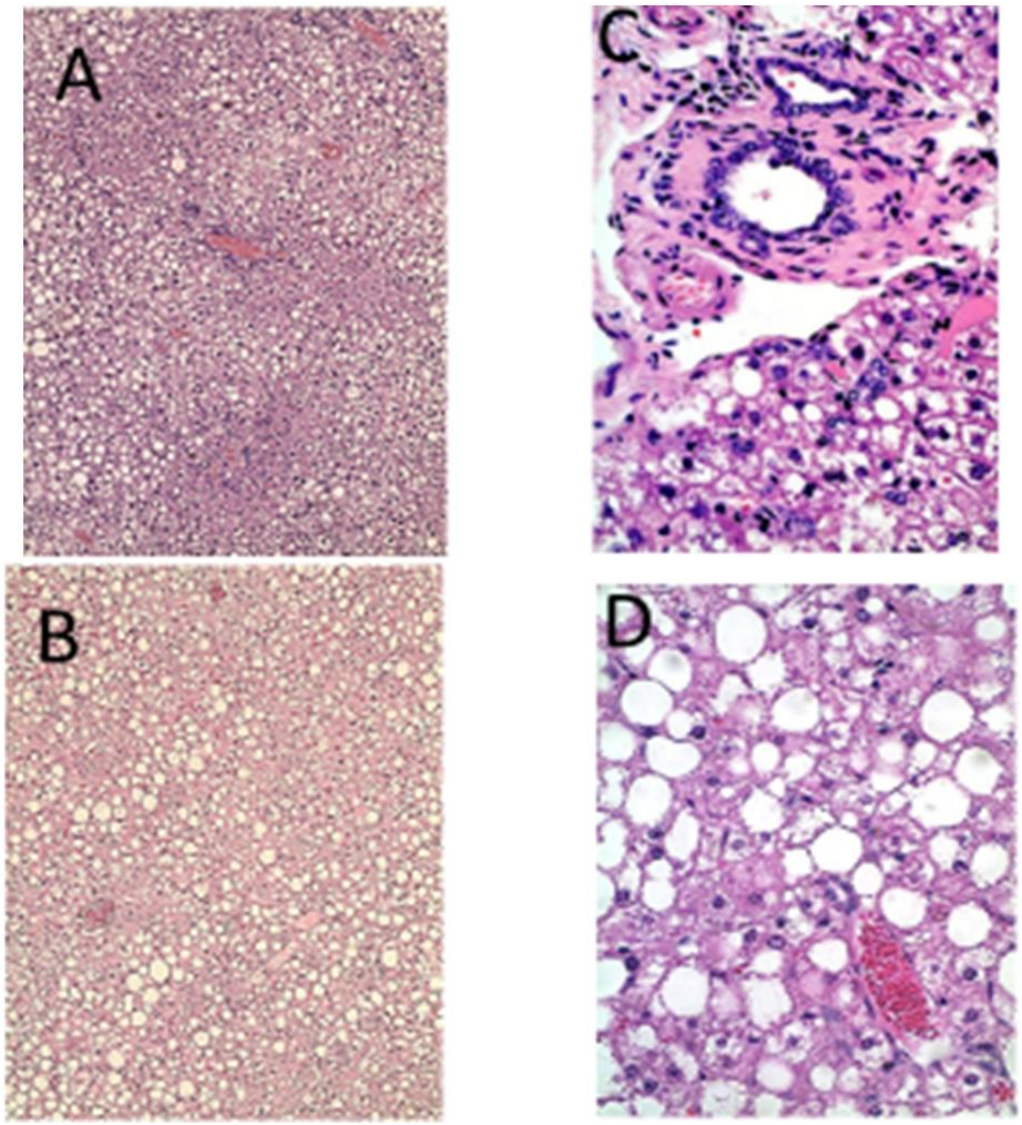

Fig. 2. Liver inflammation and vasculitis

2A, OB/OB mice untreated. Diffuse steatosis and inflammation with marked congestion of the portal veins, H&E, 100x

2B, OB/OB mice, curcumin. Steatosis and inflammation are reduced; likewise the portal vein congestion is also less extant. H&E, 100x

2C OB/OB mice untreated. Marked inflammation of two small caliber arteries contiguous to two biliary ducts showing marked fibrosis. Fat droplets and inflammation of the hepatocytes with several pyknotic nuclei and scattered macrophages. H&E 400x

2D. OB/OB mice, curcumin. Liver vasculature and bile ducts do not show the vasculitis and inflammation observed in the untreated controls. Less inflammation and nuclear pyknosis in the hepatocytes. H&E 400x

Liver damage was assessed by evaluating changes in the fat droplet presence, degree of inflammation around the bile ducts and portal veins, congestion of the portal veins, vasculitis and ductal fibrosis. A subjective rating for each slide ranging from 5 (minimal) to 50 (severe and extensive damage) was assigned to each component of the organ. The damage severity was then evaluated by statistical methods.

3.2 Cytokines and Chemokines

Adipocytokines and proteins related to inflammation are reported in Table 1. Serum

insulin levels, IL - 6, TNF-α, ALT and adiponectin were measured. There was a significant decrease in TNF-α in the curcumin treated group with a *P* value = .03 compared to controls. There was no significant difference between controls vs curcumin-treated in insulin levels, serum ALT or adiponectin levels. The inflammatory cytokine IL-6, though reduced in the curcumin group, did not reach statistical significance in the decrease from untreated controls.

Table 1. Cytokines and chemokines in Curcumin-treated vs control mice

Cytokine/ chemokine	Untreated controls mean± S.D.	Curcumin-treated mean ±S.D.
Serum insulin	3.45±0.8	2.34±1.67
IL - 6	6.41±4.47	2.96±2.42
TNF-α*	10.32±3.84	5.74±1.20
ALT	215.17± 59.22	356.8±221.7
adiponectin	13.43±3.6	7.96±3.54

Significant differences P=.05 between curcumin and controls are marked (*)

3.3 Inflammatory Cell Counts

BAL cell counts on the curcumin group showed 2.5% PMN, compared to controls' 2.9% PMN, a statistically nonsignificant difference. In the pancreas of these obese mice, there was a strong elevation of inflammatory cells in the peri-pancreatic fat of untreated controls when compared to the peri-pancreatic fat of the curcumin group, an observation further suggesting curcumin control of systemic inflammation.

3.4 Superoxide Dismutase (SOD)

Effects of curcumin intervention were measured on liver homogenate SOD, a mitochondrial anti-oxidant system with superoxide anion scavenging ability thus possessing anti-inflammatory activity. Percent inhibition of SOD activity was measured by assay of individual homogenates from the livers of the two groups. Untreated controls averaged 23.27±3.8% vs 25.92±3.3% curcumin-treated group. These data show no statistically significant difference between groups in the antioxidant enzymes of the SOD system.

Body weights and liver weights of the two experiment groups were not significantly different after 2 months of treatment, although liver fat appeared reduced with curcumin. The data are summarized in Table 2.

Table 2. Averaged group body weights and liver weights at necropsy

	Untreated mice (grams)	Curcumin treated mice (grams)	Significance
End body weight	54.25±1.8	55.14±2.9	*P*>.05
End liver weight	3.92±0.47	4.27±0.65	*P*>.05
% liver fat*	38.27±4.7%	26.5±5.2%	*P*=.01

**quantitated from digital photos at 400x, Image J software*

3.5 Blood Glucose

A difference was found between curcumin and untreated control groups in fasting blood glucose levels. Eight of 10 OB/OB mice in the curcumin treated group had blood glucose values of 217 to 396, while the OB/OB mice on the identical diet without curcumin showed 144 to 178, high for wild-type mice but not unusual in leptin knock outs. Comparison of the two groups' pooled values, however, did not show a significant difference (p =.46). Mild increases in fasting blood glucose have been previously reported in the obese (leptin K/O) mice. Further investigation will be needed to determine whether curcumin had additional effect over that of the excessive food intake of these leptin K/O mice.

4. DISCUSSION AND CONCLUSION

The decreases in hepatic inflammation, congestion, bile duct hypertrophy and vasculitis in the curcumin group vs the untreated controls suggests that curcumin is producing a benefit. The treated mice were fed a 2% (180mg/9g) curcumin diet which reduced hepatic tissue fat deposition ± 8%, improved bile duct inflammatory-related congestion and hypertrophy with reduced vasculitis and vessel related hypertrophy. Regarding the various mechanisms of curcumin activity, one of the more interesting is how curcumin acts directly on several drug transporters by binding to the same site that the inhibited compound does and thereby inhibiting its activity [19,20]. Because so many adipocytokines have potential action on liver, targeted studies would be necessary to determine mechanisms and recipients of curcumin-related efflux transporters. Although our ELISA for superoxide dismutase failed to show significant improvement with curcumin feeding, other research suggests that curcumin protects hepatocytes from high free fatty acid-

induced mitochondrial dysfunction by regulation of mitochondrial biogenesis [21].

Further study to evaluate the anti-inflammatory value of curcumin on fatty liver disease would also necessitate additional quantification of markers of inflammation in controls with fatty liver and the same measures in a curcumin fed group. Pathologists evaluated the lung inflammation (BAL cell count), pancreas (peripancreatic fat and lymphocyte count) and liver fat.

Recognizing that this is a pilot investigation, we have proposed additional pathways to investigate the input of curcumin on an obese animal model. Perhaps liver inflammatory cell counts in the untreated vs curcumin groups would furnish a good additional endpoint for evaluation. In the present study we evaluated but did not quantitatively measure peripancreatic fat deposits. Lipid input to diabetic initiation is currently a topic of scientific studies. During dissection of the organs it was noted that there was less fatty build up around most of the internal organs of the curcumin- fed mice although Table 2 reports no statistical total difference. Exact measurement of the TNFα secreting macrophages in the abdominal fat, for example, would allow more specific inflammatory endpoint analysis. An additional next step could also be a dose-response study with multiple curcumin doses and endpoints similar to those we have evaluated in the present study. Use of outbred rodents on high fat diets might furnish a more human-comparable model than this model of leptin knock-out mice. Our groups of ten were shown in pilot tests to furnish an adequate number for statistical significance.

Overall, curcumin produced physiological benefit in this model with no evident side effects. Since the model dose was based on a published human dose (mg/kg body weight), this profile offers the ability to investigate the use of curcumin and other mild anti-inflammatory agents for therapeutic intervention in fatty liver disease.

CONSENT

Not applicable.

ETHICAL APPROVAL

All experiments were performed in AAALAC-approved animal quarters on protocol 1029 approved by the University care and use committee which included approval of all diets and terminal euthanasia.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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