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Original Article

Caffeine Consumption Protects Against High Fat Diet-Induced Hepatic steatosis

Samar E. Ayoub[3], Manar G. Helal[1*], Wagdi F. Elkashef[2] and Tarek M. Ibrahim[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy & [2]Department of Pathology, Faculty of Medicine, Mansoura University, Egypt
[3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Kafr El-sheikh University, Egypt

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases and is a part of the metabolic syndrome. Currently, there are interventions that used to treat risk factors associated with NAFLD. Caffeine treatment exerted hepatoprotective, antioxidant effect and limit the hepatocellular injury. This study examined whether caffeine can ameliorate liver injury induced by high fat diet (HFD) feeding. Three groups of rats were used and treated for 16 weeks as follow: CTRL group, rats were fed a standard diet; HFD group**,** rats were fed HDF; and Caffeine 10 group**,** rats were fed HDF for 16 week in addition to caffeine (10 mg/kg) for last 8 weeks. The HFD-induced liver injury is determined biochemically and by histopathological examination. Results showed that caffeine treatment significantly decreased the elevated serum ALT, AST. Furthermore, caffeine reduced lipid profile biomarkers. In conclusion, this study revealed that caffeine treatment exerted hepatoprotective, fostering lipid metabolism and limit the hepatocellular injury induced by HFD.

Keywords: caffeine, hepatic steatosis, Rats, HFD, liver enzymes

Address for Correspondence: Dr. Manar G. Helal, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura, 35516, Egypt;
Email: manargamal2008@gmail.com

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is build-up of excess fat in the liver in the absence of alcohol consumption, the liver become vulnerable to further injury, which may result in inflammation and scarring of the liver [1]. The hallmark of NAFLD is disruption of synthesis, and metabolism of fatty acids and triglycerides (TG) with increasing predisposition of the liver to injury mediated by release of inflammatory cytokines and oxidative stress, which lead to steatohepatitis [2]. A high fat diet is usually used to induce experimental NAFL [3]. Caffeine has drawn great attention for its ability to target NAFLD treatment strategies through the ability of caffeine to increase the fat oxidation, lipolysis and weight loss is associated with a reduced risk of elevated liver enzyme levels [4]. This study aims to investigate the effect of High Fat Diet on nonalcoholic fatty liver disease for 16 weeks and determine the effect of administration of caffeine (10 mg/kg/day) on the development of disease in the last 8 weeks.

MATERIALS AND METHODS

Animals: Adult male wistar rats (n=30, 90-150 gm) were obtained from "Egyptian Organization for Biological Products and Vaccines", Agouza, Giza, Egypt. Rats were allowed to free water access throughout the whole experiment. The experimental work performed in this study comes in accordance with ethics and instructions for the use and care of experimental animals approved by the Scientific Research Ethics Committee of Faculty of Pharmacy, Mansoura University.

Drugs and Chemicals: Caffeine was obtained from Sigma-Aldrich (St Louis, MO, USA). The high fat-diet (HFD) components were purchased from local commercial sources. Other chemicals were purchased of best grades from available companies.

Experimental Design: Nonalcoholic fatty liver was induced by daily consumption of HFD which consisted of 45 % lipid (butter), 30 % carbohydrate (black honey) and 25 % protein [5], the rats were randomly assigned into three groups and treated as follows for 16 weeks:

Group (1) (n=10): rats were fed a standard diet and received the vehicle

Group (2) (n=10): rats were fed HDF and received the vehicle

Group (3) (n=10): rats were fed HDF for 16 week in addition to caffeine (10 mg/kg/day, oral) for last 8 weeks.

Rats were anesthetized and blood samples were collected for determination of liver functions and lipid profile. Liver tissues were also collected and fixed in formalin for histopathological examination and determination of TGF-β1.

Assessment of liver functions: The liver parameters include alanine aminotransferase (ALT), aspartate aminotransferase (AST) were assessed in serum by kits obtained from Biomed Diagnostics, Egy-Chem (Badr City, Egypt).

Lipid profile evaluation: Serum levels of total cholesterol, triglycerides and HDL-C were measured using diagnostic kits purchased from Biomed Diagnostics, Egy-Chem (Badr City, Egypt).

Histological examination and Immunohistochemistry analysis of TGF-β1 expression: Paraffin blocks were prepared using standard histopathological techniques. Steatosis changes were evaluated in sections (5 μm thick) stained with Masson's trichrome and hematoxylin-eosin, respectively, using the histological scoring system of Brunt and associates in 1999 [6]. After staining, all findings were evaluated using five high-power fields (400x).

Statistical Analysis: Data are expressed as mean ± S.E. in each experimental group. The results were statistically evaluated by means of one way analysis of variance, followed by Tukey–Kramer multiple comparison test using GraphPad Instat V 3.05 (GraphPad Software Inc, San Diego, CA, USA).

RESULTS

Liver Function Biomarkers: HFD causes significant ($P< 0.05$) increases in serum ALT&AST levels when compared to control group. A significant decrease in serum ALT and AST was observed in groups treated with caffeine (10 mg/kg) ***as shown in figure*** **(1)**

Lipid profile biomarkers: HFD significantly ($P< 0.05$) increased serum cholesterol& triglycerides levels and a significant decrease in serum HDL-cholesterol level when compared to control group. The high levels of serum cholesterol& triglycerides were significantly ($P< 0.05$) decreased by caffeine (10 mg/kg) as shown in ***figure*** **(2A)** and increase serum HDL-cholesterol level as shown in ***figure*** **(2B)**.

Effect on histological changes of the liver tissues in HFD-induced hepatic steatosis: HFD groups showed marked steatosis that is macrovesicular with or without foci of mild lobular or portal inflammation, Hepatocyte injuries with hepatocellular ballooning and inflammation with minimum fibrosis. Caffeine decrease steatosis,

ballooning score and inflammation score, compared with the HFD group (**fig 3A, 3B).**

Effect on hepatic expression of TGF-β1 in HFD-induced hepatic steatosis: The results showed that protein levels of TGF-β1 remarkably increased in the livers of HFD group compared to the CRTL group **(fig 4)**. Also, treatment significantly decreased the TGF-β1 expression, compared with the HFD group.

DISCUSSION

In this study, it was shown that consumption of a 45% fat content diet for 16 weeks causes accumulation of lipids within the hepatocytes of rats [7]. It was reported that in response to an augmented consumption of fat, the liver becomes rapidly after 2 weeks and highly penetrated with lipids, to reduce its lipid content in subsequent weeks (weeks 4 – 6) [8].

The present study demonstrated that 16 weeks of HFD consumption induced hepatocellular injury and hepatic steatosis that can be confirmed by serum AST and ALT activities, serum lipid disorders and liver histopathological examination.

In this study, hepatocellular injury in response to HFD can be explained by the elevated AST and ALT in the blood. These markedly elevated enzymatic activities are in agreement with results of [9]. Caffeine administration for the last 8 week explained a positive hepatoprotective effect by its ability to significant decrease serum enzymatic activities of ALT and AST. These effects are in agreement with previous studies of Modi *et al.*, (2010) [10].

The current study indicated that NAFLD was connected with higher level of triglycerides, high levels of total cholesterol and lower HDL-C. It was showed that triglyceride accumulation may be due to excessive importation of free fatty acids to the liver from adipose tissue in response to lipid overloading [11].

Also the hepatic lipids are stored in the form of triglycerides (TG), cholesterol and free fatty acids (FFA) in NAFLD [12]. All that indicate increase in triglycerides and total cholesterol level [13].

Effects obtained from this study prove that caffeine induces a significant decrease in serum TG and cholesterol resulted in significant hypoglycemic and hypolipidemic effects. It was demonstrated that caffeine induced its inhibitory effects of accumulation of fat through suppressing enzymes activities involved in the fatty acid synthesis. Also the effect of caffeine was illustrated by significant rise in serum HDL level. In addition, we need increase dose of caffeine.

In the current study, It has been reported that the grading of NAFLD includes steatosis, ballooning and lobular inflammation. Steatosis was evaluated by analyzing macrovesicular steatosis and microvesicular steatosis. Then bridging fibrosis with central to portal fibrous septa formation is seen. Our histological observations in liver are in good agreement with previous studies [14].

In the current study, immunohistochemistry technique was used to observe TGF- β1 expression in the liver tissue. It has been shown that TGF- β1 expression upregulated in HFD with activation of hepatic stellate cells (HSCs). TGF-B signaling in hepatocytes stimulated hepatic steatosis hepatocyte damage and fibrosis. TGF-B signaling also stimulated the accumulation of lipid with generation of lipogenesis-related genes [15]. Immunohistochemistry analysis indicated that caffeine treatment decrease the expression areas of TGF-B. Anti-fibrotic effect of caffeine may be through inhibition the activation of HSC [16].

CONCLUSION

Results obtained from this study confirm that caffeine may act as antihyperlipidemic hepatoprotective agent and may serve as a good protector against damage caused by NAFLD.

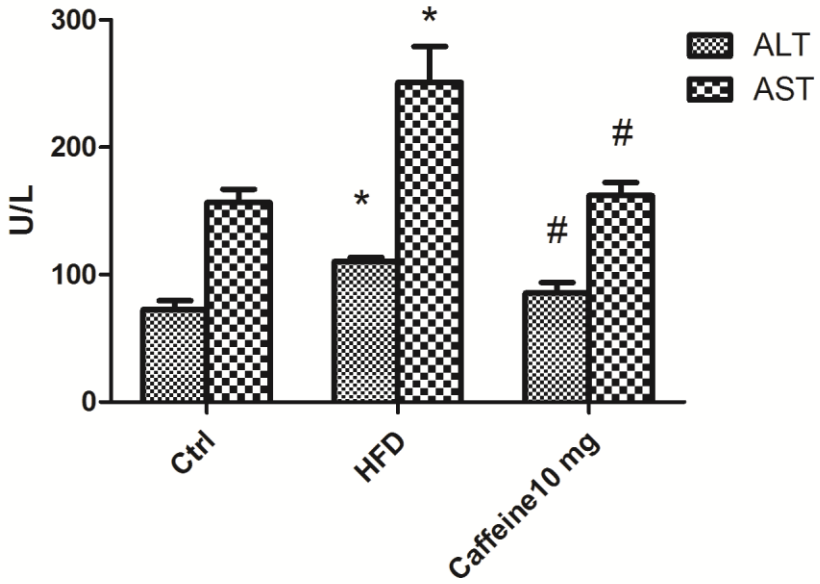

Figure ***(1): Effect of caffeine administration (10 mg/kg) on serum ALT, AST levels in HFD induced hepatosteatosis in rats.***
Values represent the mean ± SEM of 5 animals; Data were statistically evaluated by means of ***one way analysis of variance*** followed by ***Tukey-Kramer*** multiple comparisons test; * *P<0.05,* compared with control group
# *P<0.05,* compared with HFD group

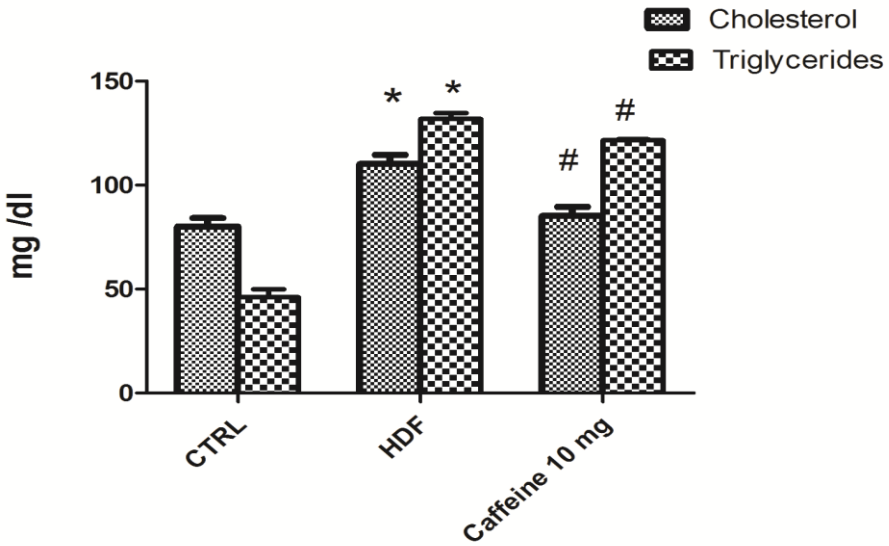

Figure (2A): Effect of caffeine consumption (10 mg/kg) on serum cholesterol and triglycerides levels in (HFD) induced hepatosteatosis in rats.
Values represent the mean ± SEM of 5 animals; Data were statistically evaluated by means of ***one way analysis of variance*** followed by ***Tukey-Kramer*** multiple comparisons test; * *P<0.05,* compared with control group; # *P<0.05,* compared with HFD group

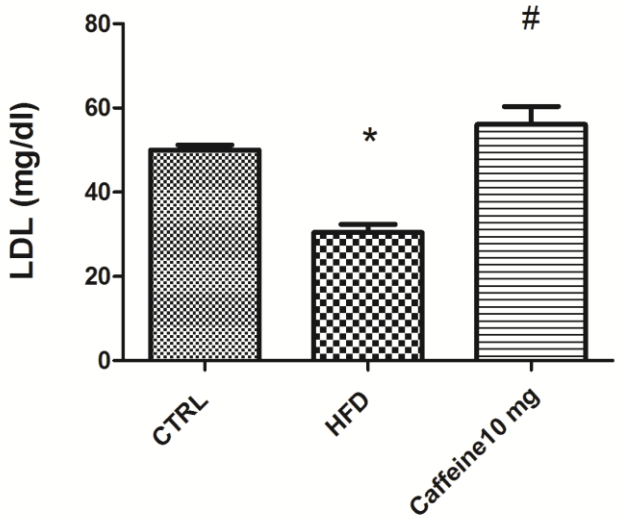

Figure (2B): Effect of caffeine consumption (10 mg/kg) on serum HDL-C level in (HFD) induced hepatosteatosis in rats.

Values represent the mean ± SEM of 5 animals; Data were statistically evaluated by means of ***one way analysis of variance*** followed by ***Tukey-Kramer*** multiple comparisons test; * *P<0.05,* compared with control group; # *P<0.05,* compared with HFD group

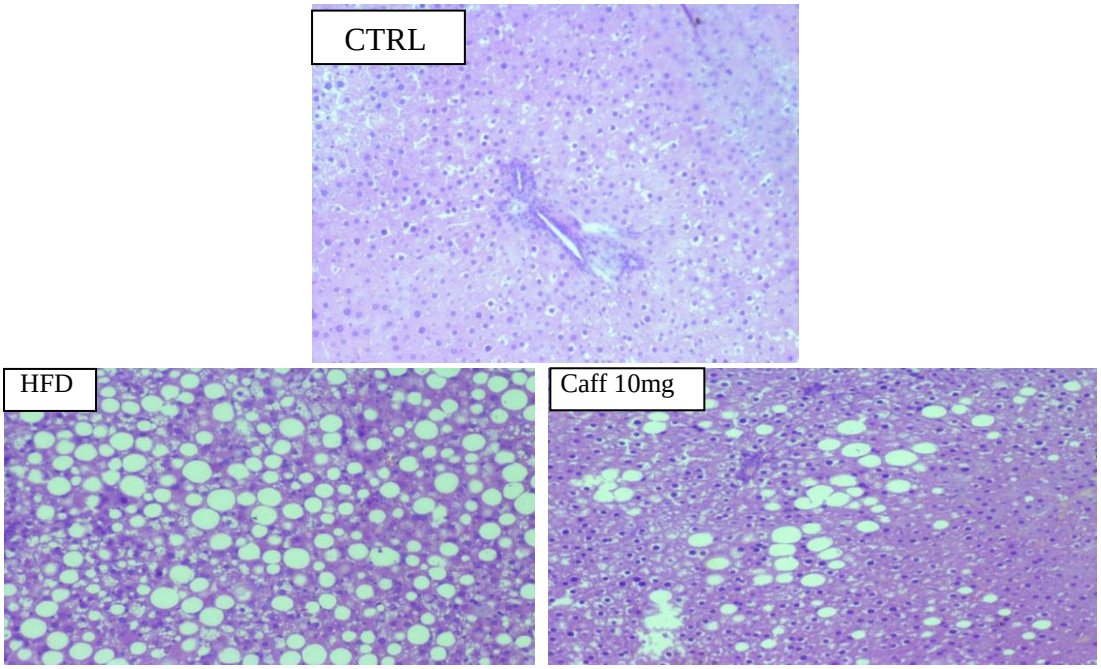

Figure (3A): Representative hepatic histopathology showing effect of caffeine consumption (10 mg/kg) on steatosis scoring (hematoxylin-eosin staining, 40ox) in HFD-induced hepatic steatosis.

(CTRL) control group, normal hepatic cells with well- preserved cytoplasm, prominent nucleus and central (HFD) HFD groups showed marked steatosis that is macrovesicular with or without foci of mild lobular or portal inflammation (Caff 10mg/kg) caffeine-treated group decrease steatosis and ballooning score.

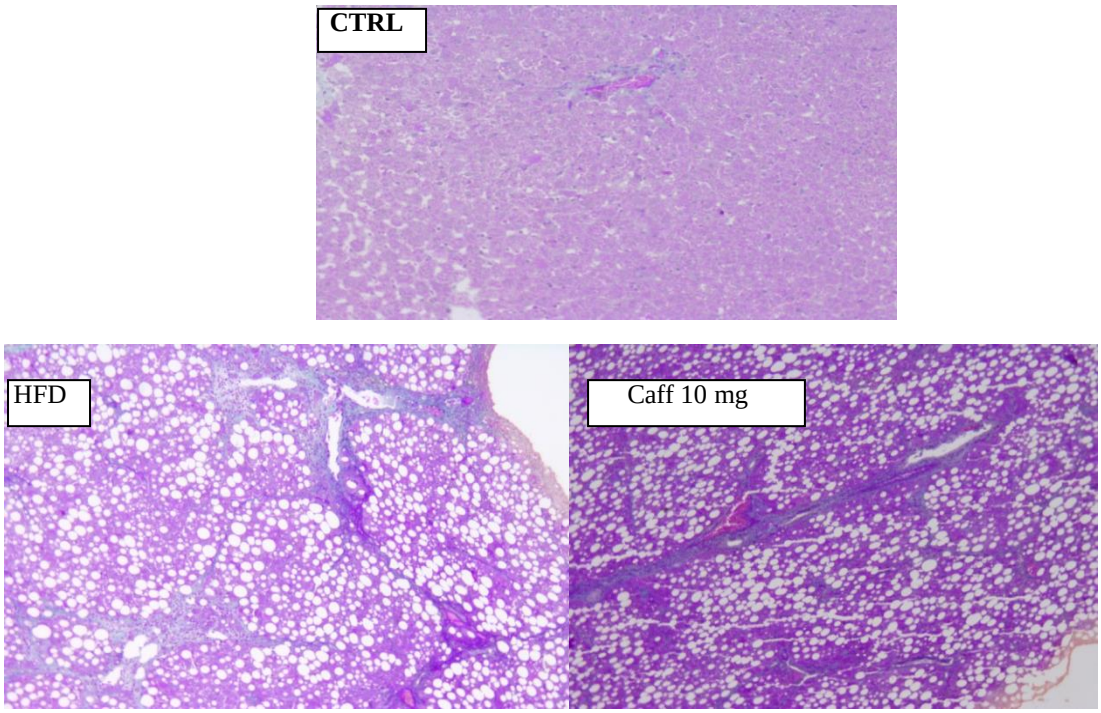

Figure (3B): Representative hepatic histopathology showing effect of caffeine consumption (10 mg/kg) on fibrosis (Masson's trichrome staining, 400x) in HFD-induced hepatic steatosis.

HFD: HFD groups showed a relatively periportal fibrosis with portal-portal septa framing liver lobules.

Caff (10mg/kg): caffeine-treated group decreased the HFD-induced fibrosis score increase.

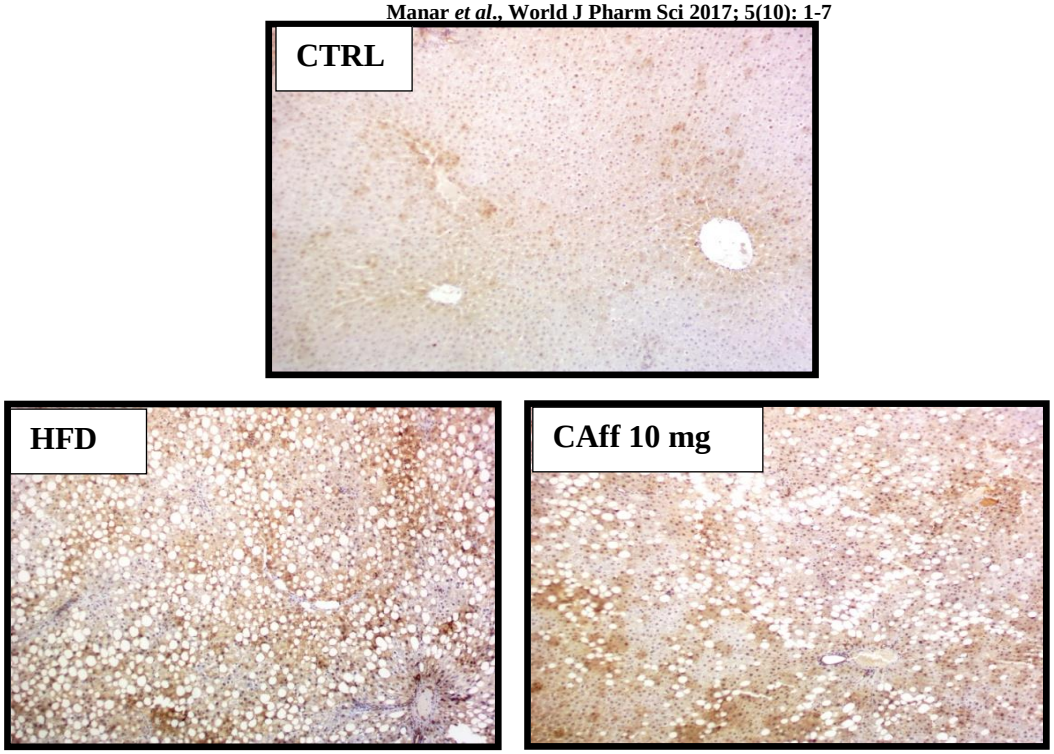

Figure (4): Representative hepatic histopathology showing HFD-induced changes in the expression of transforming growth factor-β1 (TGF-β1) protein by immunohistochemistry of CTRL, HFD and Caff (10 mg/kg).

Immunohistochemical analysis indicated that the TGF-β expression remarkably increased in the livers of HFD-fed group compared to the control group. *C*affeine-treated groups demonstrated in significant decrease the TGF-β expression.

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