

# Hypolipidemic, hepatoprotective and anti-inflammatory role of Chios Mastic gum in Streptozotocin-induced diabetic mice with fatty liver disease

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## Abstract

**Aim:** Non alcoholic fatty liver disease (NAFLD) is a major form of chronic liver disease and it is highly prevalent in patients with diabetes mellitus. Chios Mastic gum (CMG) is known for its antioxidant and its potential hypolipidemic effects. This study sought to investigate the role of CMG in metabolic profile and liver histology in an animal model of NAFLD.

**Material and Methods:** A total of 37 streptozotocin-induced diabetic 12-week-old male C57bl/6 mice were allocated into the following groups: Control group ( $n=13$ ); LdM ( $n=12$ ) animals receiving low dose mastic for 8 weeks (20 mg/Kg of body weight); HdM ( $n=12$ ) animals receiving high dose mastic (500 mg/Kg BW) for the same period. Serum lipid and glucose levels were determined at baseline and at 4 and 8 weeks (end of experiment). Serum adiponectin, resistin and interleukin-6 levels were also measured at baseline and at the end of the experiment. Histopathological examination for liver was performed.

**Results:** After 4 weeks, CMG administration resulted in decreased serum glucose and triglyceride levels in both LdM and HdM groups. At the end of the experiment, LdM presented significantly lower serum glucose and ameliorated lipidemic profile compared with control group. Adiponectin and resis-

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tin levels at the end of the experiment did not differ between control and both treated groups, while interleukin-6 was significantly lower in LdM group compared with the control group. Hepatic steatosis observed in control group was partially reversed in both CMG groups.

**Conclusions:** CMG administration in low dosages improves metabolic disturbances and exerts anti-inflammatory properties in diabetic mice while alleviating hepatic damage.

**Keywords:** chios mastic gum; diabetes; lipid metabolism; mice; non-alcoholic fatty-liver disease; interleukin-6

## 1. Introduction

Diabetes mellitus (DM) is a multi-dimensional disease, accompanied with a variety of co-morbidities such as hypertension, coronary disease, atherosclerosis, neuropathy and metabolic syndrome.<sup>1</sup> Although, different drugs are successfully used for controlling blood glucose levels, they are ineffective in reversing or even withholding the progression of certain complications.<sup>2</sup> A disease associated with the increased prevalence of DM that has raised awareness over the last decade is non-alcoholic fatty liver disease (NAFLD). Insulin resistance, hyperinsulinemia, disordered lipid metabolism, increased inflammation elements and oxidative stress, impaired fasting glucose and glucose tolerance seem to be the links between DM and NAFLD, while special environmental and genetic factors can exacerbate the progress of NAFLD at diabetic patients.<sup>3</sup> Control of the impaired glucose homeostasis and lipid concentrations inhibits micro-vascular complications and fat deposits accumulation in the liver tissue. Therefore, the main objective of medical treatment as well as prophylaxis in patients with DM and NAFLD is metabolic control.<sup>4</sup>

Chios mastic gum (GMG) is a natural resinous exudate which is excreted by *Pistacia lentiscus* var. *Chia* cv. *Anacardiaceae*. These unique mastic trees are exclusively cultivated at the southern part of Chios, which is a Greek island, situated in the Aegean Sea. Apart from the CMG's usage as a food additive, it is scientifically proven that it possess also strong therapeutic properties.<sup>5</sup> CMG has antibacterial and antifungal activity, while it can be exploited

as an anti-cancer agent.<sup>6,7</sup> CMG is also known for its anti-inflammatory properties, since it inhibits the production of inflammatory substances and markers such as nitric oxid (NO), prostaglandin E2 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and the production of superoxide and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), eliminating the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, which are dependent from Protein kinase C (PKC).<sup>8,9</sup> CMG plays a major role in the lipids metabolism as a Peroxisome proliferator-activated receptor alpha (PPAR- $\alpha$ ) agonist and especially in the reduction of human low density lipoprotein (LDL) cholesterol copper oxidation.<sup>10,11</sup> Finally, CMG, thanks to triterpenes, has also shown valuable action at the glucose metabolism, urging  $\beta$ -pancreatic cells to secrete insulin and inhibiting protein tyrosine phosphate-1B.<sup>12,13</sup>

Aim of our study was to evaluate the potential effect of CMG on glycemic and lipid control in an experimental model of DM and to investigate its protective effect in NAFLD, as a complication of diabetes.

## 2. Material and methods

### 2.1 Animal model

A total of 37 C57Bl/6 male mice 10 weeks of age and weighing 25-30 g each were bought from the Alexander Fleming Institute (Vari, Greece) and were acclimatized for 1 week before the experiment started. The mice were housed in the Laboratory for Experimental Surgery and Surgical Research, Athens University Medical School, in a controlled environment

**Table 1.** Body weight and blood clinical chemistry of control and Chios Mastic treated groups throughout the study

Mean(SD)			
	T0	T1	T2
<b>Weight (g)</b>			
Control	26.8 (2.6)	28.2 <sup>a</sup> (2.8)	28.4 <sup>a</sup> (2.9)
LdM	26 (2.7)	25.2 <sup>a</sup> (2.5)	25 <sup>a</sup> (2.5)
HdM	27.3 (2.5)	26.7 (2.3)	27 (2.5)
<b>Glucose (mg/dL)</b>			
Control	224.5 (40.4)	216.7 <sup>a</sup> (24.4)	235.1 <sup>a</sup> (30.1)
LdM	220.8 (13.5)	167.2 <sup>ab</sup> (8.6)	162.6 <sup>ab</sup> (14.6)
HdM	239.2 (27.4)	175.6 <sup>ab</sup> (16.8)	194.0 <sup>ab</sup> (45.1)
<b>Total Cholesterol (mg/dL)</b>			
Control	94.3 (15.8)	90.8 (9.8)	101.7 <sup>c</sup> (12.6)
LdM	77.3 <sup>d</sup> (8.2)	82.1 <sup>c</sup> (7.4)	77.6 <sup>d</sup> (5.4)
HdM	92.9 (6.1)	89.2 (22.1)	95.3 (13.0)
<b>LDL-C (mg/dL)</b>			
Control	41(12.5)	32.5 <sup>c</sup> (11.1)	41.9 (10.6)
LdM	38.6 (5.7)	22.9 <sup>ac</sup> (10.3)	19.9 <sup>cd</sup> (9.0)
HdM	38.6 (7)	39 (16.5)	43.8 (10.3)
<b>HDL-C (mg/dL)</b>			
Control	38.2(3)	38.2(3.9)	38.9(3.1)
LdM	29.5(3.7) <sup>d</sup>	48.8(7.2) <sup>cd</sup>	48.9(7.2) <sup>cd</sup>
HdM	38.8(4.7)	39.5(4.7) <sup>c</sup>	39.3(4.4)
<b>Triglycerides (mg/dL)</b>			
Control	75 (16.2)	98.6 (41.5) <sup>c</sup>	104.4 (31.6) <sup>c</sup>
LdM	49.2 (8.6) <sup>f</sup>	52 (12.4) <sup>b</sup>	44.1 (9.7) <sup>bc</sup>
HdM	76.4 (12.2)	61.7 (15.3) <sup>bc</sup>	61 (22.1) <sup>bc</sup>
<b>Total Proteins (mg/dL)</b>			
Control	5.03 (0.5)	5.17(0.4)	5.42 (0.2) <sup>a</sup>
LdM	5.1 (0.4)	5.46 (0.2)	5.26 (0.2)
HdM	4.95 (0.3)	5.45 (0.5) <sup>a</sup>	5.41 (0.3) <sup>a</sup>

Body Weight levels(g), glucose (mg/dL), total Cholesterol (mg/dL), LDL cholesterol(mg/dL), HDL cholesterol(mg/dL), triglycerides(mg/dL) and total protein(mg/dL) level in serum at baseline(T0), at the 4th week(T1) and at the 8th week (T2) of the experimental period period expressed as mean(SD); SD: Standard Deviation. Control: Control Group; LdM: Low Dose Mastic treated group(20mg/kg) for 8 weeks; HdM: High Dose Mastic treated group(100mg/kg) for 8 weeks.

a  $p < 0.001$  vs. baseline (T0), b  $p < 0.001$  vs. Control group, c  $p < 0.05$  vs. baseline (T0), d  $p < 0.001$  vs. Control group and HdM group, e  $p < 0.05$  vs. HdM group, f  $p < 0.05$  vs. Control group and HdM group

**Table 2.** Total adiponectin, resistin and interleukin-6 levels in all groups at the beginning (T0) and at the end of the experimental period (T2)

	Mean(SD)	
	T0	T2
<b>Adiponectin(<math>\mu\text{g/ml}</math>)</b>		
Control	14.2 (6.9)	10.5 (4.8)
LdM	14.4 (7.1)	19.8 (11.0) <sup>a</sup>
HdM	14 (6.6)	16.7 (8.3)
<b>Resistin(<math>\text{ng/ml}</math>)</b>		
Control	4.2 (2.8)	4.2 (2.7)
LdM	4.5 (2.6)	6.4 (3.5)
HdM	4.0 (2.7)	4.3 (3.9)
<b>Interleukin-6 (ng/ml)</b>		
Control	11.5 (1.7)	16.7 (5.6)
LdM	12.1 (2.4) <sup>a</sup>	13.8 (3)
HdM	8.1 (4.0)	10.0 (6.2) <sup>b</sup>

Serum adiponectin, resistin and interleukin-6 levels throughout the study period in control, Low dose Mastic (LdM) and High dose Mastic (HdM) groups expressed as mean(SD); SD: Standard deviation

a  $p < 0.05$  vs. HdM group

b  $p < 0.05$  vs. control group and LdM group

at  $20^\circ\text{C} \pm 2^\circ\text{C}$ , in cages with European standards (Tecniplast), 55% relative humidity, central ventilation (15 air changes/hour), and an artificial 12-hour light-dark cycle. Access to food and water was unrestricted for all groups. All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals".<sup>14</sup> The experimental protocol was approved by the Institutional Animal Care and Use Committee of Athens University Medical School and the Veterinary Directorate of the Athens Prefecture in agreement with the with the ethical recommendations of the European Communities Council Directive of November 24, 1986 (86/609/EEC).

### 2.2 Animal grouping and interventions

All mice became diabetic with intraperitoneal injection of streptozotocin (STZ) (40 mg/kg BW, Sigma, St. Louis, USA), confirmed by fasting serum glucose measurements 2–3 days after the administra-

tion of STZ, where glucose levels were found to be  $> 200$  mg/dL. In those mice that failed to reach this threshold, a second ip administration of STZ was performed and up to 5 days later the induction of diabetes was confirmed.

After the confirmation of the induction of diabetes, the mice were randomized into three groups; Control group ( $n = 13$ ); Low dose Mastic(LdM) group ( $n = 12$ ) animals that received low dose CMG for 8 weeks [20 mg/kg Body Weight (BW)]; High dose Mastic (HdM) group( $n = 12$ ) animals that received high dose CMG for the same period (500 mg/kg BW). The provided dosages of CMG are considered moderate to high and in agreement with the therapeutic dose, ranging from 1 to 5 g/person/day; yet no side effect of CMG has been reported in the existing literature. Furthermore, it's interesting to mention that the mastic gum is not toxic even in a dose of 28 g/person/day.<sup>5</sup> Because of the high insolubility of the CMG in water,

**Table 3.** Histopathological examination (scoring) of hepatic tissue in all groups at the end of the experimental period

Group	Liver Steatosis		Liver Focal Necrosis		Overall	
	Mean	SD	Mean	SD	Mean	SD
Control	1,46	0,51	0,46	0,66	2	0.58
LdM	0,58 <sup>a</sup>	0,51	0,33	0,49	1 <sup>a</sup>	0.80
HdM	0,41 <sup>a</sup>	0,66	0,08 <sup>b</sup>	0,29	0.5 <sup>a</sup>	0.85

3, severe; 2, moderate; 1, mild; 0, no changes in histology (scores)

<sup>a</sup>significant compared with the control group, ( $p < 0.001$ )

<sup>b</sup>marginally significant compared with the control group ( $p = 0,07$ )

its administration in the drinking water was not feasible. Thus, the total crude of CMG was ground to fine powder and then mixed with normal chow. During acclimation period food intake for each cage was recorded on a daily basis. The average amount of food consumed per mouse was then calculated in order to determine the final dose. This protocol was conducted every two weeks in order to ascertain compliance to the aforementioned dosage of CMG. In all groups normal chow (4FR25; Mucedola, Milan, Italy) was administered during the entire experiment. Mice had free access to food and water throughout the study (8-weeks). During this period, food and water consumption was daily recorded, while body weight was measured on a weekly basis.

Total CMG extract was obtained by steam distillation and was kindly donated by the Association of Chios Mastic Gum Growers (Chios, Greece), which is the exclusive worldwide producer of the mastic resin. Quality control of the CMG crude was assumed by the CMG Growers Association.

### 2.3 Blood collection- serum measurements

Blood samples were collected from mice at the establishment of diabetes (Baseline, T0), at 4 weeks (T1) and at the end of the experimental period (T2; 8 weeks). All blood samples were obtained in the morning after a 12-h fasting period, using capillary tubes introduced into the medial retro-orbital venous plexus under light ether anes-

thesia. The serum was separated by centrifugation at 3,000 rpm for 10 min.

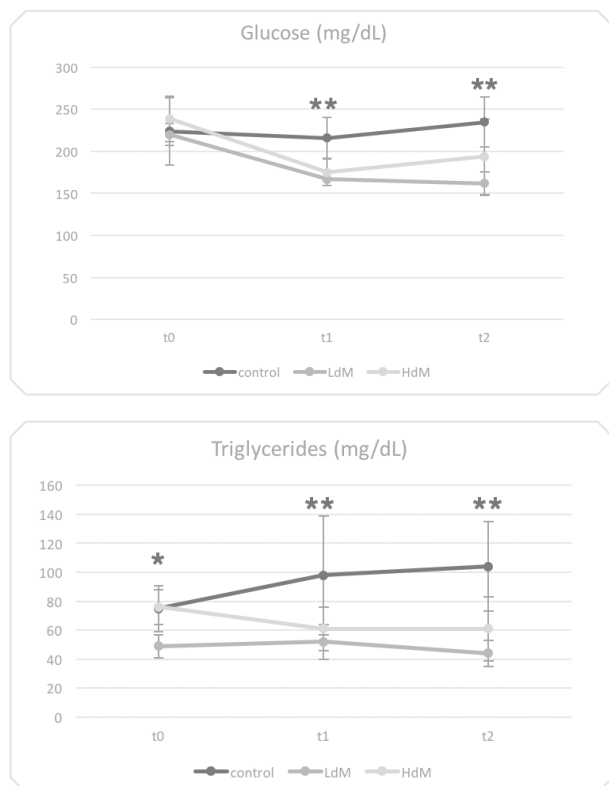
Serum concentrations of total cholesterol (Chol), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were determined using the enzymatic phenol- aminophenazone (PAP) commercial kit ("Biosis"-Biotechnological Applications, Athens, Greece). The calculation of low-density lipoprotein (LDL-C) was calculated by the Friedewald formula. Serum total protein (TP) levels were determined with commercially available kits (Biosis Biotechnological Applications, Athens, Greece). Serum glucose levels were determined using the Glycerol Phosphate Oxidase-Peroxidase (GPO-POD) method. ("Biosis"- Biotechnological Applications, Athens, GR).

### 2.4 Serum adiponectin, resistin and interleukin-6 levels

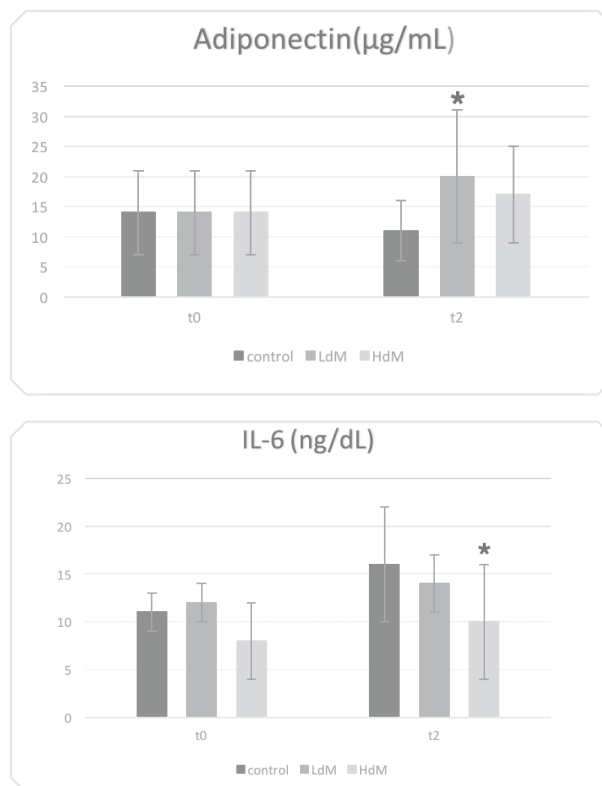
The Enzyme-Linked Immunosorbent Assay (ELISA) was used for the measurement of adiponectin, resistin and interleukin-6 (IL-6) levels using commercial available kits [Adiponectin: Biovendor, MOUSE Adiponectin Elisa, Cat. No RD293023100R (Intra-assay CV 2,3%; Inter-assay CV 4,7%); Resistin: Biovendor, MOUSE Resistin Elisa, Cat. No RD293016100R (Intra-assay CV 4,8%; Inter assay CV 5,6%); Interleukin-6: Booster Biological, MOUSE IL-6 Elisa, Cat. No EK0411 (Intra-Assay CV 6,4%; Inter-Assay CV 7,5%)].

### 2.5 Histopathological examination

Following euthanasia, liver, aorta and heart were



**Figure 1.** Glucose and triglycerides serum levels alterations throughout the study among groups; \*  $p < 0.05$ , \*\*  $p < 0.001$



**Figure 2.** Adiponectin and Interleukin-6 serum levels alterations throughout the study among groups; \*  $p < 0.05$

immediately fixed in 10% formalin at room temperature for 24 h. The tissues were then embedded in paraffin, sectioned and mounted on glass microscope slides. The sections were stained with hematoxylin eosin and examined using light microscopy by two independent researchers who were blinded to the randomization scheme. Specifically, the liver was evaluated as had been previously described by the Pathology Committee of non-alcoholic steatohepatitis Clinical Research Network. The histological features in liver were divided into five broad categories: steatosis, ballooning, portal inflammation, focal necrosis, and lobular activity. A score from 0 (absence) to 3 (severe lesion) was assigned to each parameter.<sup>15</sup>

## 2.6 Statistical analysis

Data are expressed as mean  $\pm$  1 standard deviation for continuous variables and as frequency (% percentage) for qualitative data. The normality of

the distributions was assessed with Kolmogorov-Smirnov's test and graphical methods.

Comparisons between more than two groups were performed with Analysis of Variance (ANOVA). Kruskal-Wallis's test was utilized as a non-parametric test for multiple group comparisons, using Mann-Whitney's U test for post hoc multiple testing. Comparisons between multiple time points were performed using Repeated Measures ANOVA and Friedman's test with Wilcoxon's Signed Ranks test for post-hoc comparisons.

Pearson's correlation coefficient and Spearman's rho were calculated in order to examine relationships between variables.

In all cases of multiple hypothesis testing, Benjamini-Hochberg's False Discovery Rate (FDR) was utilized in order to assess differences, as well as to control family-wise error to  $< 0.05$ .

All tests were two-sided. Differences were considered as statistically significant if the null hy-



pothesis could be rejected with > 95% confidence ( $p < 0.05$ ).

### 3. Results

#### 3.1 Body weight and metabolic profile

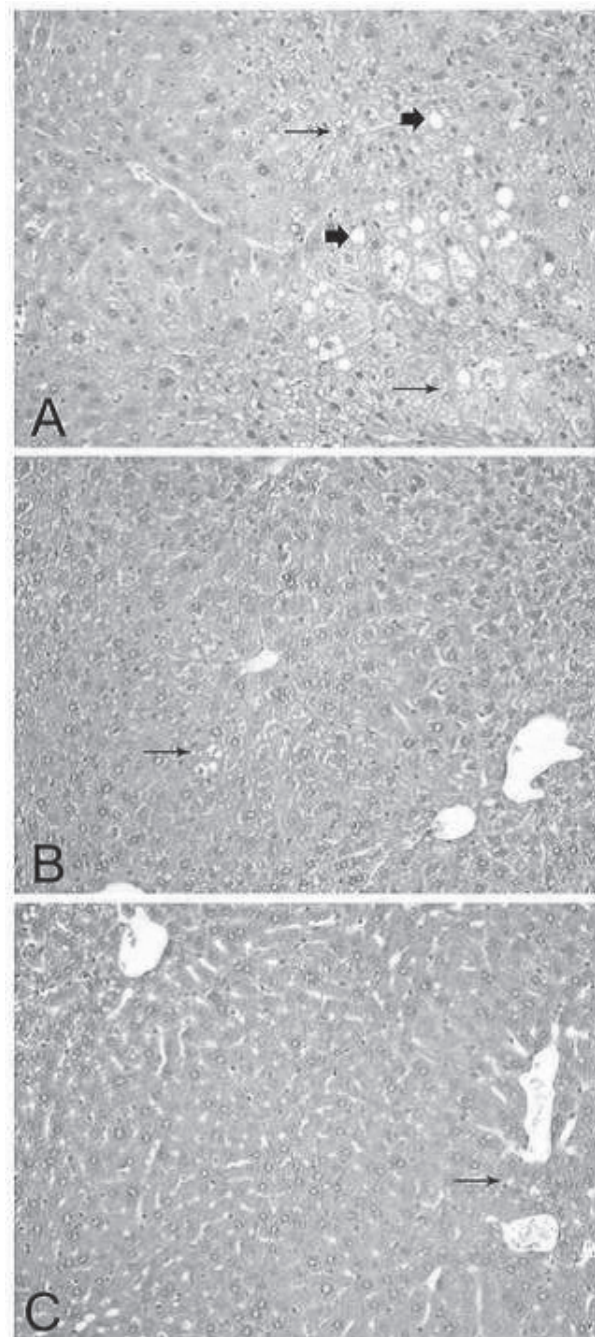
At baseline, no differences were observed in serum levels of glucose, total cholesterol, LDL-C and weight among the groups. Baseline levels of serum HDL-C and triglycerides were differed between groups ( $p < 0.05$ ) (**Table 1**). At T1, LdM group exhibited lower body weight and triglycerides as well as higher levels of HDL-C as compared with control group ( $p < 0.006$  in the first case,  $p < 0.003$  in the latter cases). At the end of the experiment, LdM group presented reduced body weight ( $p = 0.033$ ), serum total cholesterol levels ( $p < 0.001$ ), LDL-C ( $p = 0.0045$ ), triglycerides ( $p < 0.001$ ) and HDL-C levels ( $p < 0.014$ ) compared with the control group. HdM group had lower levels of both glucose and triglycerides compared with control group ( $p < 0.05$  and  $p = 0.006$ , respectively) (**Figure 1**).

#### 3.2 Serum adiponectin and IL-6

Serum adiponectin levels differed between LdM and HdM groups at the end of the experiment ( $p < 0.05$ ), yet no significant difference compared with control group was noted (**Table 2**). On the other hand, IL-6 levels were lower in HdM group compared with control group at T2 ( $p < 0.05$ ) (**Figure 2**).

#### 3.3 Histopathological examination

Hematoxylin-eosin stained liver samples obtained from all three groups showed signs of hepatic steatosis and focal necrosis (**Figure 3**). Steatosis and overall score of liver histopathology was higher in control group compared with the other two. (Steatosis: Control *vs.* LdM and control *vs.* HdM,  $P = 0.0105$  in all cases; Overall: Control *vs.* LdM and control *vs.* HdM,  $P = 0.036$  and  $P = 0.015$ , respectively) (**Table 3**). The grade of fatty liver disease was considered as "mild" in five of the nine mice in LdM group. No significant differences were detected in the grade of focal necrosis between groups. In addition, no differences were observed between LdM and HdM groups, regarding the grade of hepatic steatosis ( $p = 0.514$ ) and in the overall hepatic histopathology ( $p = 0.249$ ).



**Figure 3.** Hematoxylin and Eosin staining of liver tissue (A) moderate steatosis of liver parenchyma, both macrovesicular (thick arrows) and microvesicular (thin arrows) in control group. (B) and (C) snapshots from the CMG treated groups. Mild steatosis in both groups (thin arrows) was observed, although no significant difference existed between groups

### 4. Discussion

Currently, there is an increased interest and an ongoing search for natural agents with hypogly-

chemic potential but without the detrimental side effects presented in various antidiabetic drugs.<sup>16,17</sup> The present study investigated the impact of CMG administration on NAFLD and metabolic profile in STZ-induced diabetic rats.

In our study, both dosages of CMG decreased serum glucose levels and ameliorated hepatic steatosis. However, only low dose of CMG exhibited beneficial effects against hypertriglyceridemia.

#### 4.1 Induction of Diabetes and NAFLD

Diabetes induction was achieved by STZ administration, a well-described technique.<sup>18,19</sup> Regarding the establishment of NAFLD, STZ is among others a suitable candidate for achieving a model of liver steatosis. Specifically, STZ induces hyperglycemia and insulin resistance, which eventually leads to an increased fat deposition in the hepatic tissue.<sup>20</sup>

#### 4.2 CMG and Diabetes

Regarding serum glucose levels, a significant reduction after the 4-week treatment period for both high and low dose CMG groups which was sustained after 8 weeks of CMG treatment for LdM group, was recorded. Moreover, observation of the glucose levels throughout the experimental timeline, revealed an improvement in serum glucose levels in the two CMG crude supplemented groups. Albeit oxymoron, lower doses were accompanied with more favorable effects than higher doses. An inverse dose-dependent effect of CMG could be assumed, which cannot be furtherly interpreted since no known adverse effects of high dose CMG are described. Hence, further research is required in order to elucidate the exact molecular pathways implicated in the action of CMG, related with both its beneficial action and its potent- yet unknown- toxic effects. According to the literature, there are two compounds of CMG that are proposed to bear upon DM remission.<sup>10,21</sup> Oleanonic acid, which acts as a PPAR-gamma agonist may be implicated in the hypoglycemic activity of CMG, through a mechanism of action similar to that of glitazones.<sup>11</sup> Triterpenes, have shown valuable action on glucose metabolism, urging  $\beta$ -pancreatic cells to secrete insulin and in-

hibiting protein tyrosine phosphate-1B.<sup>22</sup> Hence, other mechanisms including the antioxidant and anti-inflammatory properties of CMG may be also related to both its hypolipidemic and hypoglycemic function.

#### 4.3 CMG and NAFLD

The implication of MCG on liver function has not been extendedly investigated. Albeit claims about the non-hepatotoxic effect of CMG administration, there is no accessible data regarding fatty liver.<sup>23</sup> The principal link between DM and NAFLD is insulin resistance at the level of the adipocytes which is responsible for down-regulation of the hormone sensitive lipase.<sup>3</sup> Subsequently, this leads first to elevated circulating free fatty acids (FFAs) and to their increased skeletal muscle and hepatic delivery and uptake, which decreases insulin action in these tissues. Insulin resistance in these tissues results in increased gluconeogenesis and glycogenolysis in liver and reduction in peripheral glucose disposal leading thus to hyperglycemia. The pancreatic beta islet cells adapt to hyperglycemia by increasing insulin secretion.<sup>24</sup> Contemporarily, hyperinsulinemia and hyperglycemia induce the upregulation of several lipogenic transcription factors, including sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate response element binding protein (ChREBP) in the liver, promoting, thus, hepatic de novo lipogenesis.<sup>25</sup>

Low-grade inflammation also appears to be involved in the pathogenesis of NAFLD and steatohepatitis.<sup>26</sup> Patients with NAFLD, either diabetic or normoglycemic, have higher circulating markers of inflammation than their respective controls.<sup>27</sup> It is now recognized that NAFLD and particularly NASH progression results from a complex interplay between insulin resistance with hyperinsulinemia, increased oxidative stress, hepatic and systemic inflammation.<sup>28</sup> Hyperinsulinemia combined with ongoing liver inflammation and hepatocyte apoptosis may induce profibrotic factors. Thus, profibrotic factors can contribute to fibrosis progression by activating hepatocyte stellate cells.<sup>24</sup>

CMG's role on NAFLD may be justified through



its glucose-lowering action and its antioxidant properties. Low glucose levels are associated with a negative feedback to SREBP-1c and ChREBP activity, halting, in this way, lipogenesis. Moreover, CMG's proven antioxidant effects contribute to lowering focal oxidative stress level in the hepatic tissue as an outcome of DM, diminishing, as such, the progress of NAFLD.<sup>21,29</sup> The fact that there were observed lower glucose levels in serum and remission of DM is in favor of a glucose-dependent mechanism of action of CMG on NAFLD. Its antioxidant and anti-inflammatory properties suggest a concomitant glucose-independent pathway. There is still to be investigated which of these two mechanisms is more efficient and prominent in the case of NAFLD and whether they are acting synergistically or interfering in certain cross-links.

#### 4.4 IL-6 and NAFLD

Among other critical pathways that have been identified as causing liver damage in patients with diabetes, both oxidative stress and inflammatory response aggravate the pathological conditions of diabetes. The inflammation-mediated liver damage constitutes a major mechanism with growing evidence.<sup>26</sup> Inflammation plays a vital role by releasing pro-inflammatory cytokines to protect the host from injury, including TNF- $\alpha$ , Interleukin -1 $\beta$  and IL-6.<sup>30,31</sup> In diabetic patients this chronic low-grade inflammatory state is linked to tissue damage, fibrosis and loss of cellular function in liver and is fundamental in the progression of NAFLD toward higher risk cirrhotic states.<sup>32</sup>

IL-6 is a liver and adipose tissue-derived proinflammatory cytokine that is implicated in hepatic and skeletal muscle. Specifically, upregulation of IL-6 is known to induce insulin resistance in hepatocytes through a NF- $\kappa$ B translocation into the nucleus, acting thus as a second hit in the pathophysiology of NAFLD.<sup>33</sup> The correlation of IL-6 with lobular inflammation grade in previous studies and the increased hepatic and circulating interleukin-6 levels in patients with nonalcoholic steatohepatitis suggest a damaging role of IL-6 in the liver tissue.<sup>32,34</sup>

Antioxidant therapy is a potential therapeutic

strategy in patients with diabetes-induced liver damage; lowering levels of cytokines may hopefully counter the effects of oxidative stress and inflammation.<sup>3</sup> A few plant-based products have been investigated as ways of protecting against and possibly reversing liver damage.<sup>35</sup> Our findings are comparable with previous studies; the decreased IL-6 levels in both mastic groups parallel to the reversed hepatic steatosis at the end of the experiment suggest strongly an IL-6 mediated antioxidant and hepatoprotective effect of CMG in NAFLD.

#### 4.5 Adiponectin, resistin and CMG

Adiponectin is an adipokine that has been recognized as a key regulator of insulin sensitivity and tissue inflammation. Current evidence supports that DM and obesity are linked to reduced levels of adiponectin.<sup>36</sup> Adiponectin also exhibits anti-inflammatory action, enhances the production of anti-inflammatory cytokines and it has been utilized as marker of insulin resistance and disturbances in lipid metabolism.<sup>37</sup> In our study, although serum adiponectin levels differed between the two groups which received CMG, this difference was not constant with the control group. Moreover, no significant difference was detected in resistin levels between the groups throughout the study. These two observations could be possibly attributed to the relatively short period of the CMG administration or due to small sample size.

#### 4.6 Lipidemic profile, weight and CMG

Current knowledge implies that CMG is efficient to prevent diabetes-induced hyperlipidemia due to its potent action as a lipoprotein lipase activator.<sup>38,13</sup> It is reported that the intraperitoneal administration of Camphene, a plant-derived monoterpene and CMG oil in normolipidemic and dyslipidemic rats ameliorated their serum lipid levels in a dose-dependent manner.<sup>12</sup> Our results are in line with these findings. Although a marked improvement in triglyceride levels after both low and high dose CMG administration was observed, the beneficial activity of CMG regarding serum total, LDL,

and HDL cholesterol levels was not detected in the high dose CMG administration. Thus, it could be assumed that the hypocholesterolemic activity of MG is dose-dependent. Moreover, animals of the LdM group exhibited lower body weight than their initial one. This observation ferments fertile ground for assumptions regarding weight-dependent mechanism of action of CMG. Yet, the weight of this group did not differ from the other two groups at any given time point. Current literature does not report on weight lowering action of CMG, however it is an aspect which could be further investigated in order to achieve a more thorough and total view of the effect of CMG.

#### 4.7 Limitations

One possible confounder that must be taken into account is that animals were housed in groups of three per cage, which did not enable the determination of the exact daily food and CMG consumption by each individual animal.

#### 4.8 Future Perspectives

Future studies should investigate in vitro and in

vivo all the different constituents of CMG separately, and examine their bioavailability in terms of selected target tissues or organs. The different beneficial actions of CMG-oil, besides its traditional uses, should also be investigated with the help of molecular biology techniques. Such high-throughput studies will help us understand further the pleiotropic actions of CMG and its constituents.

#### 5. Conclusion

CMG administered in low dosages appears effective in improving the metabolic profile and fatty liver in diabetic mice. CMG should be further investigated in future studies for its medicinal effects as a potent natural antidiabetic agent with additional hepatoprotective action. ◊

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#### Conflict of interest

All authors declare no conflict of interest.

## Περίληψη

# Προστατευτική επίδραση της Μαστίχας Χίου σε μοντέλο διαβητικής ηπατοπάθειας

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**Σκοπός:** Η μη αλκοολική λιπώδης νόσος του ήπατος είναι μία μορφή χρόνιας ηπατικής νόσου με υψηλή επίπτωση σε ασθενείς με Σακχαρώδη Διαβήτη. Η Μαστίχα Χίου είναι γνωστή για τις αντιοξειδωτικές της ιδιότητες και την πιθανή υπολιπιδαιμική της δράση. Σκοπός της παρούσας μελέτης είναι η διερεύνηση της επίδρασης της Μαστίχας Χίου στο μεταβολικό προφίλ και την ηπατική ιστολογία ενός πειραματικού μοντέλου διαβήτη με μη αλκοολική λιπώδη νόσο του ήπατος.

**Υλικό και Μέθοδος:** Χρησιμοποιήθηκαν 37 c57bl/6 ενήλικες μύες που τυχαιοποιήθηκαν στις αντίστοιχες ομάδες: Ομάδα Ελέγχου (n=13); Ομάδα Χαμηλής Δόσης [ΟΧΔ] (n=12) που έλαβε χαμηλή δόση Μαστίχας για 8 εβδομάδες (20 mg/kg σωματικού βάρους); Ομάδα Υψηλής Δόσης [ΟΥΔ] (n=12) που έλαβε υψηλή δόση Μαστίχας για 8 εβδομάδες (500 ml/kg σωματικού βάρους). Πραγματοποιήθηκε μέτρηση του λιπιδαιμικού και γλυκαιμικού προφίλ των μυών στην αρχή του πειράματος, στις 4 και 8 εβδομάδες αντίστοιχα. Ακολούθως, προσδιορίστηκαν τα επίπεδα της αντιγονεκτίνης, της ρεισοτίνης και της ιντερλευκίνης-6 στον ορό των μυών στην αρχή και το τέλος του πειράματος. Τέλος, διενεργήθη ιστοπαθολογική εξέταση του ηπατικού παρεγχύματος μετά την ευθανασία.

**Αποτελέσματα:** Μετά το πέρας των 4 εβδομάδων, η χορήγηση της Μαστίχας Χίου μείωσε τα επίπεδα γλυκόζης και τριγλυκεριδίων στον ορό ανεξαρτήτως δοσολογίας. Στο τέλος του πειράματος, η ομάδα που έλαβε χαμηλή δόση [ΟΧΔ], παρουσίασε χαμηλότερα επίπεδα γλυκόζης και βελτιωμένο μεταβολικό προφίλ σε σχέση με την Ομάδα Ελέγχου. Τα επίπεδα της αντιγονεκτίνης και της ρεισοτίνης στο τέλος του πειράματος δεν διέφεραν μεταξύ της Ομάδας Ελέγχου και των ομάδων παρέμβασης, ενώ η ιντερλευκίνη-6 ήταν χαμηλότερη στην ομάδα της χαμηλής δόσης [ΟΧΔ] σε σχέση με την ομάδα ελέγχου. Η ηπατική στεάτωση που παρατηρήθηκε στην ομάδα control είχε εν μέρει υποστρέψει στις άλλες δύο ομάδες που έλαβαν Μαστίχα.

**Συμπεράσματα:** Η χορήγηση μαστίχας Χίου σε χαμηλές δόσεις βελτιώνει τις μεταβολικές διαταραχές και ασκεί αντιφλεγμονώδεις ιδιότητες σε διαβητικούς μύες, ενώ παράλληλα μειώνει την ηπατική βλάβη.

**Λέξεις ευρητηρίου:** μαστίχα Χίου, σακχαρώδης διαβήτης, μεταβολισμός λιπιδίων, μύες, μη αλκοολική λιπώδης νόσος του ήπατος, ιντερλευκίνη 6

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