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Phytochemical Identification Components of Coffee Arabica Extracts and Its Role in Alleviation Alterations of Tetracycline Induced Non-alcoholic Steatohepatitis (NASH) in rats.

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Abstract

High doses of tetracycline (TET) caused serious health effects, including non-alcoholic steatohepatitis (NASH). We identified the bioactive content of *Coffee Arabica* extract and its possible role in the alleviation of NASH induced by TET in rats. Sixty male albino rats (150 ± 10 g) were allocated into six groups: Control, TET: it received TET orally (1 g/kg BW) for 8 days; group III (CAME): it received Coffea Arabica methanolic extract (CAME) orally (0.1 g/kg) for 4 weeks; group IV (treated): it received TET then treated with CAME for 4 weeks; group V (preventive): it received CAME for 4 weeks then received TET orally for 8 days, VI (Protected): it received TET and CAME orally for 8 days. The total phenolic in the aqueous, methanolic, and ethanolic extracts were 751.36, 1259.09, and 1146.82 mg GAE/g, respectively. The GC/MS analysis showed the highest content of chlorogenic acid. The CAME ameliorated the liver alterations in TET-intoxicated rats by reducing the indexes of liver function and lipid profile (p<0.001), enhanced antioxidant capacity. CAME preventive is the most effective in liver alteration as portal vein congestion, scattered apoptosis and inflammatory infiltration. The autophagy signals as mTOR and LC3 will be investigated for more explanation its mechanism of action.

Keywords: Non-alcoholic steatohepatitis (NASH), tetracycline, *Coffee Arabica* – GC/MS - rats.

Introduction

Non-alcoholic steatohepatitis (NASH) is an advanced fatty liver that become the most prevalent cause of liver disease globally [1]. NASH is a multifaceted disease with a complicated pathophysiology; the two-hit and multiple-hit theories, have been proposed to explain its pathophysiology [2]. Inflammation, damage, and hepatic steatosis is the main character of NASH [1]. It tends to worsen with time and high incidence of cirrhosis and hepatocellular carcinoma (HCC) [3].Worldwide, about 25% of individuals globally is predicted the leading cause of liver transplantation by 2030 [4].The most common causes of NASH are dyslipidemia, hyperglycemia, and obesity. Over 25% of patients who are extremely obese have high risk of NASH, 40% of them showed advanced stage of fibrosis [5]. Patients with NASH frequently have no symptoms at all until the illness develops into cirrhosis, at which point they suffer more severe symptoms such as jaundice, hepatic encephalopathy, and ascites and may finally to decompensated cirrhosis [6]. Tetracycline (TET) is a broad-spectrum anti-bacterial drug used in the management of infection cases, but at higher doses, it was found to cause toxicological effects such as nephrotoxicity, hepatotoxicity, and testicular damage in an animal model [7]. Experimentally, TET was used for induction of NASH in rats.

Alternative and complementary medicine from natural products as fruits, vegetables, and beverages like coffee and tea are due to their antioxidant potency components that showed an improvement in health conditions. Regular consumption of polyphenol-rich foods decreases the incidence of metabolic disorders associated with NASH [8].

Polyphenols exert antioxidant properties via removal of free radicals and protect the blood vessels, heart and decreased incidence of diabetes and cancer [9]. Coffee drinks are consumed in high rate over the world. It differ from culture to other, green or roasted with different degree. Also, its rate differ from one to another. Coffee is a powerful antioxidant agent due to its high content of alkaloids, flavonoids, and phenolics. It was reported that drinking coffee three times daily is associated with healthy status [10]. The natural antioxidants found in green coffee avoid free radicals damaging effect [11]. Arabica coffee beans have an antioxidant level ranging from 6–7%, including polyphenol compounds [12]. Up to 90% of the polyphenols in green coffee are found in chlorogenic acid (CGA), whose level in Arabica seeds varies between 6–7% [13].

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In vivo study, green *Arabica coffee* extract ameliorated the abnormalities of testicular function in diabetes rats via , attenuated inflammatory mediator release. Its action was mediated by potentiate the antioxidant capacity and reduce reactive oxygen species release [14]. In addition, it helps in clear blood clots and avoid strokes in rats [15]. Up-till now, there is no study investigating the importance of *Arabica coffee* in management of NASH. The rational of current investigation was identification of *Arabica coffee* bioactive components by GC/MS and its relevant impact in attenuation of NASH induced by tetracycline in rat model via modulation of dyslipidaemia , antioxidant capacity, liver indices and hepatocellular steatosis ,apoptosis and congestion by histopathological examination.

Materials and Methods.

Extraction of samples

The fresh Arabica coffee beans were obtained from market in Cairo, Egypt. The ground samples were soaked for 24 hours in solvents (80% methanol, 80% ethanol, and pure water) [16]. Ten grams of Arabica coffee was added was approved by the Ethics Committee of the Faculty of Science at Ain Shams University, Egypt. (ASU-SCI/BIOC/2023/4/1). to 100 ml of the each solvent inside a conical flask. Then the mixture was homogenized for three minutes in a homogenizer. Following filtration. A rotary evaporator under vacuum was used to dry the collected filtrates.

Determination of total phenolic content of Arabica coffee extract and GC-MS identification components.

The phenolic content of different solvents extracts were quantified using the Folin-Ciocalteu method [17]. The data were expressed as mg/g gallic acid. The GC-MS, Agilent Technologies 7890B GC Systems combined with the 5977A Mass Selective Detector was applied for identification components.

Determination of total antioxidant capacity of Arabica coffee extract.

The total antioxidant capacity was quantified using 2,2-diphenyl-1-picrylhydrazyl (DPPH).
[18].
The scavenging activity was calculated from the control sample using the following equation:
DPPH scavenging effect (% inhibition)=((Ac-At)/Ac)×100, where the Ac, A t : Absorbance of the control and test solution.

Animals.

Eighty male albino Wister rats, weighting 150 g±10 g, were obtained from VACSERA, Helwan, Egypt. They were maintained in normal environmental conditions .The handling of experimental animals used in the present study

Detection the suitable tetracycline dose for Induction of NASH in rat model.

In a pilot study, first group (n=5): Rats received tetracycline dissolved in water (140 mg/kg BW, i.p) for 3 days. A section of the liver was examined using haematoxylin and eosin for histopathological analysis. Liver showed normal with no steatosis or inflammation with minor modifications [19].
Second group (n=5): Rats received tetracycline orally (140 mg/kg BW) for 7 days; histopathological examination of liver showed mild micro-vesicular steatosis with no inflammation [19].
Third group (n=5): Rats received tetracycline (1 g/kg BW) for 4 days. After that, histopathological analysis showed mild micro-vesicular steatosis with portal inflammation [20].
Fourth group (n=5): rats received tetracycline (1g/kg BW) for 8 days. Histopathological analysis showed marked micro- and macro-vesicular steatosis with peri-portal and portal inflammation with minor modifications [20].This is the suitable dose selected for NASH induction model.

Animals study Design.

Six equal groups of rats were allocated as:
Group 1 (C): Rats were considered as control.
Group 2 (NASH): Rats received tetracycline orally (1 g/kg BW) for 8 days [20] with minor modifications.
Group 3 (CAME): Rats received *Coffee Arabica* methanolic extract orally (100 mg/kg BW), for 4 weeks [14].
Group 4 (treated): Rats received TET orally (1g/kg) for 8 days, then treated orally with CAME (100 mg/kg) for 4 weeks.
Group 5 (prevented): Rats received CAME orally (100 mg/kg) for 4 weeks, then received TET orally (1 g/kg) for 8 days.
Group 6 (protected): Rats co-administrated TET (1g/kg) and CAME orally for 8 days.

The rats were fasted for 24 hours. The blood were drawn under anaesthesia with thiopental in vacutainer sodium citrate tubes , centrifuged at 4000 rpm for 10 minutes. Plasma and serum were stored at -20°C for analysis.

Biochemical analysis.

Liver function tests, such as alanine aminotransferase (cat. no. 264001), aspartate aminotransferase (cat. no. 260001), and alkaline phosphatase (cat. no. 214001) activities, albumin level (cat. no. 210001), and total protein level (cat. no. 310001), were measured in serum using colorimetric method . PT (cat. no. 614000) and PTT (cat. no. 626001) were estimated in plasma using an appropriate diagnostic kit purchased from Spectrum Diagnostic, Egypt. Lipid profile tests, such as total cholesterol (cat. no. 230001), HDL-C (cat. no. 266001.), LDL-C, VLDL-

C, and triglycerides (cat. no. 314001). Oxidative stress biomarker as total antioxidant capacity (Bio Diagnostic, Egypt), cat. no. TA 2513.

Histopathological examination

The liver was removed, cleaned in isotonic sterile saline, and cut into suitable pieces in a 10% formalin solution for 24 h. Sections were cut and stained with hematoxylin and eosin, according to Drury (1983), which were examined by the CX 41RF, Olympus Corporation microscope (Tokyo, Japan), and Olympus Soft Imaging SC100 camera.

Statistical analysis

SPSS version 22.0 was utilized for statistical analysis (IBM Corp, NY, USA). Data distribution was performed \. After which continuous data were expressed as mean ± standard deviation (SD), One-Way ANOVA tests. The deviations were assessed using a Microsoft Excel sheet generated in-house, and the Chi-square (χ^2) test was utilized for categorical variables' comparison. The *p*-values were two-sided, and 0.05 was chosen as the statistically significant *p*-value.

Results.

Phytochemical identification of *Coffee Arabica* extracts.

The GC/MS analysis of *Coffee Arabica* methanolic , ethanolic and water extracts showed that a higher number of compounds were extracted from the methanol solvent than water and ethanol solvents. It showed the highest content of chlorogenic acid.

Total polyphenol content of *Arabica coffee*

The total polyphenols in the aqueous, methanolic, and ethanolic extracts were 751.36, 1259.09, and 1146.82 mg GAE/g, respectively.

Antioxidant activity of *Arabica coffee.*

The DPPH scavenging activity of water, methanol, and ethanol extracts were found 84.08%, 94.16, and 92.3%, respectively. The methanolic extract showed the highest antioxidant activity .

Biochemical analysis.

Data obtained presented a significant elevation ($p \leq 0.001$) in serum ALT, AST, and ALP in tetracycline-injected rats compared with controls. The CAME treatment recovered these changes in the treated, protected, and prevented groups. However, the preventive group is the most effective. None significant difference in the CAME group versus normal (table 1).

A statistical decrease ($P \leq 0.001$) in albumin and protein levels in tetracycline-injected rats compared with normal group. The CAME administration in the treated, prevented, and protected groups resulted in a significant elevation ($P \leq 0.001$) in albumin and protein levels versus NASH group. Coagulation indices (PT and PTT) showed a significant increase in tetracycline-injected rats ($P \leq 0.001$) versus normal rats. However, PT and PTT were reduced ($P \leq 0.001$) in the treated, preventive, and protected groups after treatment versus to the NASH group. Lipid profile showed a significant elevation ($p \leq 0.001$) in cholesterol, TG and LDL-c levels and a reduction in HDL-c in rats injected with TET versus normal rats (table 2). On the other hand, rats treated with CAME showed a significant decrease in the level's total cholesterol, triglycerides, LDL-c, and VLDL-c, accompanied by a remarkable elevation in HDL-c in comparison with the TET-treated group .The total antioxidant activity was reduced significantly ($p \leq 0.001$) in TET-injected rats versus the normal. CAME improved the activity in either treated, preventive, or protective ($p \leq 0.001$) versus untreated. The preventive group showed most effect than protected or treated (table 3).

Histopathological studies

The histological examination of the normal liver showed average portal tracts with average portal veins and hepatocytes (Figure 1a). While CAME extract showed same structure as normal (Figure 1b). The NASH group showed mildly oedematous portal tracts with mild inflammatory infiltrate, short fibrous bands, apoptosis of hepatocytes (Figure 1c,1d). Liver of the treatment group showed portal tracts with mild portal inflammatory infiltrate, scattered apoptotic hepatocytes (Figure 2a). In the prevented group, portal tracts mildly congested portal veins (Figure 2b). While the protected showed mild portal inflammatory infiltrate, and mildly dilated central veins (Figure 2c,2d). Table 7 showed the severity and incidence of histopathological changes in all groups.

Table 1. Statistical analysis (ANOVA) for liver function tests in the different groups

Parameters	Group (1) Control	Group (2) NASH	Group (3) CAME	Group (4) Treated	Group (5) Prevented	Group (6) Protected
Albumin (g%)	3.67 ±0.08^{b}	3.12 ± 0.07^{a}	3.65 ± 0.04^{b}	3.44 ± 0.03^{ab}	3.56 ± 0.05^{ab}	3.21 ± 0.06^{ab}
Total proteins (g%)	8.77 ±0.2^{b}	7.69 ± 0.21^{a}	8.73 ± 0.3^{b}	8.10 ± 0.31^{ab}	8.50 ± 0.07^{ab}	8.03 ± 0.23^{ab}
ALT (U/L)	12.5 ±1.38^{b}	37.5 ± 2.07^{a}	12.83 ± 1.47^{b}	18.5 ± 1.05^{ab}	22.67 ± 2.73^{ab}	27.83 ± 2.48^{ab}
AST (U/L)	44 ±4.24^{b}	85 ± 7.29^{a}	47 ± 2.83^{b}	55.17 ± 4.58^{ab}	51.17 ± 4.54^{ab}	59.33 ± 4.72^{ab}
ALP (U/L)	177.33 ±18.16^{b}	416.83 ±20.57^{a}	178.33 ± 15.63^{b}	335.83 ± 13.39^{ab}	224.5 ±19.89^{ab}	394 ± 11 ^{ab}
PT (sec)	13.17 ±1.17^{b}	25 ± 3.22^{a}	13.5 ± 1.87^{b}	20.17 ± 1.17^{ab}	18.17 ± 1.94^{ab}	22 ± 1.26^{ab}
PTT (sec)	32.33 ± 2.25^{b}	45.33 ± 3.39^{a}	32.33 ± 1.75^{b}	39.33 ± 3.72^{ab}	41.33 ±1.63^{ab}	42 ± 2.68^{ab}

ANOVA: analysis of variance; ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; PT: prothrombin time; PTT: partial thromboplastin.
Each value is represented as mean ± standard deviation (mean ± SD). Data with different superscripts are significantly different at $p \leq 0.05$.
[a] significance vs control group, [b] significance vs NASH group

Table 2. Statistical analysis (ANOVA) for lipid profile in the different groups

Parameters	Group (1) Control	Group (2) NASH	Group (3) CAME	Group (4) Treated	Group (5) Prevented	Group (6) Protected
TC (mg%)	133.33±11.93^{b}	197.5 ± 9.04^{a}	134.17 ± 10.15^{b}	180.83 ±9.24^{ab}	168.83 ±4.75^{ab}	186 ± 0.89^{ab}
TG (mg%)	137.67±13.32^{b}	271.67 ±20.07^{a}	138.33 ± 16.16^{b}	224 ± 14.3^{ab}	169.33 ±6.59^{ab}	248.83 ±14.99^{ab}
HDL-C (mg%)	88.33 ±8.69^{b}	54 ± 3.74^{a}	86.33 ± 3.39^{b}	67.17 ± 4.36^{ab}	75.33 ± 2.16^{ab}	61.17 ± 2.23^{ab}
LDL-C (mg%)	17.47 ±7.32^{b}	89.17 ± 8.44^{a}	20.17 ± 11.89^{b}	68.87 ± 6.63^{ab}	59.63 ± 5.7^{ab}	75.07 ± 2.52^{ab}
vLDL-C (mg%)	27.53 ±2.66^{b}	54.33 ± 4.02^{a}	27.67 ± 3.23^{b}	44.8 ± 2.86^{ab}	33.87 ± 1.32^{ab}	49.77 ± 3^{ab}

ANOVA: analysis of variance; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; vLDL-C: very low-density lipoprotein cholesterol.
Each value is represented as mean ± standard deviation (mean ± SD). Data with different superscripts are significantly different at $p \leq 0.05$.
[a] significance vs control group, [b] significance vs NASH group

Table 3. Statistical analysis (ANOVA) for serum total antioxidant capacity in the different groups

Groups	TAC (mM/ L)
Group (1) Control	1.93 ± 0.09^{b}
Group (2) NASH	0.5 ± 0.07^{a}
Group (3) CAME	1.92 ± 0.08^{b}
Group (4) Treated	0.97 ± 0.07^{ab}
Group (5) Prevented	1.43 ± 0.12^{ab}
Group (6) Protected	0.72 ± 0.07^{ab}

ANOVA: analysis of variance; TAC: total antioxidant capacity.
Each value is represented as mean ± standard deviation (mean ± SD). Data with different superscripts are significantly different at $p \leq 0.05$.
[a] significance vs control group, [b] significance vs NASH group

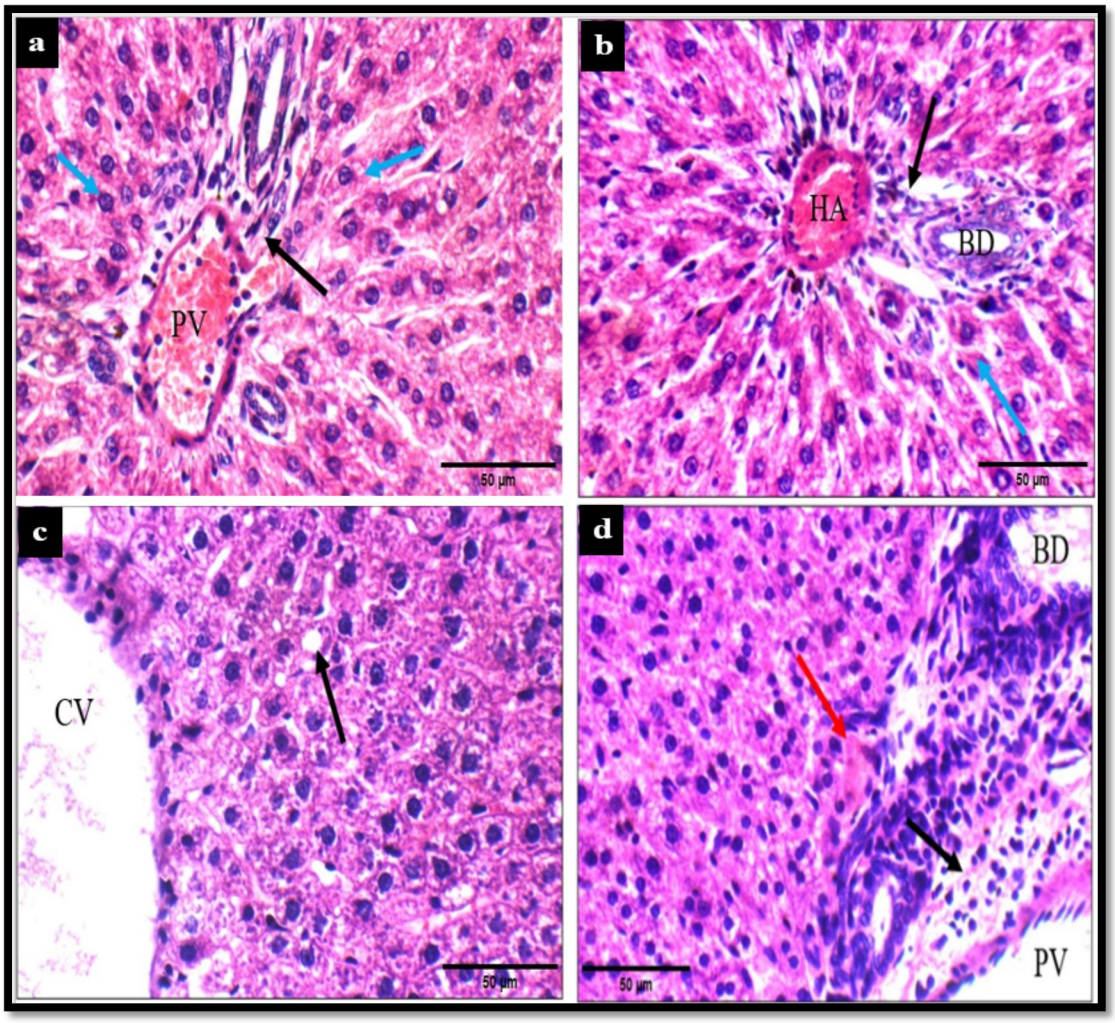

Figure 1. **(a) control group:** liver showing average portal tracts (black arrow) with average portal vein (PV), and average hepatocytes in peri-portal area (blue arrow) (H&E X 400) **(b) CAME group:** liver showing average portal tracts (black arrow) with average hepatocytes in peri-portal area (blue arrow) (H&E X 400) **(c) NASH group:** liver showing mildly dilated central veins (CV) and mild micro- and macro-vesicular steatosis in peri-portal area (black arrow) (H&E X 400). **(d) NASH group:** liver showing mildly edematous portal tracts with mild portal inflammatory infiltrate (black arrow), average portal veins (PV), and scattered apoptotic hepatocytes in peri-portal area (red arrow) (H&E X 400).

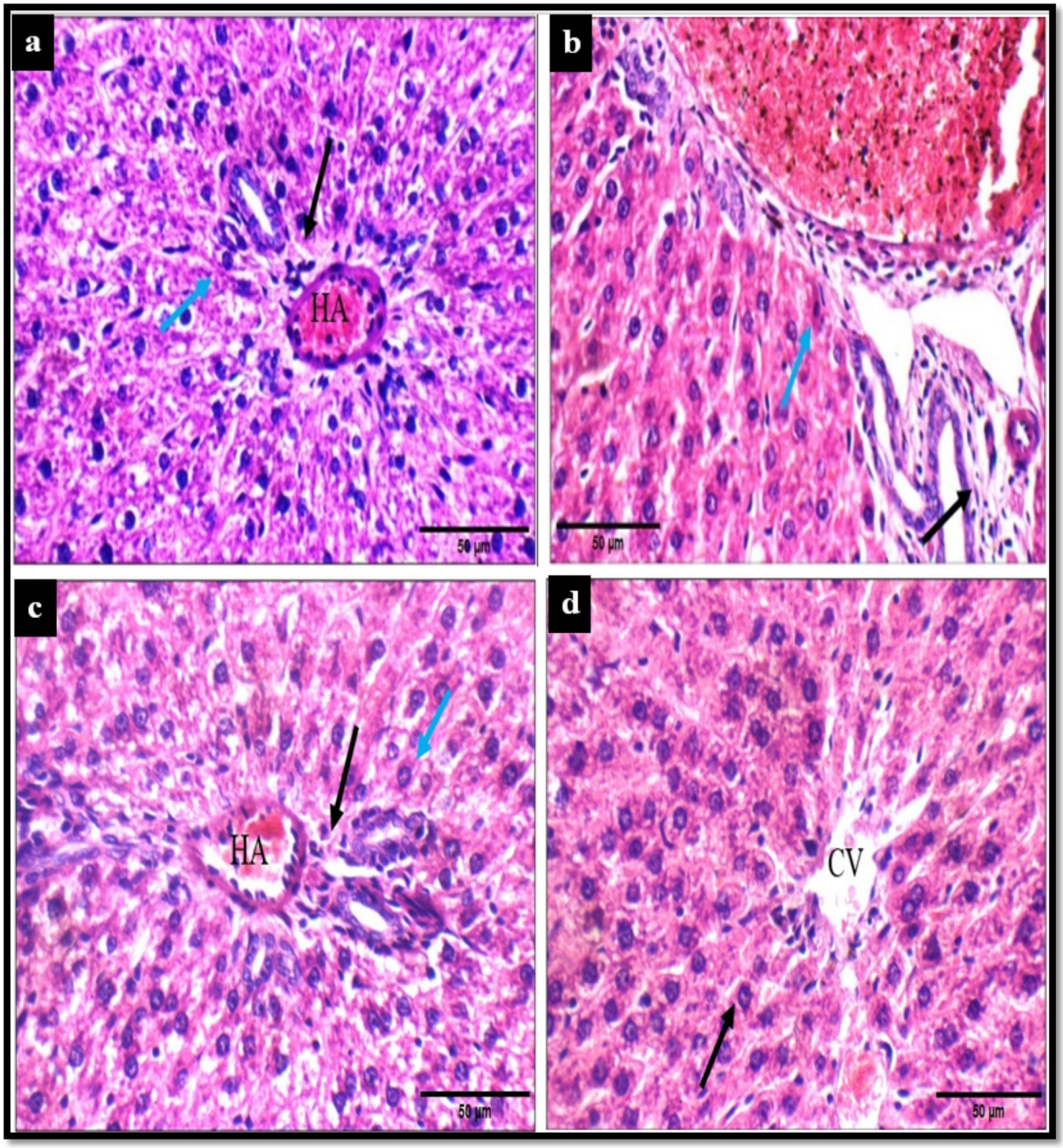

Figure 2. (a) Treated: liver showing average portal tracts (black arrow) with average portal vein (PV), and average hepatocytes in peri-portal area (blue arrow) (H&E X 400). **(b) protected:** liver average portal tracts (black arrow) with mildly congested portal veins (PV) and scattered apoptotic hepatocytes in peri-portal area (blue arrow) (H&E X 400) **(c) prevented:** liver showing average portal tracts (black arrow), and average hepatocytes in peri-portal area (blue arrow) (H&E X 400) **(d) prevented:** liver showing central vein (CV) and average hepatocytes in peri-venular area (black arrow) (H&E X 400).

Discussion.

The hallmark of NASH is accumulation of excessive fat (steatosis) in hepatocytes with or without fibrosis. Nowadays, complementary or alternative medicine is important for management of different diseases using herbal medicine. The first phase for development of a novel agent using experimental animal models for induction of NAFLD/NASH and using natural products compared with therapeutic regimes [22]. In many cases, the use of some drugs can develop steatosis (e.g tetracycline's (TET), which inhibits the action of enzymes that are involved lipoproteins release from the liver [23,24] , antiarrhythmic agents, chemotherapeutics, and [25,26].

Side effects of prolonged and high doses of tetracycline were steatosis and liver damage in animal models [27]. In addition, it is evaluated by histological staining produced from the use of TET for the management of bacterial infection. [28,29]. It was found that TET-induced NASH in an animal model [30].

Table 7. Liver histopathological results

	Portal tract				Peri-venular area			
	Portal vein	**Inflammatory infiltrate**	**Hepatocytes**	**Central vein**	**Blood sinusoids**	**Hepatocytes**	**intra-lobular Inflammatory infiltrate**	
Group (1) Control (n=5)	Average 5(100%)	No 5(100%)	Average 5(100%)	Average 5(100%)	Average 5(100%)	Average 5(100%)	No	5(100%)
Group (2) NASH (n=5)	Average 5(100%)	Mild portal & peri-portal 5(100%)	Mild steatosis & scattered apoptosis 5(100%)	Mildly dilated 5(100%)	Average 5(100%)	Mild steatosis & scattered apoptosis 5(100%)	Mild	4(80%)
Group (3) CAME (n=5)	Average 5(100%)	No 5(100%)	Average 5(100%)	Average 5(100%)	Average 5(100%)	Average 5(100%)	No	5(100%)
Group (4) Treated (n=5)	Average 5(100%)	Mild portal 3(60%)	Scattered apoptosis 4(80%)	Mildly dilated 3(60%)	Average 5(100%)	Scattered apoptosis 4(80%)	No	5(100%)
Group (5) Prevented (n=5)	Mildly congested 4(80%)	No 4(80%)	Average 4(80%)	Mildly dilated 3(60%)	Average 5(100%)	Scattered apoptosis 4(80%)	No	5(100%)
Group (6) Protected (n=5)	Mildly congested 3(60%)	Mild portal 4(80%)	Scattered apoptosis 3(60%)	Mildly dilated 4(80%)	Average 5(100%)	Scattered apoptosis 4(80%)	No	5(100%)

Where incidence represented in %

The importance of *Arabica Coffee* on health status was related to the antioxidant activity of polyphenol components and the significant contribution of chlorogenic acid (CGA) [31]. In consistency with our observation, CGA, has been reported to be the main active component of coffee, is responsible for the alleviating effect of CAME on the TET-induced liver injury in the rat model [32]. Coffee beans are a good dietary source of CGA [33]. The protecting effect of coffee polyphenols against diseases is due to the presence of chlorogenic, caffeic, ferulic, dimethoxy cinnamic acids, cinnamoyl amides, and terpenes, which have been investigated in several animal models and clinical trials [13,34, 35, 36].

Primary markers for early stages of liver injury are ALT, AST, and ALP, which are of cytosolic origin [37]. Liver injury increased the release of these enzymes into the blood [38]. The observed increased activities of ALT, AST, and ALP enzymes caused by TET due to induced injury. After administration of *Coffea Arabica* methanolic extract (CAME), it could effectively reduce the liver enzyme activities that reflected that, CAME alleviated the liver injury caused by TET. Serum albumin is the most abundant protein that has different functions in transport, osmolality, and storage .The NASH caused a reduction in the hepatic synthesis ability of albumin, and its level was reduced [39]. Moreover, this decrease in serum albumin may be due to leakage of protein via the kidney [40]. The CAME groups showed an improvement in total proteins and albumin compared to the TET group. The role of CAME may be due to its activity in protecting hepatocyte against TET of enhance protein synthesis.

There were notable alterations in serum lipid parameters. The TET-injected animals showed significant elevations in total cholesterol, TG, LDL-c, and VLDL-c, with a decrease in HDL-c compared to control animals. The groups treated with CAME exhibited an improvement in serum lipid parameters and steatosis conditions in liver tissues compared with the positive control group. The data obtained was in accordance with that of [41], who reported that daily intake of 24 g of coffee per week reduces levels of blood cholesterol and LDL-c,

while it is against the results of [42], who reported that coffee intake daily does not impact serum lipids. In other studies, to investigate the beneficial effects of CGA in insulin resistance attenuation and reduce the incidence of type II diabetes in experimental and human studies [43]. Sudeep et al. [44] reported that administration of CGA reduces lipid profile in hyperlipidemia Wister rats.

It was reported that animal models and NASH patients showed increased production of free radicals that caused lipid peroxidation products and lowered the antioxidant capacity of the body [45,46]. The normal defense against free radicals in the body includes total antioxidant capacity (TAC), CAT, SOD, and reduced glutathione (GSH) [47]. Compared with the TET-injected group, the level of TAC in the serum of the CAME-treated groups was significantly elevated but not returned to the baseline level, indicating that CAME could improve the antioxidant capacity and alleviate the damage of TET to the antioxidant enzyme system.

The histological findings in this study revealed that the TET-treated group showed mild steatosis of hepatocytes and causing fatty liver degeneration. This is in agreement of study by [48], a single oral dose of TET (1 g/kg) injected into male mice caused hepatocellular damage. Histopathological evaluation showed micro vesicular steatosis after 24 h, characterized by increased cholesterol and triglyceride biosynthesis and a decrease in β-oxidation of fatty acids. The mild steatosis in our intoxicated animals might be attributed to the low dose of tetracycline that was injected into the animals. The histopathological results were in the same direction as the biochemical results.

It is noticed that the CAME administration in different three-way (groups 4, 5, and 6) manners exerted a beneficial effect on rat livers, where coffee ameliorated all the studied parameters. The consumption of coffee daily is recommended to reduce the progression of liver diseases due to its antioxidant properties. According to the review by [49].Meta-analyses reported that consumption of coffee reduced incidence of liver disease patients and lower mortality rate [50,51].In conclusion, CAME attenuated the incidence of NASH by antioxidant activity, reducing serum lipid profile, improving hepatocyte integrity, and preventing the accumulation of fat droplets in the livers of rats. This evidence supports the potential benefits of Arabica coffee in reducing the pathological progress of NASH. Further study will be done to investigate the role of autophagy on NASH and the impact of Arabica coffee extract by measuring m-TOR signal-mediated autophagy.

Limitation of study:

Due to high costs of kits for purification and studying the mechanism of action of purified compound from coffee Arabica and using large number of rats for explore the signal pathway.

List of abbreviations:

Non-alcoholic steatohepatitis (NASH), hepatocellular carcinoma (HCC), Tetracycline (TET), chlorogenic acid (CGA), Gas chromatography/Mass spectrum (GC/MS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Coffee Arabica methanolic extract (CAME),

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