

Original Research Article

Modulation of atherogenesis, blood coagulation, and immunological parameters by *Ocimum basilicum* L. in high-fat diet-induced Nonalcoholic fatty liver disease rat model

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Abstract

Objective: This study investigates the impact of *Ocimum basilicum* in modulating atherogenesis, blood coagulation, and immunological parameters in high-fat diet-induced Non-alcoholic fatty liver disease (NAFLD) in rats.

Materials and Methods: Thirty albino Wistar rats were divided into six groups. Group I (control; male) received a standard rat diet. Group II (NAFLD control; male) was fed a high-fat diet (HFD), groups III and IV were respectively male and female groups fed HFD alongside 100 mg/kg *O. basilicum* aerial part hydromethanol extract (OCB), group V (male) received 200 mg/kg OCB, and group VI (male) was treated with 20 mg/kg of atorvastatin. Rats were fed HFD for eight weeks, while plant administration commenced in the fifth week and lasted 21 days. The animals were sacrificed under anesthesia, and blood samples were collected via the jugular vein and processed for assays.

Results: Activated partial thromboplastin time was significantly ($p < 0.05$) prolonged in groups administered with OCB compared to NAFLD control. In contrast, there was no difference in prothrombin time (PT) except in the male group treated with 100 mg/kg OCB having prolonged PT. The elevated levels of serum C-reactive proteins, interleukin-6, tissue necrotic factor- α , and angiotensin-converting enzyme, and plasma and liver lipids due to HFD induction, were mitigated by OCB treatment. Liver histology showed the capability of OCB to reverse hepatocyte damage caused by high fats and oxidative stress at an administered dosage of 100 mg/kg.

Conclusion: This study showed that *O. basilicum* possesses anticoagulant, anti-inflammatory, and anti-atherogenic activity and could thus be useful in the treatment and management of NAFLD and its complications.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is now recognized as the most prevalent chronic liver disorder worldwide, affecting an estimated 25–30% of the global adult population (Vernon et al. 2011; Younossi et al. 2019). It is characterized by excessive fat accumulation in more than 5–10% of hepatocytes in individuals with little or no alcohol intake (Bellentani 2017). This abnormal lipid deposition disrupts liver architecture and promotes inflammatory and fibrotic processes which can progress to cirrhosis (Mohebbati et al. 2024) and hepatocellular carcinoma if left unchecked.

NAFLD is strongly linked to metabolic derangements, particularly hyperlipidemia and insulin resistance, which exacerbate hepatic lipid deposition and lipotoxicity (Neuschwander-Tetri 2017). Additionally, continuous intake of high-fat diets can result in metabolic syndromes that promote oxidative stress and mitochondrial dysfunction, triggering chronic inflammation and the activation of pro-inflammatory cytokines such as Tissue necrotic factor-alpha (TNF- α) and Interleukin-6 (IL-6) (Esler & Bence 2019; Mirea et al. 2020). This inflammatory cascade is central to the transition from simple steatosis to non-alcoholic steatohepatitis (NASH) and fibrosis (Rinaldi et al. 2021).

Beyond liver-specific consequences, NAFLD is associated with systemic complications including endothelial dysfunction and a prothrombotic state, which markedly increase cardiovascular and thrombotic risk (Ogresta et al. 2022; Iglesias-Morcillo et al. 2023). Atherogenic indices including the Atherogenic Coefficient (AC) Atherogenic Index of Plasma (AIP), and Coronary Risk Index (CRI), serve as indicators of cardiovascular risk and are closely associated with NAFLD severity (Sookoian and Pirola 2013). Current preventive strategies primarily involve lifestyle modifications; however, there is no approved

pharmacological therapy, highlighting the urgent need for novel therapeutic agents capable of targeting multiple pathogenic mechanisms, including dyslipidemia, oxidative stress, and inflammation.

Natural products are gaining attention as potential sources of multi-target therapeutics for NAFLD, particularly those with antioxidant and anti-inflammatory properties. *Ocimum basilicum* (sweet basil), a widely used culinary and medicinal herb in the Lamiaceae family, has long been utilized in traditional medicine for the treatment of inflammatory disorders, digestive ailments, and infections (Gupta et al. 2010; Henry et al. 2018). It is an annual or perennial plant with dark green oval leaves that can grow up to 30-150 cm long. The plant is used in various countries for local medicinal and therapeutic purposes.

According to traditional healers' beliefs, *O. basilicum* possesses therapeutic properties in the management of inflammation-related illnesses affecting the digestive, respiratory, urinary, and reproductive systems (Aminian et al. 2022; Azizah et al. 2023). Phytochemical analyses have revealed its richness in bioactive compounds such as flavonoids, phenolics, and essential oils which confer antioxidant, anti-inflammatory, and immunomodulatory activities (Kumar et al. 2016; Kamelnia et al. 2023). Neuroprotective effects of *O. basilicum* hydroethanol extract and fractions have also been reported (Mansouri et al. 2022). Despite these promising properties, there is limited experimental evidence on the role of *O. basilicum* in modulating metabolic and hepatic dysfunction associated with NAFLD. Specifically, its impact on lipid metabolism, atherogenic indices, and oxidative-inflammatory pathways in NAFLD has not been fully elucidated.

Therefore, this study investigates the potential of *O. basilicum* hydromethanol extract as a natural therapeutic agent against NAFLD with the objectives to evaluate its effects on lipid profile, atherogenic indices, oxidative stress

markers, and liver histopathology in a high-fat diet-induced NAFLD rat model. By addressing these gaps, the study seeks to establish whether *O. basilicum* can provide a multi-targeted approach for treating or mitigating NAFLD and its associated cardiovascular risks.

Materials and Methods

Reagents and drugs

The chemicals, reagents, and solvents used were of analytical grade. The activated partial thromboplastin time (aPTT) and prothrombin time (PT) reagents were obtained from Agappe Laboratories, India. ELISA kits (Elabscience, UK) were used for the determination of cytokines levels, while Randox (UK) kits were used for lipid profile assays.

Plant material and extraction

The plant, *Ocimum basilicum* Linnaeus, Lamiaceae, was plucked directly from a local farm at Ibafo, Ogun state, Nigeria (6°43'58.3"N, 3°25'50.1"E) in April 2023. A voucher specimen was identified at the Department of Botany at the University of Lagos (UNILAG), Nigeria by Dr. George Nodza and deposited in the herbarium with reference number 10040 on the 20th of June 2023. The aerial part, shoots and stem were sorted and dried in a hot air oven at 40°C, to a crunchable texture, and then pulverized into a fine powder using a laboratory blender. Fifty grams (50 g) of the powdered plant were soaked in 50% methanol (200 mL of methanol and 200 ml of distilled water; 8:1 (w/v)) in an airtight jar and intermittently shaken over the space of a week (cold maceration) to allow better interaction between the plant and solvent. At the end of one week, the mixture was filtered, concentrated *in vacuo*, and further dried to achieve a sticky and viscous texture using a hot air oven at 50°C, after which, it was weighed, kept in an airtight container, and refrigerated at 4°C. The percentage yield of the plant extract was 66%.

Acute toxicity study

Acute toxicity of the extract was assessed using the Organization of Economic Co-operation and Development (OECD) guidelines for testing chemicals (Acute Oral Toxicity- section 423). Three female Wistar rats were administered with single oral doses (10 - 1000 mg/kg body weight) of *O. basilicum* aerial part extract (OCB), after overnight fasting. Rats were observed every 15 min for 5 hr for post-administration symptoms, and subsequently twice daily for 14 days (OECD, 2001).

Preparation of the high-fat diet

A high-fat diet (HFD) was prepared as described by Madkhali (2020) with some modifications; a normal pellet (standard rat feed; 60.42%) was ground and mixed thoroughly with tallow (30.21%), egg yolk (4.53%), and milk powder (4.83%) using cellulose (CMC) as a binder. The proximate analysis conducted on the HFD is shown in Table 1.

Table 1. Proximate analysis of formulated high-fat diet

Components	Amount (g/100g) %
Moisture	6.6
Ash content	2.6
Crude fiber	9.0
Carbohydrates	33.8
Fats	43.2
Protein	4.8

Experimental animals

Thirty adult Wistar albino rats were obtained from the Animal facility of Mountain Top University, Ogun State, Nigeria, and used in the study. Animals were acclimatized for a week and were housed in well-ventilated cages in a controlled environment, with access to food and water *ad libitum*, under a 12 hr light/12 hr dark cycle, throughout the study. The study was approved by the University Ethical Committee for Animal Studies and ethic approval (with reference number CMUL/ACUREC/09/22/926) was obtained

from College of Medicine, University of Lagos, Nigeria. The principles and requirements for the care and use of laboratory animals (NIH guidelines; National Research Council (US) committee update, 8th edition, 2011) were strictly followed.

The rats were weighed and grouped into six cages, having 5 rats each. The males were carefully separated from the females.

Group 1: Control, Group 2: NAFLD control, Group 3: NAFLD (M) + 100 mg/kg of OCB, Group 4: NAFLD (F) + 100 mg/kg of leaf extract, Group 5: NAFLD + 200 mg/kg, and Group 6: NAFLD + Standard (Atorvastatin 20 mg/kg).

The HFD was fed *ad libitum* to the rats for 8 weeks to induce NAFLD (Bravo et al., 2011), and at the 5th week of feeding, plant extract and drug administration commenced. The administration was done orally, once daily for 21 days. Experimental rats were weighed on specific days using a highly sensitive ANTOM compact electronic weighing balance (Mumbai, India).

On completion of the administration period, the rats were euthanized under ether anaesthesia through cervical dislocation. Blood samples were collected through the jugular puncture into plain sample bottles (for inflammatory parameters), and Eppendorf tubes containing tri-sodium citrate (1:9) for blood coagulation parameters, and were then centrifuged at 1500 g for 5 min and 2500 g for 10 min, respectively. The serum and plasma were separated from whole blood and refrigerated at -4°C until assay. The schematic illustration of the protocol is presented in Figure 1.

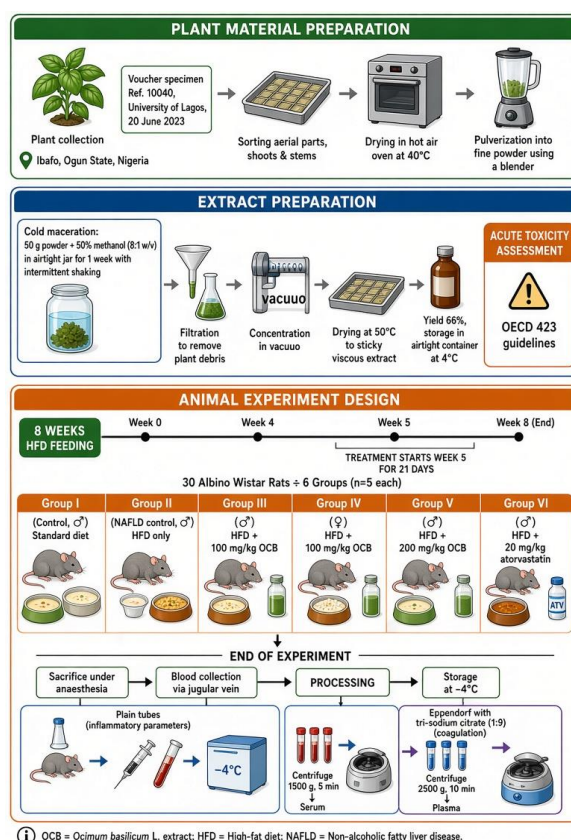
Sample size and dose justification

The doses of 100 and 200 mg/kg body weight were selected based on previous studies where similar phytochemical-rich plant extracts demonstrated significant pharmacological activity and safety in rodent models (Saha et al., 2012; Udofia et al., 2019). The sample size was determined

a priori with the primary outcome set as hepatic steatosis severity. Diet-induced NAFLD in rats typically yields large group differences (≈ 1.5 – 2.0 SD) versus controls on hepatic Triglycerides (TG), and histology. Using a two-sided test ($\alpha = 0.05$, $1 - \beta = 0.80$) and a conservative standardized effect $d = 1.8$, the per-group requirement is:

$$n = \frac{2 \cdot (Z_{\{\alpha/2\}} + Z_{\{\beta\}})^2}{d^2} = \frac{2 \cdot (1.96 + 0.84)^2}{1.8^2} \approx 4.9 = 5 \text{ animals/group.}$$

This size also accommodates the planned one-way ANOVA across 6 groups with pre-specified contrasts (e.g. HFD vs control; treatments vs HFD), for which, multiplicity control reduces per-contrast power; maintaining $n = 5$ preserves $\geq 80\%$ power for effects ≥ 1.8 SD while keeping animal use minimal. Moreover, NAFLD model is a low risk experiment with very low or no attrition envisaged. The approach thus aligns with ARRIVE 2.0 guidance to justify numbers and minimize animal use consistent with scientific objectives (3Rs).



① OCB = *Ocimum basilicum* L. extract; HFD = High-fat diet; NAFLD = Non-alcoholic fatty liver disease.

Figure 1. Schematic illustration of the experimental protocol

Oral glucose tolerance test

The animals were fasted overnight (12 hr) and administered with a 2% glucose solution orally. A baseline blood glucose measurement was done at the start (0 min) of the experiment, while the blood glucose was further measured at 30, 60, and 120-min intervals after receiving the glucose solution.

Determination of blood coagulation parameters

aPTT and PT were determined in the rats' plasma using AGGAPE test kits, following the manufacturer's instructions. Briefly, the PT reagent was reconstituted with distilled water and prewarmed in a water bath at 37°C for 10 min. After, 0.1 ml of plasma was properly pipetted into an individual clean test tube at 37°C and then incubated for 3 min. At the end of the 3 min, exactly 0.1 ml of pre-warmed reagent was forcibly pipetted into a test tube containing the plasma sample, a timer was started simultaneously and clotting time was recorded in seconds.

For aPTT determination, the aPTT reagent and CaCl₂ were gently swirled and warmed at 37°C for 10 min. Exactly 0.1 ml of the test sample was pipetted into a clean test tube at 37°C, followed by prewarmed aPTT reagent (0.1 ml), gently mixed and incubated for 3 min at 37°C. After, 0.1 ml of prewarmed CaCl₂ was forcibly added to the mixture while a timer was started simultaneously. The clotting time (as APTT) was then recorded in seconds.

Assay for inflammatory parameters

The levels of C-reactive protein (CRP) in the serum of the experimental animals were determined using a CRP latex reagent according to the manufacturer's instructions, while cytokine levels of IL-6 and TNF-α were determined using ELISA kits.

Determination of lipids and atherogenic parameters

The levels of triglycerides, total cholesterol, and HDL_Cholesterol (HDL_C) in the plasma and liver of experimental rats were measured using Randox assay kits, following the guidelines provided in the kit's instruction manual. LDL_Cholesterol (LDLC) was calculated from measured values of total cholesterol, triglycerides, and HDL_C;

$$[LDL_C] = [Total\ cholesterol] - [HDL_C] - [Trig/5]$$
 (Friedwald 1972).

All values are expressed in mg/dl. The serum Angiotensin Converting Enzyme (ACE) was quantitatively determined spectrophotometrically using an assay kit as described in the manufacturer's protocol.

Atherogenic indices – Atherogenic coefficient (AC) and Atherogenic index of the plasma (AIP) were calculated according to the formula:

$$AC = \frac{TC-HDL-c}{HDL-c}, \quad AIP = \log \frac{TG}{HDL-c}$$
 (Ayodele et al. 2023; Tan et al. 2004)

Histology of the liver

Histopathology examination of the liver of the experimental rats was done according to the method described by Krause (2001).

After sacrifice, the liver was excised, rinsed in ice-cold saline to remove blood and debris, and blotted dry. Part of the left lobe of the liver was immediately fixed in 10% neutral buffered formalin for histopathological examination. The remaining lobes were homogenized, centrifuged, and the supernatant was stored at -80°C until further use for lipid profile assays. To assess pathophysiological alterations, formalin-fixed liver samples were processed, embedded in paraffin, sectioned (4–5 μm), and stained with hematoxylin and eosin (H&E). Microscopic evaluation focused on the architecture of hepatic lobules, hepatocyte morphology, sinusoidal arrangement, and the presence of necrosis, steatosis, inflammation, or fibrosis, which are indicative of liver injury.

Statistical analysis

The data obtained from these investigations were statistically analyzed

using GraphPad Prism 9.5.1 (GraphPad Software, Inc., USA). The results are expressed as mean $\pm$ standard error of the mean (SEM) and considered to be significant at a p-value less than 0.05. The comparison of groups was conducted through a one-way Analysis of Variance (ANOVA) and Tukey's test.

Results

Effect of *O. basilicum* extract on the body weight of the experimental rats

The body weights of the experimental rats consistently increased over the weeks of the study. However, at eight weeks, the

body weights of groups administered with the extract and standard drug were significantly ($p < 0.05$) lower than those of the NAFLD only group. The group administered 200 mg/kg OCB recorded the lowest increase in body weight (Table 2).

Oral glucose tolerance

There was an initial increase in blood glucose concentrations of all the HFD-induced groups after glucose administration compared to the control. However, the blood glucose concentrations were significantly ($p < 0.05$) lowered in the OCB-treated groups compared to the NAFLD only from 30 to 60 min after glucose administration (Figure 2).

Table 2. Change in body weight of experimental rats

Group	Initial body weight (g)	Final body weight (g)	% Change in weight
Control	161.4 $\pm$ 8.33	212.0 $\pm$ 13.07	31.4 $\pm$ 1.13
NAFLD only	141.0 $\pm$ 7.57	209.6 $\pm$ 9.93	48.7 $\pm$ 0.67***
NAFLD(m) + OCB 100 mg/kg	161.0 $\pm$ 4.76	225.4 $\pm$ 9.83	40.0 $\pm$ 1.54*
NAFLD(f) + OCB 100 mg/kg	162.0 $\pm$ 9.76	231.5 $\pm$ 18.77	42.9 $\pm$ 0.98**
NAFLD + OCB 200 mg/kg	147.0 $\pm$ 6.87	203.4 $\pm$ 10.93	38.4 $\pm$ 1.79*
NAFLD + ATV 20 mg/kg	152.4 $\pm$ 9.59	218.8 $\pm$ 9.63	43.6 $\pm$ 0.82**

Values are Mean $\pm$ SEM, n=5, % Change in body weight = change in body weight/Initial body weight $\times$ 100. Values were statistically significant at * $p < 0.05$, ** $p < 0.01$, ns; *** $p < 0.001$, vs control. NAFLD(m)- Male rats induced with NAFLD, NAFLD(f)- Female rats induced with NAFLD, ATV- Atorvastatin, OCB- *O. basilicum* extract.

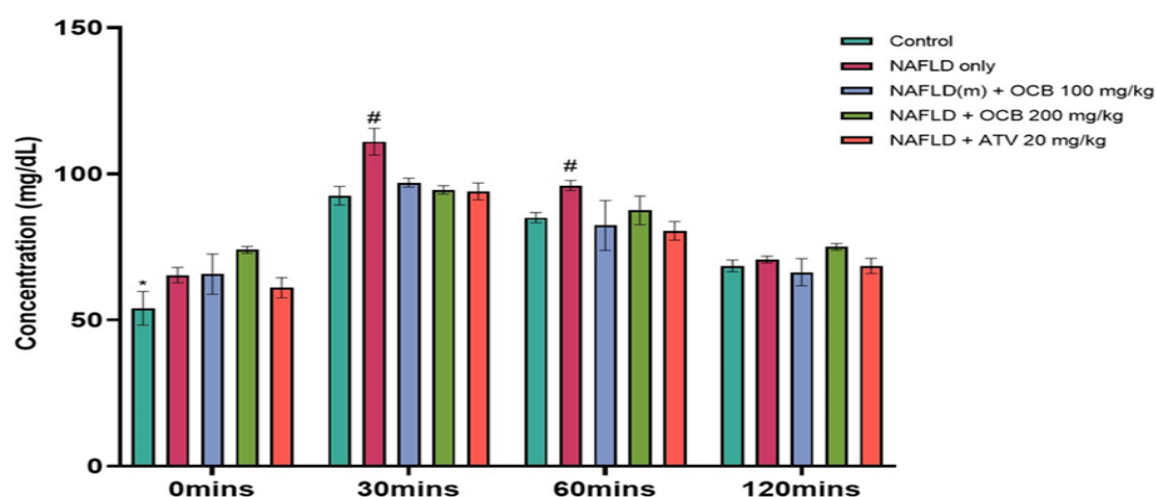


Figure 2. Oral glucose tolerance in experimental groups. NAFLD(m)- male rats induced with NAFLD, ATV- Atorvastatin, OCB- *O. basilicum* extract. Data are presented as Mean $\pm$ SEM; n=5. #($p < 0.05$) significant compared with treatment groups and control; *($p < 0.05$) lower than test groups.

Anticoagulant effects *O. basilicum* extract

The result presented in Figure 3a shows a significantly prolonged ($p<0.05$) prothrombin time (PT) in the male group treated with 100 mg/kg OCB against the control and NAFLD-only groups. There were no significant differences ($p>0.05$) in PT among the groups administered 100 mg/kg (female), 200 mg/kg, and atorvastatin. However, longer PTs were recorded in these groups compared to the control group.

According to Figure 3b, aPTT was significantly shortened in the NAFLD-only group compared to the control. The times were significantly ($p<0.05$) increased in the treatment groups compared to the control groups, with the male group treated with

OCB 100 mg/kg and atorvastatin recording the highest time.

Anti-inflammatory effect of *O. basilicum* extract

The CRP levels were elevated in the NAFLD control group compared to the control group. This increase was significantly ($p<0.05$) mitigated in the groups treated with different concentrations of OCB and atorvastatin (20 mg/kg). The highest reduction was observed in the groups treated with 100 mg/kg OCB with no difference recorded between the male and female groups (Figure 4a). Similarly, the levels of $\text{TNF-}\alpha$ and IL-6 were significantly ($p<0.01$) higher in NAFLD only group compared to other groups (Figure 4b and c).

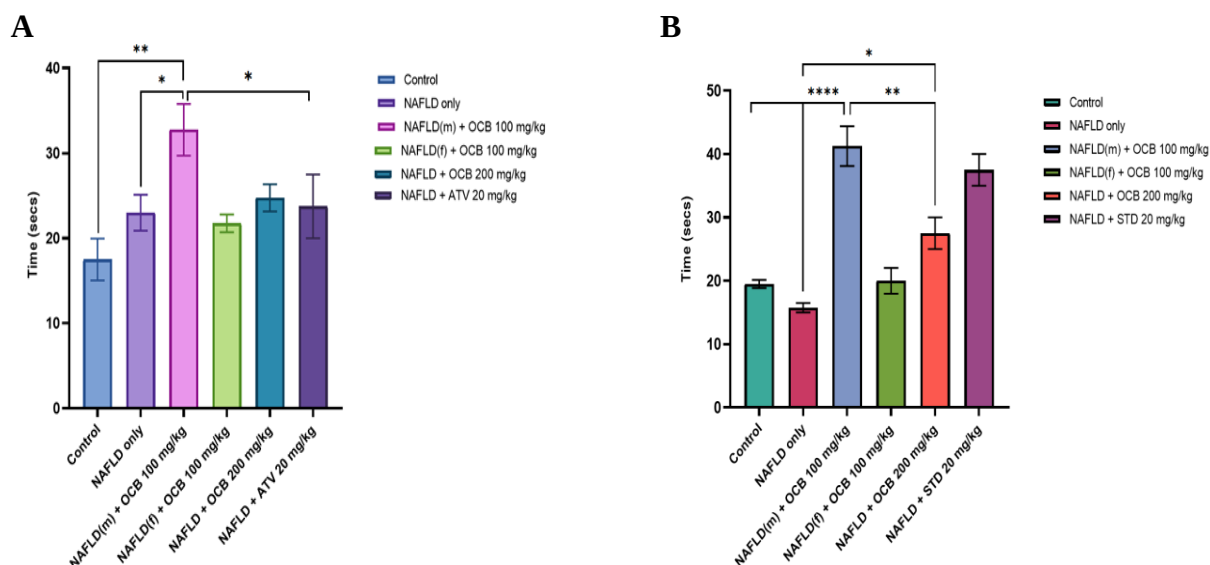


Figure 3. Effect of OCB on prothrombin time (A) and activated partial thromboplastin time (B) of high-fat diet-induced rats. NAFLD(m)- male rats induced with NAFLD, NAFLD(f)- female rats induced with NAFLD, ATV- Atorvastatin, OCB- *O. basilicum* extract. Data are presented as Mean $\pm$ SEM; $n=4$. * $p<0.05$ significant; ** $p<0.01$ significant; *** $p<0.001$ significant as indicated by the lines.

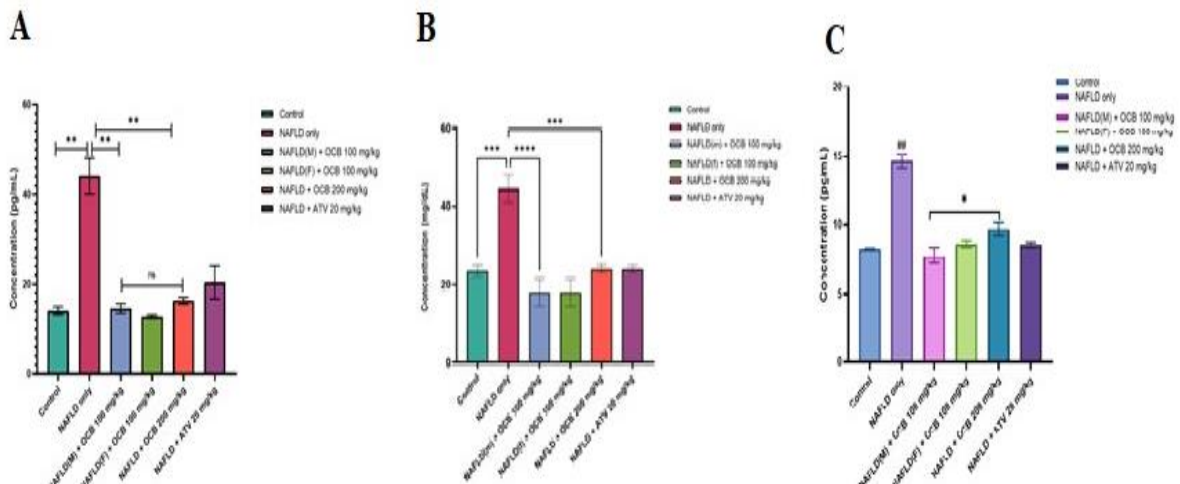


Figure 4. Effects of OCB on the concentrations of CRP (A), TNF- α (B), and IL-6 (C) in high-fat diet-induced rats. NAFLD(M)- Male rats induced with NAFLD, NAFLD(F)- Female rats induced with NAFLD, ATV- Atorvastatin, OCB- *O. basilicum* extract. Data are presented as Mean $\pm$ SEM; n=4. *p<0.05 significant; **p<0.01, ***p<0.01, ****p<0.001 significant as indicated by lines; ##p<0.01 significantly higher than other groups.

Hypolipidemic and anti-atherogenic effects of *O. basilicum*

The results of the plasma and liver lipid profile, angiotensin-converting enzyme (ACE), and atherogenic indices (AC and AIP) in the experimental rats are presented in Table 3. There was significantly increased (p<0.05) plasma and liver total cholesterol, triglycerides, and LDL_C levels in the negative control (NAFLD only) compared to the control rats. Significant reductions of the parameters were recorded in groups treated with OCB and atorvastatin, except for plasma cholesterol, in which the reduction was not

significant. ACE concentration was significantly (p<0.05) increased in the NAFLD-only when compared to the control, and similar reductions were observed in the treated groups compared with the NAFLD-only group. AC and AIP were lowered in the rats upon administration of OCB and atorvastatin.

Histopathology of the liver of experimental rats

The photomicrographs of the liver tissues of the experimental rats are shown with descriptions in Figure 5.

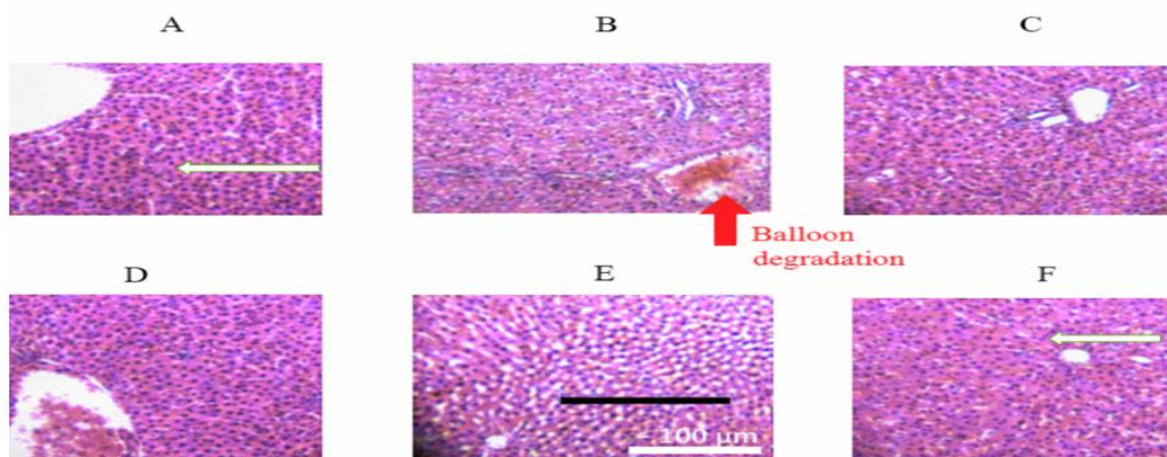


Figure 5. Photomicrograph of the liver of the experimental rats. A; Control- Normal liver architecture (white arrow), B; NAFLD control- Balloon degeneration (liver parenchymal cell death) and blood vessel congestion, C; NAFLD(m) + OCB 100 mg/kg- Normal liver architecture, D; NAFLD(f) + 100 mg/kg- Hepatocytes are normal, Blood vessel congestion, E; NAFLD + OCB 200 mg/kg- Parenchymal cell death/ necrosis (black arrow), F; NAFLD + ATV- Normal liver architecture. Objective magnification: x 40

Modulatory effects of *Ocimum basilicum* in diet-induced NAFLD

Table 3. Lipid profile and atherogenic parameters in experimental rats

Groups	Total Cholesterol		Triglycerides		HDL_C		LDL_C		ACE	AC	AIP
	(mg/dl)		(mg/dl)		(mg/dl)		(mg/dl)		IU/ml		
	<i>Plasma</i>	<i>Liver</i>	<i>Plasma</i>	<i>Liver</i>	<i>Plasma</i>	<i>Liver</i>	<i>Plasma</i>	<i>Liver</i>			
Control	187.60 ± 3.64 ^a	204.70±5.72 ^a	165.10± 5.92 ^b	190.90±2.25 ^a	51.88±1.26 ^a	55.63±5.53 ^b	102.70±2.53 ^b	110.89±1.64 ^a	69.40± 5.75 ^a	0.11± 0.05 ^a	0.90± 0.05 ^a
NAFLD only	200.40 ± 3.13 ^b	284.10±19.56 ^d	218.50± 12.42 ^c	269.30±3.34 ^c	50.68±1.86 ^a	48.55±1.75 ^a	106.02±1.42 ^b	181.69±9.42 ^d	108.40± 6.58 ^c	0.24± 0.11 ^c	1.03± 0.17 ^b
NAFLD(m) + OCB 100 mg/kg	194.60 ± 6.85 ^b	241.10±7.19 ^c	170.90± 3.24 ^b	211.20±11.93 ^b	61.18±4.49 ^b	65.58±4.11 ^{bc}	99.24±1.85 ^b	133.28±2.24 ^b	77.50± 4.46 ^b	0.11± 0.02 ^a	0.91± 0.04 ^a
NAFLD(f) + OCB 100 mg/kg	195.90 ± 3.27 ^b	226.00±16.31 ^b	146.70± 4.34 ^a	196.20±11.61 ^a	56.50±4.20 ^b	61.05±3.40 ^b	60.86±5.61 ^a	125.71±3.31 ^b	84.37± 2.05 ^b	0.16± 0.07 ^b	0.86± 0.08 ^a
NAFLD + OCB 200 mg/kg	189.60 ± 3.17 ^b	243.50±2.67 ^c	187.00± 2.18 ^{bc}	208.50±18.42 ^b	54.38±3.99 ^a	60.95±5.11 ^b	97.82±2.42 ^b	140.85±4.67 ^{bc}	93.21± 1.57 ^b	0.09± 0.01 ^a	0.94± 0.03 ^a
NAFLD + ATV	200.40 ± 4.97 ^b	245.10±8.29 ^c	184.00± 3.64 ^{bc}	216.20±9.23 ^b	53.58±2.21 ^a	58.65±3.63 ^b	93.62±2.45 ^b	114.31±2.49 ^a	54.03± 8.15 ^a	0.10± 0.02 ^a	0.94± 0.04 ^a

Values are Mean±SEM, n=5, Different letters (superscript) within the column indicate significant differences (p<0.05). ACE-Angiotensin converting enzyme, AC – Atherogenic Coefficient, AIP- Atherogenic Index of Plasma, NAFLD(m)- male rats induced with NAFLD, NAFLD(f)- female rats induced with NAFLD OCB- *Ocimum basilicum* extract, ATV- Atorvastatin.

Discussion

The study investigated the therapeutic effects of *O. basilicum* by possible modulation of atherogenic, blood coagulation, and immunological parameters in NAFLD induction in rats. In this study, a high-fat diet (HFD) was used to induce NAFLD. According to Madkhali (2020) slightly modified method, HFD was prepared using a powdered normal pellet diet, animal fat, egg yolk, and milk powder, and fed to the rats for 8 weeks. Proximate analysis revealed that the formulated diet contained 43.2% fat, 33.8% carbohydrates, and 4.8% protein (Table 1), providing a high-energy intake primarily from fats to promote hepatic lipid accumulation and metabolic stress. Such macronutrient profiles have been demonstrated to induce progressive hepatic steatosis, obesity, insulin resistance, and dyslipidemia, which are key hallmarks of human NAFLD (Im et al. 2021; Vacca et al. 2024). The fat content of the formulated diet aligns with reports that while standard rat diets contain less than 10% of kcal fat, high-fat diets contain 30%-50% of kcal fat (Kucera and Cervinkova 2014). Compared with methionine–choline-deficient (MCD) diets which induce rapid hepatic injury and fibrosis but cause weight loss and improved insulin sensitivity, HFD models better replicate the metabolic syndrome context associated with human disease (Van-Herck et al. 2017). Furthermore, extended feeding of high-fat diets similar in fat proportion (40–45%) has been reported to produce stepwise progression from simple steatosis to steatohepatitis and early fibrosis in experimental animals (Fang et al. 2022).

HFD prepared in this study facilitated an unusual increase in body weights across all groups fed with the diet, confirming the experimental model. Previous studies have shown and confirmed that excess fat intake is responsible for increased body weight, which leads to a high risk of obesity, insulin resistance, NAFLD, and other related metabolic disorders (Costa et al. 2011).

The fasting blood glucose levels persisted above 100 mg/dl in the NAFLD control group, this may signify a prediabetic state as noted by ElSayed et al. 2023. However, the groups administered with *O. basilicum* extract (OCB) were able to counteract this effect which points to likely anti-hyperglycemic activity of the plant.

The shorter activated partial thromboplastin time (aPTT) observed in NAFLD-induced groups in the present study indicates that NAFLD induces a procoagulant state in the experimental rats. This aligns with the report of Caldwell et al (2004) that NAFLD induces a procoagulant state and inhibits fibrinolysis. The significantly ($p < 0.05$) longer prothrombin time (PT) observed in the male group treated with 100 mg/kg OCB, coupled with the longer aPTT in treatment groups compared with the control groups indicates the anticoagulant effect of OCB. The findings of this study are in agreement with the results from a previous study which reported the anticoagulant attributes of *O. basilicum* in a dose-dependent manner (Tohti et al. 2006). Since the effect is most pronounced on the aPTT, the plant may exert its anticoagulant effect by modulating the concentrations or activities of the factors of intrinsic (contact) and common coagulation pathways (factors II, V, VIII, IX, X, XI, and XII (Ayodele et al. 2019; Santoro et al. 2023).

CRP is introduced as the most sensitive inflammatory indicator and a strong independent predictor of cardiovascular risk (Michishita et al., 2008). In this study, the CRP levels were significantly increased with HFD induction in the rats when compared with the control. Although Foroughi et al. (2016) reported that there is no correlation between CRP levels and fatty liver, however, the significant ($p < 0.05$) reductions in CRP levels in groups treated with OCB 100 mg/kg compared to the control groups suggest possible anti-inflammatory activities of the plant. Tumor necrosis factor-alpha (TNF- α)

and IL-6 are key pro-inflammatory cytokines that play a fundamental role in inflammation (Uchihara et al. 2006). This study demonstrates that the hydromethanolic extract of *O. basilicum* decreased TNF- α and IL-6 levels. This finding is in agreement with previous results which showed that methanolic extracts of *O. basilicum* decrease TNF- α levels (Takeuchi et al. 2020). The decrease in the tested cytokines further indicates the anti-inflammatory effects of *O. basilicum*.

Angiotensin Converting Enzyme (ACE) is a pivotal player within the renin-angiotensin-aldosterone system (RAAS), and elevated ACE activity has been linked to heightened vasoconstriction, inflammation, and oxidative stress, all contributing to the progression of cardiovascular conditions (Santos et al. 2018) which align with the study's focus on lipids and atherogenic indices. The significant increase in ACE activity in NAFLD control when compared to the control suggests that the HFD may have contributed to elevated ACE levels. Similarly, elevated lipid profiles (total cholesterol, triglycerides, and LDL_C) and reduced HDL_C recorded in the HFD-induced groups compared to the normal rats validate the pro-atherogenic capability of HFD.

The ability of the *O. basilicum* extract, in similarity with the standard drug (atorvastatin) to counteract the elevated ACE and lipids in the treatment groups, suggests the hypolipidemic and anti-atherogenic effect of the plant. OCB may exert its hypolipidemic activity in a similar mechanism to statin drugs. Atorvastatin has been implicated in modulating the LDL_C concentration by increasing the number of LDL receptors on the surface of liver cells, thus increasing its hepatic uptake and clearance of LDL_C (Dagli-Hernandez et al. 2022; McIver et al. 2024). OCB may also possibly function in a similar mechanism. The atherogenic indices in the experimental animals suggest that the methanol extract of *O. basilicum* may have

a beneficial impact in reducing the atherogenic risk as evidenced by the lower AC and AIP in the male and female groups administered with OCB when compared to the negative control group.

From the histopathology results, it was seen that HFD fed to experimental rats led to balloon degradation caused by hepatocellular injury seen in NAFLD, this is in line with a study by Brunt et al. (2021), which noted the presence of ballooning in NAFLD liver biopsy. Ballooning injury is a major feature of importance in NAFLD, as its presence is associated with an increased risk of progression to cirrhosis. It was also observed that *O. basilicum* extract (100 mg/kg) ameliorated the effect of the fatty diet on the liver; this may be due to the hepatoprotective properties of the plant. In a previous study by Renzulli et al. (2004), members of the Lamiaceae family demonstrated capabilities to reverse damage caused by high cholesterol levels and oxidative stress, which is in line with the findings of this study. The liver tissue necrosis observed in the group treated with 200 mg/kg is indicative of the toxicity of *O. basilicum* extract at a higher dose.

A limitation of this study is assessing only two doses (100 and 200 mg/kg) of the plant extract. These doses were selected based on previous reports indicating efficacy and safety for phytochemical-rich extracts. However, including intermediate or additional doses could have provided a more comprehensive understanding of the dose-response relationship. Future research is needed to establish a complete dose-response curve and identify the optimal effective dose (ED₅₀).

Furthermore, the control and NAFLD groups, as well as treated groups III, V, and VI, comprised only male rats, whereas treated group IV comprised female rats, corresponding to male group III at a tested concentration of 100 mg/kg. This was done to check for differences in male and female animals' responses to the plant extract. However, the difference in sex distribution may limit direct comparisons across groups

and the generalizability of the findings to both sexes. Future studies should use animals of the same sex across all experimental groups or include both male and female animals in each group to enable a more comprehensive evaluation of sex-specific responses.

In conclusion, NAFLD is a complex disorder intricately linked to hyperlipidemia, coagulation abnormalities, and systemic inflammation. The findings of this study suggest that *O. basilicum* possesses anticoagulant, anti-inflammatory, hypolipidemic, and anti-atherogenic activity, which accounts for its ethnomedicinal use in the treatment of inflammation and cardiovascular conditions. The plant could be a potential therapeutic source in the phytopharmaceutical development of novel drugs for treatment and management of NAFLD and its complications.

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Conflicts of interest

Authors declare no competing interests.

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Ethical Considerations

Ethical Committee for Animal Studies and ethic approval (with reference number CMUL/ACUREC/09/22/926) was obtained from College of Medicine, University of Lagos, Nigeria.

Code of Ethics

CMUL/ACUREC/09/22/926

Authors' Contributions

Opeyemi Ayodele: Conceptualization, Methodology, Supervision, Data curation, review, and editing. Praise Odabi: Investigation, Data curation, Writing-original draft. Esther Ishiegbu: Investigation, Writing-original draft. Olawunmi Oyerinde: Methodology, Supervision, Writing-review and editing.

Abbreviations

aPTT- Activated partial thromboplastin time, ATV- Atorvastatin, CRP- C- reactive protein, NAFLD- Non-alcoholic fatty liver disease, NAFLD(M)- Male rats induced with NAFLD, NAFLD(F)- Female rats induced with NAFLD, OCB- *O.basilicum* extract, PT- Prothrombin time, TNF- α – Tumor necrosis factor alpha

References

- Aminian AR, Mohebbati R, Boskabady MH (2022) The Effect of *Ocimum basilicum* L. and Its Main Ingredients on Respiratory Disorders: An Experimental, Preclinical, and Clinical Review. *Front Pharmacol.* 12: 805391. <https://doi.org/10.3389/fphar.2021.805391>
- Ayodele OO, Onajobi FD, Okoro EE, Osoniyi O (2023) *Crassocephalum crepidioides* (Asteraceae) Benth S. Moore Leaves Fractions Attenuate Dyslipidemia and Atherogenic Indices in Diabetic Rats. *Pharmacogn Res.* 15(4):761-770. DOI: 10.5530/pres.15.4.080.
- Ayodele OO, Onajobi FD, Osoniyi O (2019) In vitro anticoagulant effect of *Crassocephalum crepidioides* leaf methanol extract and fractions on human blood. *J. Exp. Pharmacol.* 11:99–107. <https://doi.org/10.2147/JEP.S218261>
- Azizah NS, Irawan B, Kusmoro J, et al. (2023) Sweet Basil (*Ocimum basilicum* L.) —A Review of Its Botany, Phytochemistry, Pharmacological Activities, and Biotechnological Development. *Plants* 12: 4148. <https://doi.org/10.3390/plants12244148>.
- Bellentani S (2017) The epidemiology of non-alcoholic fatty liver disease. *Liver Int.* 37(Suppl. 1):81–84.
- Bravo E, Palleschi S, Aspichueta P, et al. (2011) High fat diet-induced nonalcoholic fatty

- liver disease in rats is associated with hyperhomocysteinemia caused by down regulation of the transsulphuration pathway. *Lipids Health Dis.*, 10:60. doi: 10.1186/1476-511X-10-60.
- Brunt EM, Kleiner DE, Carpenter DH, Rinella M, Harrison SA, Loomba R (2021) NAFLD: Reporting Histologic Findings in Clinical Practice. *Hepatology*. 73(5), 2028–2038. <https://doi.org/10.1002/hep.31599>
- Costa RR, Villela NR, Souza MDGC, et al. (2011) High fat diet induces central obesity, insulin resistance and microvascular dysfunction in hamsters. *Microvasc. Res.* 82: 416–422. <https://doi.org/10.1016/j.mvr.2011.08.007>.
- Dagli-Hernandez C, Zhou Y, Lauschke VM, et al. (2022) Pharmacogenomics of statins: lipid response and other outcomes in Brazilian cohorts. *Pharmacol Rep.* 74(1):47-66.
- ElSayed NA, Aleppo G, Aroda VR, et al. (2023) Classification and Diagnosis of Diabetes: Standards of Care in Diabetes-2023. *Diabetes Care.* 46(Suppl 1): S19–S40. <https://doi.org/10.2337/dc23-S002>
- Esler WP, Bence KK (2019) Metabolic Targets in Nonalcoholic Fatty Liver Disease. *Cell Mol Gastroenterol Hepatol.* 8(2):247–267. <https://doi.org/10.1016/j.jcmgh.2019.04.007>.
- Fang T, Wang H, Pan X, et al. (2022) Mouse models of nonalcoholic fatty liver disease (NAFLD): pathomechanisms and pharmacotherapies. *Int J Biol Sci.* 6;18(15):5681-5697. doi: 10.7150/ijbs.65044.
- Foroughi M, Maghsoudi Z, Khayatzadeh S, et al. (2016) Relationship between non-alcoholic fatty liver disease and inflammation in patients with non-alcoholic fatty liver. *Adv. Biomed. Res.* 5: 28. <https://doi.org/10.4103/2277-9175.176368>
- Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 18(6): 499–502.
- Gong H, Zhong H, Hui-Mei Xu, Xiong-Chang L, Liang-Ping L, De-Kui Z (2023) Insight into increased risk of portal vein thrombosis in nonalcoholic fatty liver disease. *Eur. J. Intern. Med.* 114: 23-34. <https://doi.org/10.1016/j.ejim.2023.06.011>.
- Gupta S, George M, Singhal M (2010) Leaves extract of *Murraya koenigii* Linn for anti-inflammatory and analgesic activity in animal models. *J. Adv. Pharm. Res.* 1(1): 68–77.
- Henry Ivanz A, Boy AJH, Rutilla AJH, et al. (2018) Recommended Medicinal Plants as Source of Natural Products: A Review. *DCM.* 1(2): 131-142. [https://doi.org/10.1016/S2589-3777\(19\)30018-7](https://doi.org/10.1016/S2589-3777(19)30018-7).
- Iglesias Morcillo M, Freuer D, Peters, A. et al., (2023) Association between fatty liver index and blood coagulation markers: a population-based study. *Lipids Health Dis.* 22: 83. <https://doi.org/10.1186/s12944-023-01854-8>.
- Im YR, Hunter H, de Gracia Hahn D, et al. (2021) A Systematic Review of Animal Models of NAFLD Finds High-Fat, High-Fructose Diets Most Closely Resemble Human NAFLD. *Hepatology*, 74(4):1884-1901. doi: 10.1002/hep.31897.
- Kamelnia E, Mohebbati R, Kamelnia R, El-Seedi HR, Boskabady MH (2023) Anti-inflammatory, immunomodulatory and anti-oxidant effects of *Ocimum basilicum* L. and its main constituents: A review. *Iran. J. Basic Med. Sci.* 26(6): 617–627. <https://doi.org/10.22038/IJBMS.2023.67466.14783>.
- Krause WJ (2001) *The Art of Examining and Interpreting Histologic Preparations: A Student Handbook.* New York: Parthenon Publishing Group.
- Kumar V, Marković T, Emerald M, Dey, A (2016) Herbs: Composition and Dietary Importance. *Encyclopedia of Food and Health*, 332–337. doi:10.1016/b978-0-12-384947-2.00376-7.
- Kucera O, Cervinkova Z (2014) Experimental models of non-alcoholic fatty liver disease in rats. *World J Gastroenterol.* 14;20(26):8364-76. doi: 10.3748/wjg.v20.i26.8364.
- Madkhali HA (2020) Morin attenuates high-fat diet induced-obesity related vascular endothelial dysfunction in Wistar albino rats. *Saudi Pharm J.* 28(3): 300–307. <https://doi.org/10.1016/j.jsps.2020.01.009>.
- Mansouri S, Hosseini M, Alipour F, et al. (2022) Neuroprotective effects of the

- fractions of *Ocimum basilicum* in seizures induced by pentylenetetrazole in mice. *Avicenna J Phytomed.* 12(6): 614-626. <https://dx.doi.org/10.22038/AJP.2022.20470>.
- McIver LA, Siddique MS (2024) Atorvastatin. Feb 4. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. PMID: 28613530.
- MDidea "Basil and Its Basic Botanical Info" retrieved from <https://www.mdidea.com/products/proper/proper08601.html> in July 31, 2017
- Michishita R, Shono N, Inoue T, Tsuruta T, Node, K (2008) Associations of monocytes, neutrophil count, and C-reactive protein with maximal oxygen uptake in overweight women. *J. Cardiol.* 52: 247–253.
- Mirea AM, Stienstra R, Kanneganti TD, et al. (2020) Mice deficient in the IL-1 β activation genes *Prtn3*, *Elane*, and *Casp1* are protected against the development of obesity-induced NAFLD. *Inflammation.* 43: 1054-1064. <https://doi.org/10.1007/s10753-020-01190-4>.
- Mohebbati R, Momeni-Moghaddam M.A, Asghari R, et al. (2024) The comparison of the effects of nano-silymarin and silymarin on high-fat diet-induced fatty liver of adult male rats. *Avicenna J Phytomed.* 14(3): 365-375. <https://dx.doi.org/10.22038/AJP.2024.23734>.
- Neuschwander-Tetri BA (2017) Non-alcoholic fatty liver disease. *BMC Med.* 15(1), 1-6. <https://doi.org/10.1186/s12916-017-0806-8>.
- OECD EPR (2001) Organization for Economic Co-operation and Development, Paris, 2001. URL <http://www.oecd-ilibrary.org/content/book/9789264189867-en>.
- Ogresta D, Mrzljak A, Cigrovski Berkovic M, Bilic-Curcic I, Stojisavljevic-Shapeski S, Virovic-Jukic L (2022) Coagulation and Endothelial Dysfunction Associated with NAFLD: Current Status and Therapeutic Implications. *J. Clin. Transl. Hepatol.* 10(2): 339–355. <https://doi.org/10.14218/JCTH.2021.00268>.
- Renzulli C, Galvano F, Pierdomenico L, Speroni E, Guerra M (2004) Effects of rosmarinic acid against aflatoxin B1 and ochratoxin-A-induced cell damage in a human hepatoma cell line (Hep G2). *J. Appl. Toxicol.* 24: 289–296. doi: 10.1002/jat.982.
- Rinaldi L, Pafundi PC, Galiero R, et al. (2021) Mechanisms of Non-Alcoholic Fatty Liver Disease in the Metabolic Syndrome. A Narrative Review. *Antioxidants (Basel):* 10(2), 270. <https://doi.org/10.3390/antiox10020270>.
- Saha S., Mukhopadhyay MK, Ghosh PD, Nath D (2012) Effect of Methanolic Leaf Extract of *Ocimum basilicum* L. on Benzene-Induced Hematotoxicity in Mice. *eCAM.* 2012, 176385. <https://doi.org/10.1155/2012/176385>.
- Santoro RC, Molinari AC, Leotta M, Martini T (2023) Isolated Prolongation of Activated Partial Thromboplastin Time: Not Just Bleeding Risk!. *Medicina (Kaunas).* 59(6): 1169. <https://doi.org/10.3390/medicina59061169>
- Santos RAS, Sampaio WO, Alzamora AC, et al. (2017) The ACE2/angiotensin-(1–7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1–7). *Physiol Rev.* 98(1): 505–553. doi: 10.1152/physrev.00023.2016.
- Sookoian S, Pirola C.J (2008) Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: a systematic review. *J. Hepatol.* 49(4): 600-607. doi: 10.1016/j.jhep.2008.06.012.
- Takeuchi H, Takahashi-Muto C, Nagase M, Kassai M, Tanaka-Yachi R, Kiyose C (2020) Anti-inflammatory effects of extracts of sweet basil (*Ocimum basilicum* L) on a co-culture of 3T3-L1 Adipocytes and RAW264 7 macrophages. *J. Oleo Sci.* 69(5):487-493. doi: 10.5650/jos.ess19321.
- Tan MH, Johns D, Glazer NB (2004) Pioglitazone reduces atherogenic index of plasma in patients with type 2 diabetes. *Clin Chem.* 50(7):1184-8. doi: 10.1373/clinchem. 2004.031757, PMID 15117857.
- Tessari P, Coracina A, Cosma A, Tiengo A (2009) Hepatic lipid metabolism and non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis.* 19: 291–302. doi: 10.1016/j.numecd.2008.12.015.
- Tohti I, Tursun M, Umar A, Turdi S, Imin H, Moore, N (2006) Aqueous extracts of *Ocimum basilicum* L. (sweet basil) decrease platelet aggregation induced by ADP and thrombin in vitro and rats arterio-venous shunt thrombosis in vivo. *Thromb.*

Modulatory effects of *Ocimum basilicum* in diet-induced NAFLD

- Res. 118(6): 733–739.
doi:10.1016/j.thromres.2005.12.0).
- Udoha MU, Okolie NJ, Ijioma S.N (2019) Chronic Oral Administration of *Ocimum gratissimum* Leaf Extract Induced Gastrointestinal Necrosis in Treated Albino Rats. EJMP, 27(3): 1-9.
DOI:10.9734/EJMP/2019/v27i330113
- Uchihara M, Izumi N (2006) High-sensitivity C-reactive protein (hs-CRP): A promising biomarker for the screening of non-alcoholic steatohepatitis (NASH). Nihon Rinsho. 64: 1133–1138.
- Vacca M, Kamzolas I, Harder, LM, et al. (2024) An unbiased ranking of murine dietary models based on their proximity to human metabolic dysfunction-associated steatotic liver disease (MASLD). Nat metab. 6(6):1178–1196.
<https://doi.org/10.1038/s42255-024-01043-6>.
- Van Herck MA, Vonghia L, Francque SM (2017) Animal Models of Nonalcoholic Fatty Liver Disease-A Starter's Guide. Nutrients. 27;9(10):1072. doi: 10.3390/nu9101072.
- Varani J, McClintock SD, Knibbs RN, Harber I, Zeidan D, Jawad-Makki MAH, Aslam MN (2022) Liver Protein Expression in NASH Mice on a High-Fat Diet: Response to Multi-Mineral Intervention. Front Nutr. 11;9:859292. doi: 10.3389/fnut.2022.859292.
- Vernon G, Baranova A, Younossi ZM (2011) Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 34(3):274-285. doi: 10.1111/j.1365-2036.2011.04724.x.