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# Avocado Oil Alleviates Oxidative Stress and Improves Biochemical and Histopathological Alterations in Diabetic Rats

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**Abstract**

Diabetes mellitus is a widespread persistent metabolic disorder necessitating appropriate management to prevent its complications. The utilization of natural products in dietary adjustments is increasingly popular for the regulation of hyperglycemia. Our research aimed to assess the impact of avocado cold-pressed oil (ACPO) on the pancreas, kidneys, and liver of STZ-induced diabetic rats. An analysis of the components and antioxidant capacity of ACPO was conducted. Forty-two Male Wistar rats were divided into six groups for the study. Group 1 (G1) served as the normal control, G2 received oral administration of avocado cold pressed oil (ACPO), G3 received oral administration of metformin, G4 (considered the positive control) was administered a single intraperitoneal dose of STZ (40 mg/kg), and G5 comprised diabetic rats treated with ACPO orally. The group identified as G6 comprises diabetic rats who were orally administered metformin. The ACPO composition contained 25.77% saturated fatty acids, with the remaining 72.82% unsaturated fatty acids, predominantly oleic acid. The radical scavenging activity of ACPO was favorable, as evidenced by its antioxidant effects against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO). Moreover, ACPO led to a required decrease in blood glucose levels and improvement in liver and kidney function parameters with only numerical reductions in lipid profile parameters. Additionally, ACPO mitigated histopathological abnormalities in the pancreas, kidneys, and liver, enhanced insulin expression in islets of Langerhans, and attenuated oxidative stress by suppressing induced nitric oxide synthase (iNOS) expression in these organs. To summarize, the polyphenolic compounds present in ACPO played a crucial role in managing hyperglycemia, enhancing liver and kidney function, ameliorating histopathological lesions, and mitigating oxidative stress associated with diabetes mellitus.

**Keywords**: avocado cold pressed oil; diabetes mellitus; histopathology; immunohistochemistry

## 1. Introduction

The description of diabetes mellitus entails a metabolic disorder with elevated blood glucose levels that persist for an extended duration. The origin of this condition arises from impairments in either the secretion of insulin, its action, or a combination of both mechanisms. Prolonged hyperglycemia is associated with persistent damage, compromised function, and eventual organ failure, remarkably impacting the pancreas, kidneys, and liver **[1]**. A critical factor in the onset and progression of diabetes is the pancreas, particularly the beta cells in the islets of Langerhans **[2].** Diabetes type II is distinguished by the co-occurrence of insulin intolerance and a relative inadequacy in insulin secretion, which results in hyperglycemia **[3].** Chronic hyperglycemia triggers oxidative stress and inflammation **[3].** Furthermore, the capacity of the advanced glycation end products to build up in the pancreatic tissue initiates fibrotic processes and hinders insulin secretion **[4].** Besides, the increased demand for insulin in the state of insulin intolerance, as observed in diabetes type II, beta-cell exhaustion can further diminish insulin secretion **[5].**

The liver establishes a characteristic role in the breakdown of glucose and is highly affected by diabetes mellitus. Up to 70% of diabetic patients encounter fatty liver affection, a prevalent complication of the disease **[6].** The pathogenesis of fatty liver affection in diabetes involves insulin intolerance, which leads to elevated liver flux from free fatty acids and subsequent hepatic steatosis **[7].** Fatty liver affection is characterized by inflammation and liver cell damage due to endoplasmic reticulum stress and oxidative stress **[6].** These stress responses activate inflammatory pathways, leading to the recruitment of immune cells and further exacerbating liver injury **[8].** Glycation end products formed during chronic hyperglycemia also induce liver

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damage by activating pro-inflammatory signaling pathways and inducing fibrosis **[6,8].** Fatty liver affection may lead to adverse consequences in the liver as cirrhosis, and sometimes liver carcinoma **[9].** Diabetic nephropathy, a widespread microvascular adverse consequence that occurs due to diabetes mellitus, is identified by glomerular hyperfiltration, albuminuria, and a profound decline in renal function that ultimately contributes to renal disease from the terminal stage **[10].** Prolonged hyperglycemia triggers glomerular enlargement, enhances the synthesis of extracellular matrix proteins, and accelerates the creation of glycation end products, resulting in glomerulosclerosis and tubulointerstitial fibrosis **[11].** The potential therapeutic effect of natural compounds on diabetes mellitus and its complications has gained interest recently **[12,13].** Avocado oil, extracted from the seed of the fruits, is of interest not only for its gastronomic applications but also for its prospective health advantages. Its distinctive nutritional composition plays a vital role in its medicinal attributes. ACPO consists primarily of monounsaturated fatty acids (MUFA), particularly oleic acid which accounts for approximately 50-70% of its entire fatty acid composition **[14].** There is a prominent similarity in the fatty acid profile of both avocado oil and olive oil and they are associated with health improvements, including improved lipid profiles and insulin sensitivity **[15].** In addition to MUFAs, avocado oil also contains polyunsaturated fatty acids (PUFA), such as linoleic acid, and small amounts of saturated fatty acids. Moreover, an extensive range of bioactive substances, such as phytosterols, tocopherols (known as vitamin E), and carotenoids (like lutein and zeaxanthin), are abundant in avocado oil **[14].** These constituents exhibit antioxidant, anti-inflammatory, and potential disease-fighting characteristics **[16**]. Therefore, this investigation was conducted to elucidate the impact of ACPO administration on controlling diabetes and its adverse consequences in rats compared with a reference drug using biochemical analysis, histopathological, and immunohistochemical examinations.

## 1. Materials and Methods

### 2.1. Chemicals

The reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide, and Folin-Ciocalteu were supplied by Sigma-Aldrich®. To dissolve streptozotocin (STZ), which was acquired from Chem Cruz® (sc-200719), United States, a PH 4.5 citrate buffer was utilized. We used commercially available 500 mg metformin pills (Minapharm Pharmaceuticals SAE, Egypt). Every chemical was instantly dissolved before use.

### 2.2. Preparation of avocado cold pressed oil (ACPO)

The avocado oil was extracted by cold press method using a hydraulic press unit for pressing and extracting natural oils, National Research Center (NRC), Cairo, Egypt.

### 2.3. Analysis of avocado oil

**Preparation of fatty acid methyl ester (FAME)**

After incorporating 0.1 g of the sample with 2 mL of isooctane and 0.1 KOH (2M) in methanol, the mixture was vortexed until turbidity developed. Upon adding 2 mL of 40% NaCl, the top layer was submerged. A gram of anhydrous sodium sulfate down coating was used. Two milliliters of isooctane were used to dissolve the sap layer, or down layer. Following ISO 12966-2:2017(E), an international standard organization second partition that presents instructions for the production of methyl esters of fatty acids from both animal and vegetable oil sources, the dissolved layer was then filtered, inserted into the vial, and injected through Gas chromatography-mass spectrometry (GC-MS) **[17].**

**Gas chromatography-mass spectrometry analysis (GC-MS)**

The gas chromatography-mass spectrometry (GC-MS) system was established by Agilent Technologies and installed at the Chromatography laboratory of Cairo University Research Park (CURP) in Cairo, Egypt. It consisted of a gas chromatograph (7890B) and a mass spectrometer detector (GC/MS Triple quad 7890b). Particularly, a 19091S-433: 1 HP-5MS UI column with an internal diameter of 30 m x 250 µm and a film thickness of 0.25 µm. With a spitless injection volume of 2 µL and a flow rate of 2.25 mL/min, helium was used as the carrier gas for the analytical procedures. The temperature program included two minutes at 40 °C in the beginning, then an increase of 4 °C per minute to 280 °C for another two minutes. The injector was maintained at 250°C but the detector was consistently adjusted at 230°C. Mass spectra were generated through electron ionization (EI) at -70 eV, encompassing a spectral range from 50 to 500 m/z, with a scan time of 20ms and a solvent delay of 5 minutes. The mass temperature and Quad were stabilized at 150°C each. The identification of distinct components was established by juxtaposing the spectrum fragmentation pattern against the data archived in the Wiley and NIST Mass Spectral Library **[18,19].**

**Antioxidant Activity of a 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Nitric Oxide (NO) Radical Scavenging Assay**

The antioxidative properties of ACPO were assessed through the implementation of a 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay, through the procedures outlined by Tang and Suleria **[20].** The assessment involved measuring absorbance at 517 nm, with DPPH radical scavenging activity determined using a specific formula. The formula for calculation of inhibition (%) is Abs A0 – Abs AO /AbsA0, where Abs A0 reveals the control absorbance, and Abs AO denotes the treated sample absorbance

with multiple concentrations of the essential oil under investigation. The fundamental basis of the analysis is to determine the generation of NO redox compounds by sodium nitroprusside (SNP) in an aqueous medium, resulting in a pH alteration at physiological levels to provide detectable nitrite ions using Greiss reagent (1% sulfanilamide in 5% ortho-H3PO4 and 0.1% naphthyl ethylene diamine dihydrochloride) **[21].** The method was conducted as outlined by Sreejayan and Rao **[22].** That absorbance was detected at 540 nm in contrast to the matching blank solution. Using this formula, NO radical scavenging activity was identified. The NO· scavenging effect (%) is determined through this formula [(A0-A1)/A0) × 100], where A0 conveys the control sample absorbance and A1 the treated sample absorbance at several tested extract concentrations.

**Total Phenolic Content (TPC)**

Using a Folin-Ciocalteu reagent, the method outlined by Siddhuraju and Becker **[23]** was used to precisely identify the phenolic compounds (TPC) of avocado cold-pressed oil (ACPO). At 750 nm, the absorbance was detected. Gallic acid was used as the standard in the standardization and adjustment of the calibration curve. The following calculation was used to determine the results, which are shown as milligrams of gallic acid equivalent (mg GAE/g OIL), or milligrams of gallic acid per gram of oil. TPC = Gallic acid × V × m M (mg GAE/g OIL). The calibration curve yielded the standard concentration of gallic acid, or C gallic acid; the dilution factor, V; the whole oil weight (g); and the DW oil concentration, M.

### 2.4. Acute toxicity of ACPO

Six groups of four male rats were used for measuring the oral LD50 of ACPO. Via an orogastric tube, rats were administered six successive dilutions of the cold-pressed oil extract in the vehicle (control), 2, 4, 6, 8, and 10 g/kg **[24].** Mortality and additional acute toxicity indicators were tracked for seventy-two hours then two weeks later.

### 2.5. Animals

Forty- two adult male Wistar rats weighing between 150 and 200 grams were acquired from the Research Institute of Ophthalmology in Egypt. They were housed in plastic cages with an iron mesh cover, maintaining a temperature of 20 to 25ºC under a 12-hour light/12-hour dark cycle. The rats were given unlimited access to water and commercial pellets (Al-Fahed Company Group, Egypt) with a protein content of 21%. One week of acclimatization of the rats preceded the initiation of the experiment. The Institutional Animal Care and Use Committee (IACUC) established ethical approval for all experimental procedures; the code number for this committee is Vet CU 25122023878.

### 2.6. Experimental design

The rats were assigned into six groups, each group had 7 rats. The first group was a normal control group (NC group). The second group of rats was administered 0.1 mL of avocado oil daily according to LD 50 (1/10 LD50) by stomach tube for 45 days (oil control (OC) group). The third group of rats was administered metformin (ref. drug) 500 mg/kg orally for 45 days (reference control (RC) group). In the fourth, fifth, and sixth groups, they were injected with STZ intraperitoneal at a single dose of 40 mg/kg after 2 weeks of the experiment onset **[25].** The fasting blood glucose concentration of all rats was assessed after 48 hours from STZ injection, then every week by blood glucometer (elite, YASEE Bio Medical Inc.Shandong,266000, China), and eventually at the end of the experiment by a semi-automated chemistry analyzer (Genrui wp21b, China). The fourth group of rats were diabetic rats injected with STZ (positive control (PC) group). The fifth group of diabetic rats was treated with 0.1 mL avocado oil daily prior to STZ injection for two weeks and continued till the end of the experiment (45 days) (oil treated (OT) group). The sixth group of diabetic rats were treated with 500 mg/kg metformin and continued till the end of the experiment (reference drug-treated (RT) group). The preliminary and final body weights of rats were assessed. The rats were humanely euthanized after 45 days from the onset of the experiment through decapitation with 2% isoflurane as a tranquilizer for inhalation anesthesia.

### 2.7. Clinical signs and mortalities

All groups are daily observed during the experiment for detection of any abnormal clinical signs and recording the mortalities due to induction of diabetes mellitus by STZ.

### 2.8. Determination of Body weight and relative pancreas, kidneys, and liver weights

The rats body weights were recorded at the end of the experiment. Each rat pancreas, kidneys, and liver were extracted, weighed, and soaked up liquid. The organ/final body weight ratio was then determined. The following formula was used to determine the rat relative tissue weights:

Relative pancreas weight (g/100g) = Entire pancreas weight/ Eventual body weight × 100

Relative kidneys weight (g/100g) = Entire kidneys weight/Eventual body weight × 100

Relative liver weight (g/100g) = Entire liver weight/ Eventual body weight × 100

### 2.9. Biochemical analysis

**Determination of glucose level in plasma**

The blood was collected in sodium fluoride-containing tubes. Glucose level was detected in plasma using commercial kits acquired from (Bio-Diagnostics company, Cairo, Egypt) through enzymatic and colorimetric methods **[26]** whereas glucose oxidase (GOD) ameliorated the conversion of glucose into gluconic acid. The glucose level was determined by a semi-automated chemistry analyzer (Genrui wp21b, China).

**Measurement of kidneys and liver function parameters and lipid profile**

Serum aspartate aminotransferase (AST) and alanine transaminase (ALT) were measured using techniques published by Reitman and Frankel **[27]** via commercial kits purchased from (Spectrum and Biomed companies, Cairo, Egypt) respectively. Serum urea and creatinine were identified guided by the prior approaches **[28,29]** using commercial kits purchased from (Bio-Diagnostics company, Cairo, Egypt). Serum triglyceride and total cholesterol were measured colorimetrically based on previous methods **[30,31]** using commercial kits purchased from (Bio-Diagnostics company, Cairo**,** Egypt). All these parameters were measured by a semi-automated chemistry analyzer (Genrui wp21b, China).

### 2.10. Histopathology

Samples from the pancreas, kidneys, and liver were preserved in 10% neutral buffer formalin as a fixative. The samples were prepared by ascending concentrations of ethanol and xylene, made into sections (3–4 μm thick), and stained by standard hematoxylin and eosin stain according to Suvarna et al. **[32]**. Tissue slides were photographed by an Olympus XC30 camera and a light microscope (Tokyo, Japan).

### 2.11. Immunohistochemistry

According to Campbell and Macfarlane **[33]**, pancreatic insulin immunohistochemistry (1:100) was carried out, and pancreas, kidneys, and liver paraffin-embedded tissue sections were used for iNOS (1:100) **[34]**. As directed by the kit manufacturer (Elabscience biotech, USA), slides were coated with anti-insulin antibodies (E-AB-70202, Elabscience, USA) and anti-iNOS (E-AB-15323, Elabscience, USA) before being subjected to the Poly-HRP Anti Rabbit/Mouse IgG Detection System. To develop color, 3,3′-Diaminobenzidine was used. Using five images of each group of rats at a magnification of 200x, the area percentage of beta cells in the pancreatic islet was calculated using Image J software.

### 2.12. Statistical analysis

The statistical assessment was conducted using the statistical software SPSS, version 26.0 (SPSS Inc., Chicago, IL, USA). The data findings were thoroughly analyzed and interpreted using one-way analysis of variance and post-hoc tests (The Duncan and Tamhane test). The data findings were demonstrated as mean ± standard error. When $P < 0.05$, it was considered significant.

## 3. Results and Discussion

### 3.1. Analysis of avocado oil GC-MS analysis

GC/MS assessment of avocado oil revealed the documentation of five compounds, the saturated fatty acid constitutes 25.77 represented by 14-methyl Pentadecanoic acid, methyl ester while the unsaturated fatty acid constituted 72.82% represented by 9-Hexadecenoic acid, methyl ester (Palmitoleic acid 11.17%), 9-Octadecenoic acid methyl ester. (Oleic acid 42.66%), 9,12-Octadecadienoic acid (Linoleic acid 14.86%) and 9,12,15-Octadecatrienoic acid (Linolenic acid 4.13%) **(Fig.1, Table. 1).** The analysis of avocado oil revealed its richness with bioactive components such as phytosterols and polyphenols as documented before. These compounds induce antidiabetic effects by improving insulin intolerance and suppressing Redox imbalance and inflammation **[36],** which are considered important factors in the progression of diabetes **[37, 38].** Avocado oil contained 26.14% of saturated fatty acids while the remaining 73.86% was unsaturated fatty acids, mainly oleic and palmitic. These findings were compatible with those published previously **[39].**

Avocado plants contain both saturated and unsaturated fatty acids, such as linolenic acid, palmitoleic acid, linoleic acid, palmitic acid, oleic acid, and stearic acid. These fatty acids have been reported in avocado oil by Wang et al. **[40]** and Ramos-Aguilar et al. **[41].** Provided that these fatty acids are shown to have anti-diabetic features through pancreatic cell regeneration, it is feasible that they are the actual cause of the hypoglycemic effect that we observed in our research study. Additionally, the antioxidant potential of avocado oil as assessed by DPPH and NO revealed potent and desirable radical scavenging potential corresponding to Flores et al. **[42]** and Abd Elkader et al. **[43].**

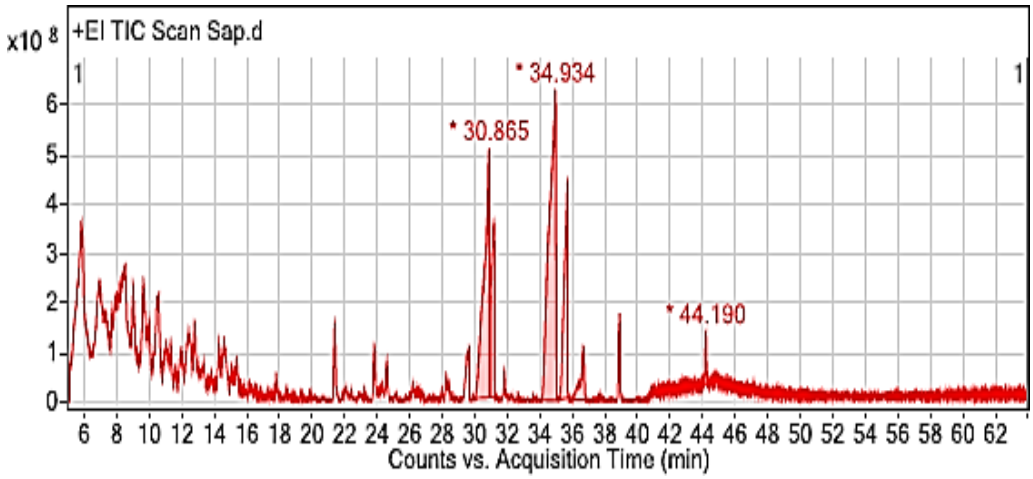


**Fig. 1. Total ion chromatogram of GC/MS analysis of FAME composition of Avocado**

**Table. 1. Results of GC-MS analysis of saponifiable matter of avocado oil**

| Peak | Rt(min) | Compounds | Chemical Formula | M+ | BP | Area %. |
|---|---|---|---|---|---|---|
| 1 | 30.86 | 14-methy Pentadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270 | 74 | 25.77 |
| 2 | 31.17 | 9-Hexadecenoic acid, methyl ester (Palmitoleic acid) | $C_{17}H_{32}O_2$ | 268 | 55 | 11.17 |
| 3 | 34.93 | 9-Octadecenoic acid methyl ester. (Oleic acid) | $C_{19}H_{36}O_2$ | 296 | 55 | 42.66 |
| 4 | 35.29 | 9,12-Octadecadienoic acid . (Linoleic acid) | $C_{19}H_{34}O_2$ | 294 | 67 | 14.86 |
| 5 | 35.95 | 9,12,15-Octadecatrienoic acid (Linolenic acid) | $C_{21}H_{36}O_2$ | 292 | 79 | 4.13 |
| | | Total identified compounds | | | | 98.59 |
| | | Saturated fatty acids | | | | 25.77 |
| | | Unsaturated fatty acids | | | | 72..82 |

$R_t$= retention time, $M^+$: molecular weight, BP: base peak

**Antioxidant activity of avocado cold pressed oil**

The antioxidant potential of the investigated ACPO was assessed by two invitro models of screening (DPPH and NO potent antioxidant techniques) and contrasted with a reference standard consisting of butylated hydroxyl toluene (BHT) and ascorbic acid (V.C.).The antioxidant potential of the avocado cold pressed oil was performed and showed concentration dependents **(Tables 2 & 3).** At 12000 µg/mL, ACPO showed significant % inhibition for DPPH• and NO• scavenging activity as 75.63 ± 1.08 % DPPH as well as 79.36± 0.69% for NO• compared with Vit C. and BHT (96.09 ± 0.14, 98.32 ±1.69%, and 92.94 ± 0.281, 95.18 ± 0.191%, respectively).Moreover, the IC50 (µg/mL) were1127±0.12, 735±0.87 and 459±0.32 for DPPH• and 1020 ±0.58, 491±0.43 and 479±0.76 µg/ml for NO•, for ACPO, V.C and BHT, respectively.

**Table 2. The IC50 and percentage of DPPH free radical inhibition of avocado cold- pressed oil (ACPO) at different concentrations (µg/mL) in relation to BHT and vitamin C (standards).**

| Conc µg/ml | Avocado cold pressed Oil (ACPO) | VIT.C | BHT |
|---|---|---|---|
| 250 µg/ml | 18.56 ± 0.35e | 23.73 ± 0.53 e | 40.46 ±2.52 e |
| 500 µg/ml | 34.46 ± 0.61d | 41.08 ± 0.52 d | 56.10 ±2.90 d |
| 1000 µg/ml | 48.7 ± 0.7 c | 64.57 ± 0.59 c | 67.88 ±2.11c |
| 1500 µg/ml | 60.9 ± 0.46b | 81.85 ± 0.46 b | 88.18 ±1.01b |
| 2000 µg/ml | 75.63 ± 1.08a | 96.09 ± 0.14 a | 98.32 ±1.69 a |
| IC50 | 1127±0.12 | 735±0.87 | 459±0.32 |

The mean ± standard error is used to express values. Substantial changes in concentrations are indicated by dissimilar letters ($p \leq 0.05$).

**Table 3. The percentage and IC50 of inhibition of avocado cold-pressed oil (ACPO) in NO free radical at several concentrations (µg/mL) in relation to standard BHT and vitamin C.**

| Conc µg/ml | ACPO | VIT.C | BHT |
|---|---|---|---|
| 250 µg/ml | 22.20 ± 0.41$^{e}$ | 33.32 ± 0.321$^{e}$ | 35.73 ± 0.393$^{e}$ |
| 500 µg/ml | 36.30 ± 0.92 $^{d}$ | 53.61 ± 0.415$^{d}$ | 55.18 ± 0.397$^{d}$ |
| 1000 µg/ml | 51.06 ± 0.37 $^{c}$ | 67.83 ± 0.505$^{c}$ | 76.98 ± 0.500$^{c}$ |
| 1500 µg/ml | 64.70 ± 1.04$^{b}$ | 75.25 ± 0.317$^{b}$ | 87.97 ± 0.091$^{b}$ |
| 2000 µg/ml | 79.36 ± 0.69 $^{a}$ | 92.94 ± 0.281$^{a}$ | 95.18 ± 0.191$^{a}$ |
| IC50 | 1020 ±0.58 | 491±0.43 | 479±0.76 |

The mean ± standard error is used to express values. Diverse letters have substantial differences between concentrations ($p \leq 0.05$).

**Total phenolic content (TPC) in ACPO**

The Data findings of TPC revealed that the ACPO had the greatest TPC 63.54 ± 0.50 (mg GAE/g Oil).

### 3.2. Observation of clinical signs and mortalities

The STZ-diabetic rats showed signs of frequent urination, dumpy litter, and remarkable thirst with a gradual reduction in weight and increased appetite. After one week of induction of diabetes by STZ, one rat from the positive control group was dead.

### 3.3. The weight and relative weight of the pancreas, kidneys, and liver

There was no significant difference in the relative organ weight between groups **(Table .4).**

**Table 4. The weight and relative weight of the pancreas, kidneys, and liver**

| Groups | Pancreas weight (g) | Kidney weight (g) | Liver weight (g) | Relative Pancreas weight (g/100 g) | Relative kidney weight (g/100 g) | Relative liver weight (g/100 g) |
|---|---|---|---|---|---|---|
| NC | 0.29±0.024 | 1.01±0.07 | 3.44±0.26 $^{a}$ | 0.14±0.01 | 0.51±0.02 $^{a,b}$ | 1.73±0.10 $^{a}$ |
| OC | 0.51±0.08 | 1.11±0.04 | 4.45±0.43 $^{a,b}$ | 0.21±0.03 | 0.46±0.01$^{a}$ | 1.83±0.17 $^{a}$ |
| RC | 0.38±0.04 | 1.05±0.04 | 3.57±0.23 $^{a}$ | 0.19±0.01 | 0.52±0.01 $^{a,b}$ | 1.78±0.06 $^{a}$ |
| PC | 0.37±0.05 | 1.13±0.12 | 3.85±0.31 $^{a}$ | 0.19±0.02 | 0.59±0.01 $^{b}$ | 2.03±0.13 $^{a,b}$ |
| OT | 0.45±0.21 | 1.24±0.16 | 5.70±0.93 $^{b}$ | 0.19±0.08 | 0.56±0.04 $^{b}$ | 2.55±0.28 $^{b}$ |
| RT | 0.40±0.10 | 1.05±0.05 | 4.03±0.18 $^{a}$ | 0.23±0.03 | 0.58±0.04 $^{b}$ | 2.22±0.17 $^{a,b}$ |

Data findings are demonstrated as means ± SEM. Values in the same column bearing diverse lowercase letters indicate substantial differences between groups. (Means are significant compared to the control groups at a $P < 0.05$, n=5 rats)**.**

### 3.4. Biochemical analysis

Plasma glucose level was significantly increased in the positive control group (PC), while in oil-treated (OT) and reference drug-treated (RT) groups there was a significant decrease compared to the PC group **(Fig. 2).** Like the earlier study **[44],** ours found that giving avocado oil to diabetic rats dramatically reduced their plasma glucose levels. This may be explained by its capacity to increase the uptake and utilization of glucose in the peripheral tissues, reduce the production of glucose in the liver, and improve the function of beta cells in the pancreas **[45,46,47].** Serum urea and creatinine were increased in the PC group and lowered remarkably in the OT group. RT group showed only a decrease in creatinine. Avocado oil improved kidney function parameters by lowering serum urea and creatinine concentrations likewise to the previous study **[48].** ALT and AST were elevated significantly in the PC group whereas it was significantly decreased in the RT and OT groups respectively **(Fig. 3).** Furthermore, avocado oil significantly enhanced liver function parameters by decreasing serum ALT and AST levels. A previous study demonstrated the hepatoprotective effect of avocado oil and that it significantly decreased liver damage and improved liver function in diabetic rats. Moreover, it reduced lipid peroxidation and enhanced the antioxidant enzyme systems in the liver **[15].** Serum triglyceride was elevated in all diabetic groups however it was decreased significantly in the RT group compared to the PC group and was lowered numerically in the OT

group. Serum cholesterol was also increased in diabetic groups and decreased partially without significance in OT and RT groups **(Fig. 3).** The latter agreed with a previous study **[49].**

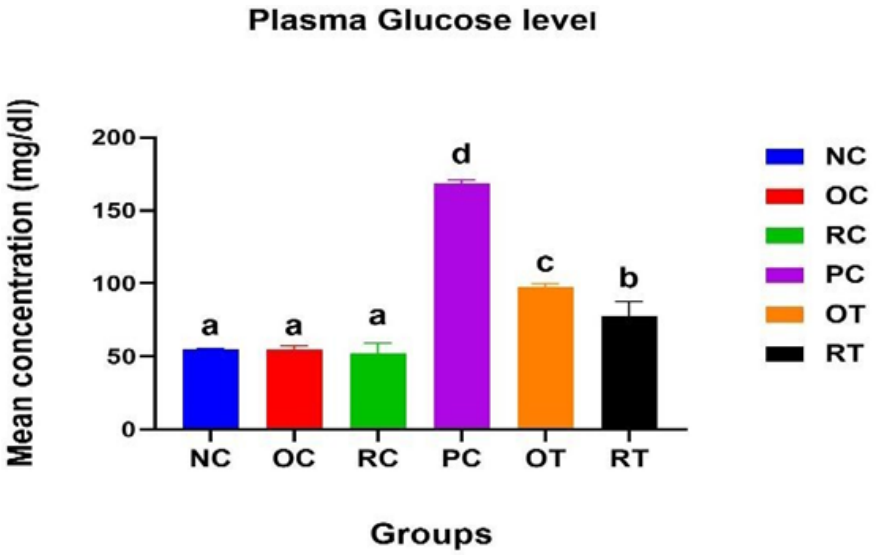


**Fig. 2. Plasma glucose levels in different groups.** Values are expressed as mean ± SEM. Columns that contain various lowercase letters are significant to each other. (Means are significant in contrast to the control groups at $P < 0.05$, n=5 rats). (NC) Normal control group (OC) Avocado oil control group. (RC) Reference drug control group. (PC) Positive control group. (OT) Avocado oil treated group. (RT) Reference drug- treated group.

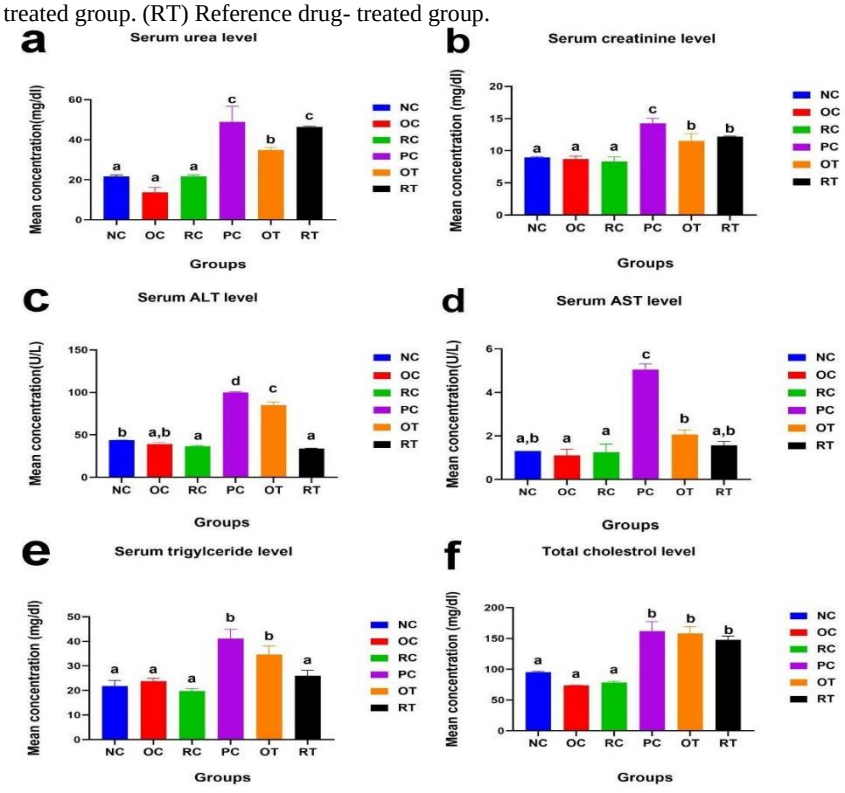


**Fig. 3. Charts of kidneys, liver function parameters, and lipid profile**. (a) serum urea level, (b) serum creatinine level, (c) serum ALT levels, (d) serum AST levels, (e) serum triglyceride level, and (f) serum total cholesterol level. Values are represented as mean ± SEM. Columns that have dissimilar lowercase letters are significant to each other. (Means are significant in comparison to the control groups at $P < 0.05$, n=5 rats). (NC) Normal control group (OC) Avocado oil control group. (RC) Reference drug control group. (PC) Positive control group. (OT) Avocado oil-treated group. (RT) Reference drug-treated group.

**3.5. Histopathology**

Histopathological scrutiny of the pancreas revealed the typical structure of pancreatic acini and islets of Langerhans in the NC, OC, and RC groups **(Fig. 4a, b & c).** In adverse, pancreatic tissue of PC rats exhibited vacuolar degeneration of pancreatic acinar epithelium **(Fig. 4d)** in addition to small-sized, markedly vacuolated islets of Langerhans with irregular border, congestion of pancreatic blood vessels associated with few inflammatory cell infiltrations **(Fig. 4e)**, hyperplasia in the pancreatic duct epithelium and thickening in its wall **(Fig. 4f).** On the other hand, the pancreas microscopy in OT and RT groups showed moderate sized islets of Langerhans with fewer degenerated cells **(Fig. 4g & h).** The avocado oil exerted a protective effect on beta cells of islet of Langerhans according to the results of histological examination of STZ-diabetic rats. Oxidative stress elevation plays a crucial role in beta cell destruction **[50]**. The study outlined by Dankyi et al. **[51]** revealed that avocado peel extracts have remarkable α-amylase and α-glucosidase inhibitory effects (enzymes associated with diabetes type II), and the suppression of these enzymes was correlated to the prevention of oxidative stress in the pancreas and was suggested to be a possible mechanism for the anti-diabetes characteristics of avocado extracts.

Concerning kidneys, microscopically, renal tissue of NC, OC, and RC groups revealed typical histological structure **(Fig. 5a, b, c).** In contrariwise, the kidneys of PC rats showed thickening of the glomerular basement membrane and mesangial expansion, renal lipidosis of tubular epithelial cells in which vacuoles were observed in many tubules **(Fig. 5d).** In the OT group, there was thickening of the glomerular basement membrane, congested glomerular tufts with little degeneration of the epithelial lining renal tubules **(Fig. 5e).** Furthermore, renal sections of RT group showed mild thickening of the glomerular basement membrane with mild tubular renal lipidosis **(Fig. 5f).** Administration of avocado oil enhanced the histopathological findings in the kidneys of the diabetic rats used in this study. Concurrently, it was found that avocado oil supplementation in obese diabetic mice has improved renal function and reduced markers of kidney damage, including a reduction in urinary albumin excretion in addition to inflammation and oxidative stress in the kidneys **[52]**. In a prior investigation, avocado oil improved the renal vascular function of hypertensive rats while also suppressing oxidative stress, mitochondrial dysfunction, and renal damage **[48]**.

Microscopy of the liver in NC, OC, RC groups revealed a typical histological structure **(Fig. 6a, b, c).** However, it revealed hepatocellular vacuolar degeneration, mononuclear inflammatory cell infiltration, periportal solitary hepatocellular necrosis **(Fig. 6d, e & f)** as well as slight hyperplasia in the portal area in the PC group. Otherwise, in liver microscopy of OT group, hepatocytes had minor vacuolar degeneration, and the central vein was slightly congested **(Fig. 6g).** Meanwhile, the hepatic tissue of rats from RT group exhibited slight Kupffer cell proliferation and binucleation of hepatocytes **(Fig. 6h).** According to the results of the previous study **[53]**, avocado oil also enhanced liver histopathology and reduced the harmful effects of STZ on the liver. This might be because avocado oil can increase the activity of the electron transport chain in the mitochondria of the liver, brain, and kidney, which lowers the levels of ROS and oxidative stress in the mitochondria **[54, 55].**

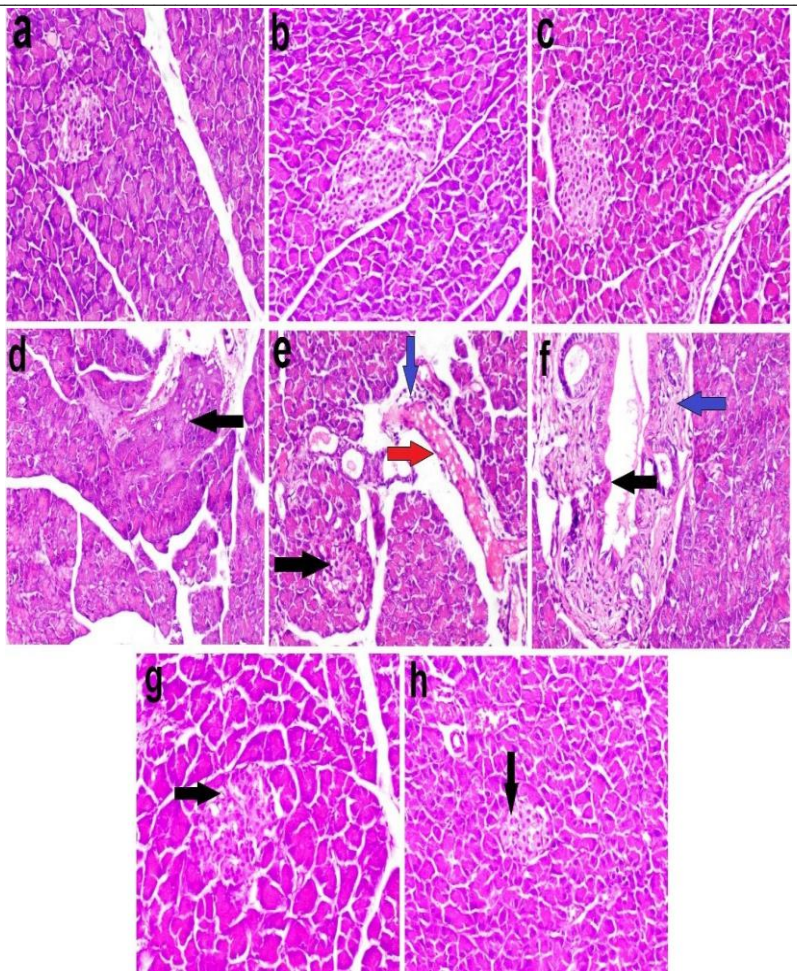


**Fig. 4. Histopathology of pancreas in all groups.** (a) NC, (b) OC, and (c) RC groups showed typical histological structure, (d), (e) & (f) PC group; (d) showed vacuolar degeneration of pancreatic acinar epithelium (black arrow), (e) showed small-sized, markedly vacuolated islets of Langerhans (black arrow), congested blood vessel (red arrow), and few inflammatory cells infiltration (blue arrow), and (f) showed hyperplasia in the pancreatic duct (black arrow) and thickening in its wall (blue arrow), (g) OT and (h) RT groups showed vacuolar degeneration of few cells of islets of Langerhans (black arrow). (Hematoxylin and eosin stain, 200X).

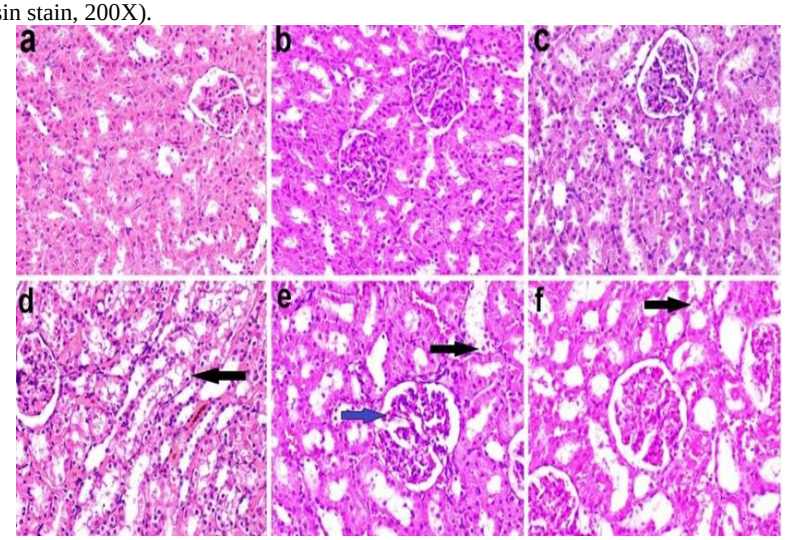


**Fig. 5. Histopathology of renal cortex in all groups.** (a) NC, (b) OC , and (c) RC groups exhibited appropriate histological structure , (d) PC group showed severe renal lipidosis of tubular epithelial cells (black arrow), (e) OT group showed congested glomerular tuft (blue arrow), some renal tubules had slight degeneration in the epithelial lining (black arrow) ,and (f) RT group showed mild renal lipidosis in some tubular epithelium (black arrow).(Hematoxylin and eosin stain 200X).

### 3.6. Immunohistochemical findings of insulin and iNOS expression

The immune expression of insulin was demonstrated in the islets of Langerhans in the pancreatic beta cells. It was severely expressed in NC, OC, and RC groups **(Fig. 7a, b & c,),** weakly expressed in the PC group **(Fig. 7d),** and moderately expressed in OT and RT groups **(Fig. 7e & f).** The immune expression of iNOS was demonstrated in the pancreatic acini. It was weakly expressed in NC, OC, and RC groups **(Fig. 7g, h & i),** severely expressed in the PC group **(Fig. 7j)**, and moderately expressed in both OT and RT groups **(Fig. 7k & l).** The immune expression of iNOS in the kidneys. It was mildly expressed in the NC, OC, and RC groups **(Fig. 8a, b & c)** and severely expressed in the epithelial lining of renal tubules as well as in endothelial lining the glomerular tufts **(Fig. 8d).** The OT group revealed mild expression of iNOS in the glomerular tufts, and epithelial lining some renal tubules in contrast to the PC group **(Fig. 8e)**, while the reference drug-treated group revealed high expression of iNOS in both glomerular tufts and epithelial cells of renal tubules **(Fig. 8f).**

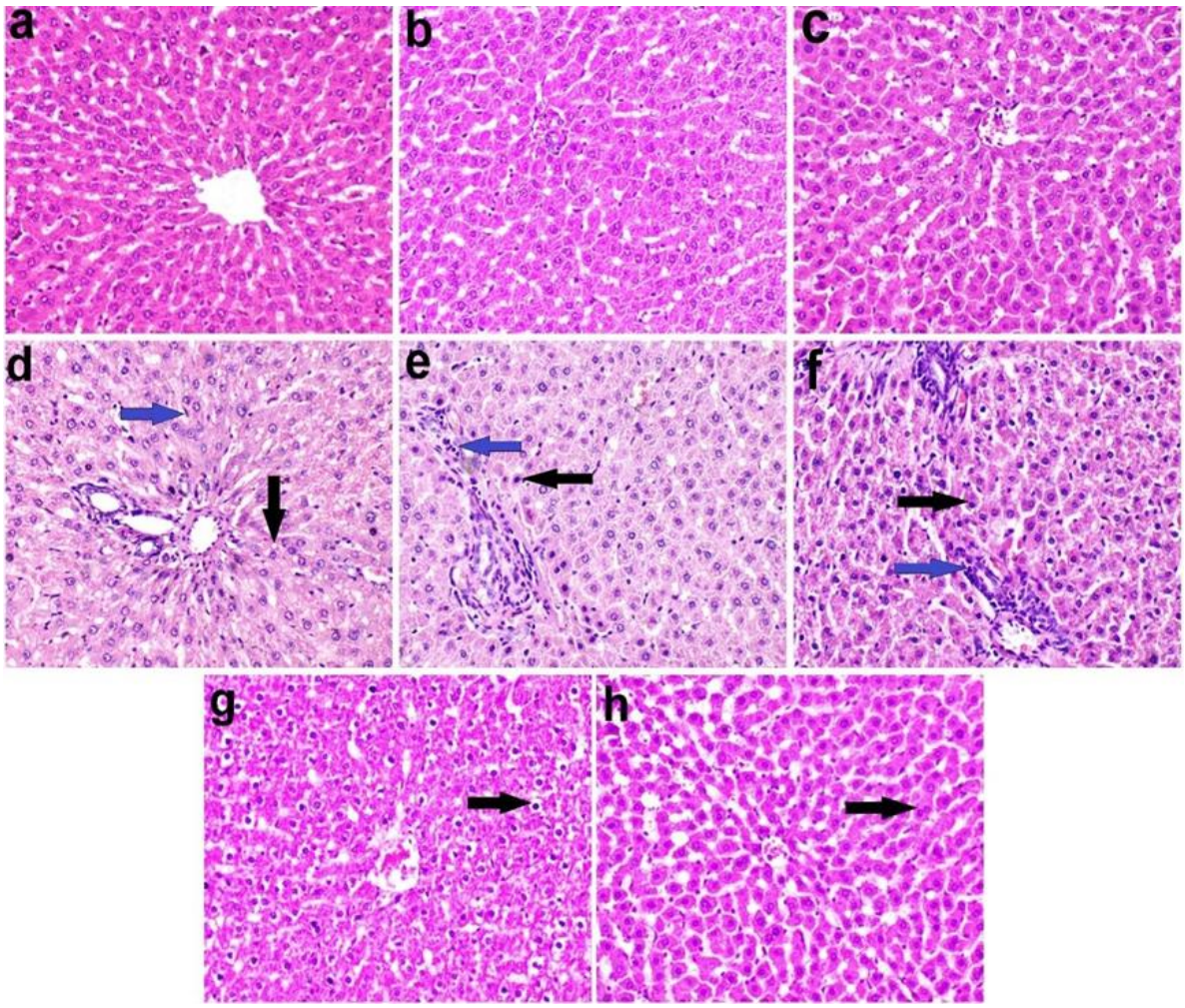


**Fig. 6. Histopathology of liver in all groups.** (a) NC, (b) OC, and (c) RC groups showed normal histological structure, (d), (e) & (f) PC group; (d) showed hepatocellular vacuolar degeneration (black arrow) and Kupffer cell activation (blue arrow). (e) & (f) showed periportal solitary hepatocellular necrosis (black arrow) and portal mononuclear inflammatory cell infiltration (blue arrow). (g) OT group showed mild vacuolar degeneration of hepatocytes (black arrow). (h) RT group showed binucleation of hepatocytes (black arrow). (Hematoxylin and eosin stain, 200X).

The immune expression of iNOS in the liver. It was mildly expressed in the NC, OC, and RC groups **(Fig. 8g, h & i)** and severely expressed in the cytoplasm of hepatocytes of the positive control (PC) group **(Fig. 8j).** The OT group revealed mild expression of iNOS **(Fig. 8k)**, while the reference drug-treated group revealed moderate expression of iNOS in the cytoplasm of hepatocytes **(Fig. 8l).** The area percent of insulin and iNOS expression in different organs are presented in **Fig. 9**. In the PC group, a notable reduction of the percentage of the area of insulin-positive beta cells was observed in contrast to the normal

control group. There is improvement in the OT group. However, in the RT group, the percentage of area of beta cells increased significantly and even partially restored. Furthermore, there was a substantial reduction in iNOS expression in avocado oil-treated (OT) and reference drug-treated (RT) groups in contrast to the positive control (PC) group.

Inducible nitric oxide synthetase (iNOS) is an enzyme that is triggered in cells by pro-inflammatory cytokines and/or bacterial lipopolysaccharide (LPS) producing a high amount of NO (micromolar range) **[56]**. However, its overexpression can result in deleterious effects due to high NO generation **[56]**. The immune expression of iNOS in the pancreas, kidneys, and liver in case of avocado oil-treated group was decreased, which indicates reduced peroxynitrite formation, a free radical that induces oxidative stress. The antioxidant effect of avocado oil may be due to the level of monounsaturated fatty acids especially oleic that proved to be effective in counteracting the suppression of the inflammatory cytokine TNF- α on insulin production in rat pancreatic beta cell line INS-1 **[57].**

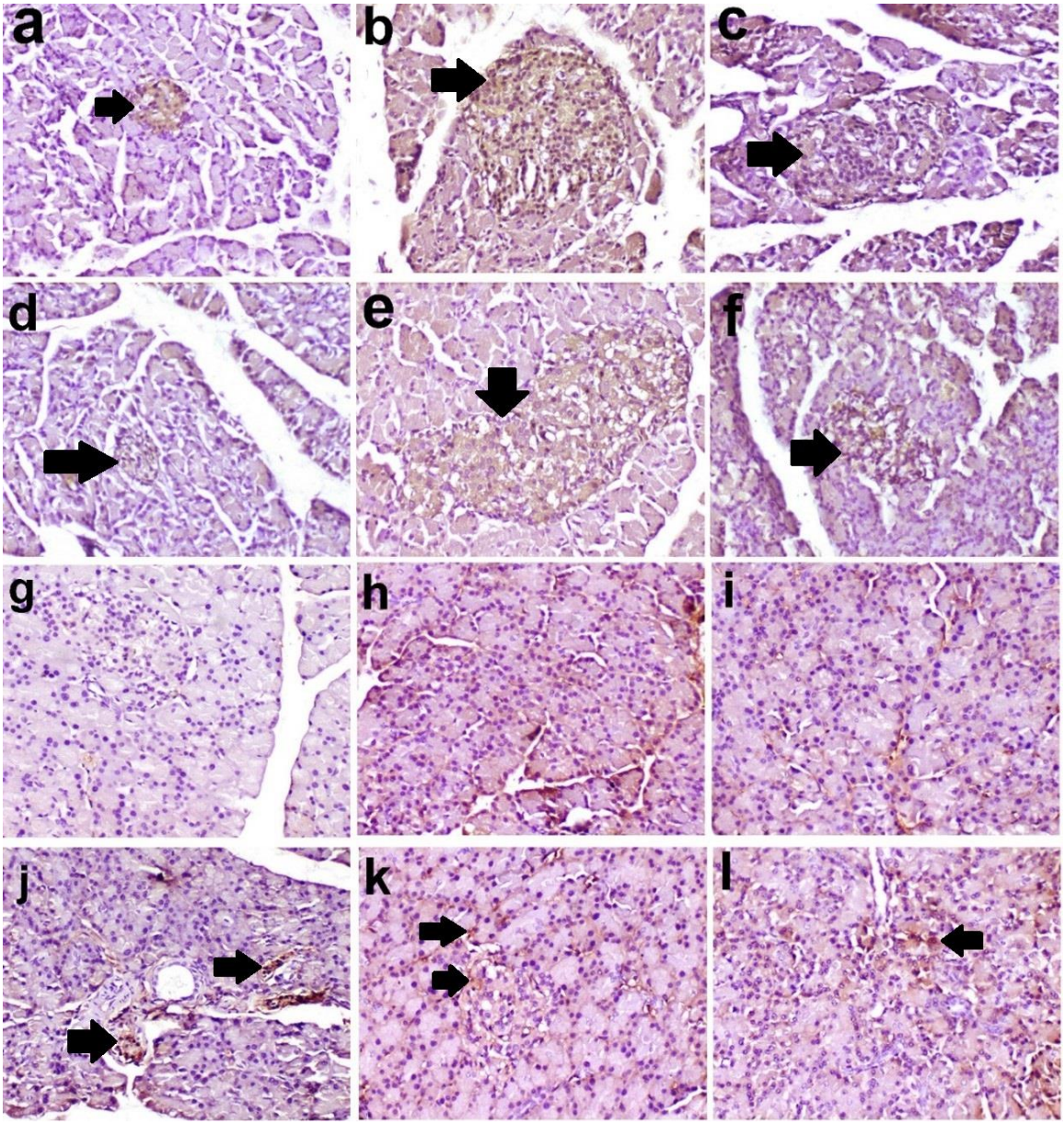


**Fig. 7 (a-f): Insulin Immunohistochemistry in beta cells of islets of Langerhans in the pancreas of rats.** (a) NC, (b) OC, and (c) RC groups showed well-arranged positive cells, (d) the PC group showed few positive cells, (e) OT group showed many well-arranged positive cells, and (f) RT group showed moderately well- arranged positive cells in islets of Langerhans. **(g-l) Immunohistochemistry of iNOS in the pancreatic acini.** (g) NC, (h) OC, and (i) RC groups showed mild expression, (j) PC group showed severe expression, (k) OT, and (l) RT groups showed moderate expression. Black arrows referred to immune reaction of iNOS in pancreas. (DAB and hematoxylin, 200X).

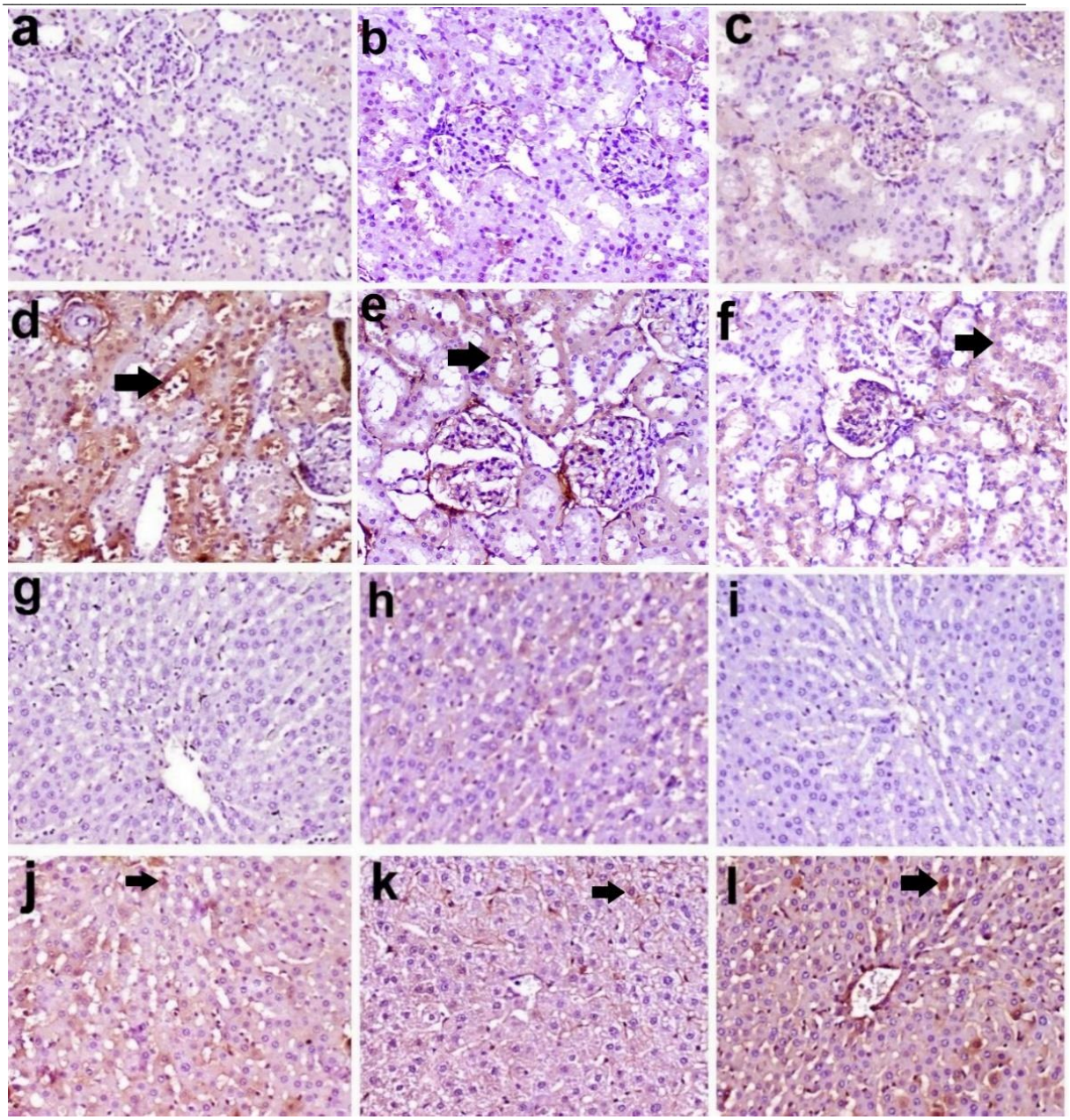


**Fig. 8 (a-f) Immunohistochemistry of iNOS in the kidney.** (a) NC, (b) OC, and (c) RC groups showed weak expression, (d) PC group showed severe expression in the epithelial lining of renal tubules, (e) OT group showed mild expression in the basement membrane of Bowman's capsule, the glomerular tuft, and in the epithelial lining of renal tubules, and (f) RT group showed moderate expression of iNOS in both glomerular tuft and epithelial cells of renal tubules. **(g-l) Immunohistochemistry of iNOS in liver.** (g) NC, (h) OC, and (i) RC groups showed weak expression in hepatocytes, (j) PC group showed severe expression in hepatocytes, (k) OT group showed mild expression in hepatocytes, (l) RT group showed moderate expression of iNOS in hepatocytes. Black arrows referred to immune reaction of iNOS.
(DAB and hematoxylin ,200X).

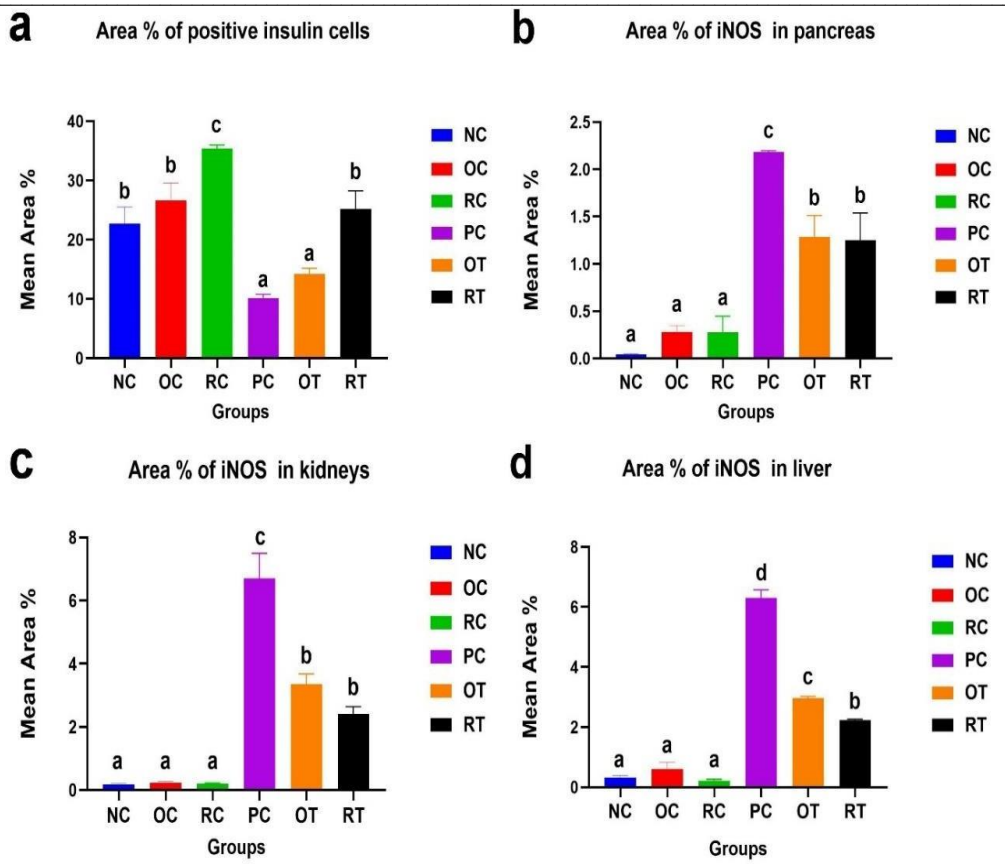


**Fig. 9**. (a) Image analysis of the percentage of area of positive insulin cells in the islets of Langerhans**.** (b) The percentage of the area of iNOS in the pancreas. (c) The percentage of the area of iNOS in the kidney. (d) The percentage of the area of iNOS in the liver. Values are identified as mean ± SEM. Columns having dissimilar lowercase letters are significant to each other. (n=5 rats, Means are significant in contrast to the control groups at $P < 0.05$). (NC) Normal control group (OC) Avocado oil control group. (RC) Reference drug control group. (PC) Positive control group. (OT) Avocado oil-treated group. (RT) Reference drug-treated group.

**4. Conclusion**

In conclusion, avocado oil possessed an anti-diabetic potential as it improved the biochemical markers and histopathology of the pancreas, kidneys, and liver. This could be attributed to the antioxidant potential of avocado oil. Additional studies can further elucidate the mechanism of avocado oil on diabetes.

**5. Conflict of Interest**

The authors declare that there is no conflict of interest.

**6. Formatting of funding sources**

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Not applicable.

**8. References**

1. **Daryabor, G., Atashzar, M. R., Kabelitz, D., Meri, S., & Kalantar, K. (2020).** The Effects of Type 2 Diabetes Mellitus on Organ Metabolism and the Immune System. Frontiers in immunology, 11, 1582. https://doi.org/10.3389/fimmu.2020.01582.

2. **Ohiagu, F., Chikezie, P., & Chikezie, C. (2021).** Pathophysiology of diabetes mellitus complications: Metabolic events and control. Biomedical Research and Therapy, 8(3), 4243-4257. https://doi.org/10.15419/bmrat.v8i3.663.

3. **Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020).** Pathophysiology of Type 2 Diabetes Mellitus. International journal of molecular sciences, 21(17), 6275. https://doi.org/10.3390/ijms21176275.

4. **Yaribeygi, H., Sathyapalan, T., Atkin, S. L., & Sahebkar, A. (2020)**. Molecular mechanisms linking oxidative stress and diabetes mellitus. Oxidative medicine and cellular longevity, 2020(1), doi:10.1155/2020/8609213.

5. **Khin, P. P., Lee, J. H., & Jun, H. S. (2023).** Pancreatic beta-cell dysfunction in type 2 diabetes. European Journal of Inflammation, 21, 1721727X231154152.

6. **Kosmalski, M., Ziółkowska, S., Czarny, P., Szemraj, J., & Pietras, T. (2022).** The coexistence of nonalcoholic fatty liver disease and type 2 diabetes mellitus. Journal of Clinical Medicine, 11(5), 1375.

7. **Evangelia Makri, Antonis Goulas, Stergios A. Polyzos. (2021).** Epidemiology, Pathogenesis, Diagnosis and Emerging Treatment of Nonalcoholic Fatty Liver Disease, Archives of Medical Research, 52(1), 25-
37. https://doi.org/10.1016/j.arcmed.2020.11.010.

8. **Khneizer, G., Rizvi, S., & Gawrieh, S. (2021).** Non-alcoholic Fatty Liver Disease and Diabetes Mellitus. Advances in experimental medicine and biology, 1307, 417–440. https://doi.org/10.1007/5584_2020_532

9. **Grgurevic, Ivica, Tonci Bozin, Mislav Mikus, Michal Kukla, and James O'Beirne. (2021).** Hepatocellular Carcinoma in Non-Alcoholic Fatty Liver Disease: From Epidemiology to Diagnostic Approach, Cancers 13, (22): 5844. https://doi.org/10.3390/cancers13225844

10. **Xie, D., Ma, T., Cui, H., Li, J., Zhang, A., Sheng, Z., & Xie, Y. (2023).** Global burden and influencing factors of chronic kidney disease due to type 2 diabetes in adults aged 20-59 years, 1990-2019. Scientific reports, 13(1),
20234. https://doi.org/10.1038/s41598-023-47091-y.

11. **Sinha, S. K., & Nicholas, S. B. (2023).** Pathomechanisms of Diabetic Kidney Disease. Journal of clinical medicine, 12(23), 7349. https://doi.org/10.3390/jcm12237349.

12. **Aly, T.A.A., Mohamed, S.M., Khattab, M.S., Abido, A.M.M., Abdel- Rahim, E.A., Al-Farga, A., Sarpong, F., Aqlan, F. (2023).** Clover microgreen incorporation in diet-controlled diabetes and counteracted aflatoxicosis of rats. Food Science and Nutrition. 11(12):7605-7617. doi: 10.1002/fsn3.3680.

13. **Mohamed, S.M., Aly, T.A.A., Khattab, M.S., Abdel-Rahim, E.A., Al- Farga, A. (2023).** Pathological and biochemical evaluation of radish microgreen on diabetes and aflatoxicosis in rats. Environmental Science and Pollution Research Int. 30(43):98389-98399. doi: 10.1007/s11356-023- 29334-7.

14. **Ahmed, O. M., Fahim, H. I., Mohamed, E. E., & Abdel-Moneim, A. (2022)**. Protective effects of Persea americana fruit and seed extracts against chemically induced liver cancer in rats by enhancing their antioxidant, anti-inflammatory, and apoptotic activities. Environmental science and pollution research international, 29(29), 43858–43873. https://doi.org/10.1007/s11356-022-18902-y.

15. **Lucas J. Stolp, Dharma R. Kodali, (2022),** Chapter 2 - Naturally occurring high-oleic oils: Avocado, macadamia, and olive oils,Editor(s): Frank J. Flider,High Oleic Oils,AOCS Press, Pages 7-52, ISBN 9780128229125. https://doi.org/10.1016/B978-0-12-822912-5.00003-4.

16. **Kamiloglu, S., Capanoglu, E., Jafari, S.M. (2022).** An Overview of Food Bioactive Compounds and Their Health-Promoting Features. In: Jafari, S.M., Capanoglu, E. (eds) Retention of Bioactives in Food Processing. Food Bioactive Ingredients. Springer, Cham. https://doi.org/10.1007/978-3-030- 96885-4_1.

17. **I.O.f. Standardization, Animal and Vegetable Fats and Oils: Gas Chromatography of Fatty Acid Methyl Esters: Part 2: Preparation of Methyl Esters of Fatty Acids, International Organization for Standardization, 2011.**

18. **Younis, I. Y., Khattab, A. R., Selim, N. M., Sobeh, M., Elhawary, S. S., & Bishbishy, M. H. E. (2022)**. Metabolomics-based profiling of 4 avocado varieties using HPLC-MS/MS and GC/MS and evaluation of their antidiabetic activity. Scientific reports, 12(1), 4966.
https://doi.org/10.1038/s41598-022-08479-4

19. **Uarrota, V. G., Hernández, I., Ponce, E., Bauer, C. M., Maraschin, M., & Pedreschi, R. (2022).** Metabolic profiling and biochemical analysis of stored Hass avocado fruit by GC-MS and UHPLC-UV-VIS revealed oxidative stress as the main driver of 'blackspot'physiological disorder. International Journal of Food Science & Technology, 57(12), 7896-7916.

20. **Tang, J., Dunshea, F. R., & Suleria, H. A. R. (2019).** LC-ESI-QTOF/MS Characterization of Phenolic Compounds from Medicinal Plants (Hops and Juniper Berries) and Their Antioxidant Activity. Foods (Basel, Switzerland), 9(1), 7. https://doi.org/10.3390/foods9010007

21. **Balakrishnan N, Panda A, Raj N, Shrivastava A, Prathani R. (2009).** The evaluation of nitric oxide scavenging activity of Acalypha indica Linn root. Asian Journal of Research in Chemistry 2:148–150.

22. **Sreejayan, & Rao, M. N. (1997).** Nitric oxide scavenging by curcuminoids. The Journal of pharmacy and pharmacology, 49(1), 105–107. https://doi.org/10.1111/j.2042-7158.1997.tb06761.x

23. **Siddhuraju, P., & Becker, K. (2003).** Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. Journal of agricultural and food chemistry, 51(8), 2144–2155. https://doi.org/10.1021/jf020444

24. **Ozolua, R. I., Anaka, O. N., Okpo, S. O., & Idogun, S. E. (2009).** Acute and sub-acute toxicological assessment of the aqueous seed extract of Persea americana mill (Lauraceae) in rats. African journal of traditional, complementary, and alternative medicines : AJTCAM, 6(4), 573–578. https://doi.org/10.4314/ajtcam.v6i4.57214

25. **Mythili, M. D., Vyas, R., Akila, G., & Gunasekaran, S. (2004).** Effect of streptozotocin on the ultrastructure of rat pancreatic islets. Microscopy research and technique, 63(5), 274–281. https://doi.org/10.1002/jemt.20039

26. **Trinder P. (1969).** Determination of blood glucose using an oxidase- peroxidase system with a non-carcinogenic chromogen. Journal of clinical pathology, 22(2), 158–161. https://doi.org/10.1136/jcp.22.2.158

27. **Reitman, S., & Frankel, S. (1957).** A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology, 28(1), 56–63. https://doi.org/10.1093/ajcp/28.1.56

28. **Fawcett, J. K., & scott, J. E. (1960).** A rapid and precise method for the determination of urea. Journal of clinical pathology, 13(2), 156–159. https://doi.org/10.1136/jcp.13.2.156

29. **Moore, J. F., & Sharer, J. D. (2017).** Methods for Quantitative Creatinine Determination. Current protocols in human genetics, 93, A.3O.1–A.3O.7. https://doi.org/10.1002/cphg.38

30. **Fossati, P., & Prencipe, L. (1982).** Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical chemistry, 28(10), 2077–2080.

31. **Richmond W. (1973).** Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clinical chemistry, 19(12), 1350–1356.

32. **Suvarna, S. K., Layton, C., & Bancroft, J. D. (2019).** Bancroft's Theory and Practice of Histological Techniques. Churchill Livingstone Elsevier.

33. **Campbell, S.C., Macfarlane, W.M. (2003)**. Detection of Insulin Production by Immunohistochemistry. In: Özcan, S. (eds) Diabetes Mellitus. Methods in Molecular Biology™, vol 83. Humana Press. https://doi.org/10.1385/1- 59259-377-1:047

34. **Martín-Burriel, I., Roome, N. O., Dorchies, O., & Prenez, A. (2004).** Histopathological and molecular changes during apoptosis produced by 7H- dibenzo[c,g]-carbazole in mouse liver. Toxicologic pathology, 32(2), 202–

211. https://doi.org/10.1080/01926230490274353

35. **Hsu, W. H., Hsiao, P. J., Lin, P. C., Chen, S. C., Lee, M. Y., & Shin, S.J. (2017).** Effect of metformin on kidney function in patients with type 2 diabetes mellitus and moderate chronic kidney disease. Oncotarget, 9(4), 5416–5423. https://doi.org/10.18632/oncotarget.23387.

36. **Marra, A., Manousakis, V., Zervas, G. P., Koutis, N., Finos, M. A., Adamantidi, T., ... & Tsoupras, A. (2024).**

Avocado and Its By-Products as Natural Sources of Valuable Anti-Inflammatory and Antioxidant Bioactives for Functional Foods and Cosmetics with Health PromotingProperties. AppliedSciences, 14(14), https://doi.org/10.3390/app 14145978

37. **Gao, Y., Xun, R., Xia, J., Xia, H., & Sun, G. (2023).** Effects of phytosterol supplementation on lipid profiles in patients with hypercholesterolemia: a systematic review and meta-analysis of randomized controlled trials. Food & function, 14(7), 2969–2997. https://doi.org/10.1039/d2fo03663k.

38. **Kupnik, K., Primožič, M., Kokol, V., Knez, Ž., & Leitgeb, M. (2023).** Enzymatic, Antioxidant, and Antimicrobial Activities of Bioactive Compounds from Avocado (Persea americana L.) Seeds. Plants (Basel, Switzerland), 12(5), 1201. https://doi.org/10.3390/plants12051201.

39. **Bhuyan, D. J., Alsherbiny, M. A., Perera, S., Low, M., Basu, A., Devi, O. A., Barooah, M. S., Li, C. G., & Papoutsis, K. (2019).** The Odyssey of Bioactive Compounds in Avocado (Persea americana) and Their Health Benefits. Antioxidants (Basel, Switzerland), 8(10),426. https://doi.org/10.3390/antiox8100426

**40. Wang, Q., Liu, R., Chang, M., Zhang, H., Jin, Q., & Wang, X. (2020).** Dietary oleic acid supplementation and blood inflammatory markers: a systematic review and meta-analysis of randomized controlled trials. Critical Reviews in Food Science and Nutrition, 62(9), 2508–2525. https://doi.org/10.1080/10408398.2020.1854673

**41. Ramos-Aguilar, A. L., Ornelas-Paz, J., Tapia-Vargas, L. M., Gardea- Béjar, A. A., Yahia, E. M., Ornelas-Paz, J. J., Ruiz-Cruz, S., Rios- Velasco, C., & Escalante-Minakata, P. (2021).** Effect of cultivar on the content of selected phytochemicals in avocado peels. Food research international (Ottawa, Ont.), 140, 110024. https://doi.org/10.1016/j.foodres.2020.110024

42. **Flores, M., Saravia, C., Vergara, C. E., Avila, F., Valdés, H., & Ortiz- Viedma, J. (2019).** Avocado Oil: Characteristics, Properties, and Applications. Molecules (Basel, Switzerland), 24(11), 2172. https://doi.org/10.3390/molecules24112172

43. **Abd Elkader, A. M., Labib, S., Taha, T. F., Althobaiti, F., Aldhahrani, A., Salem, H. M., Saad, A., & Ibrahim, F. M. (2022).** Phytogenic compounds from avocado (Persea americana L.) extracts; antioxidant activity, amylase inhibitory activity, therapeutic potential of type 2 diabetes. Saudi journal of biological sciences, 29(3), 1428–1433. https://doi.org/10.1016/j.sjbs.2021.11.031

44. **Aigbiremolen A, Ativie R, Aisuodionoe M, Odigie O, Igweh J, Egwaoje M. (2018).** Effect of aqueous extract of Persea americana seed on blood glucose in Alloxan-induced diabetic Wistar rats. Asian J Med Health 9(3):1– 10

45**. Câmara, J. S., Albuquerque, B. R., Aguiar, J., Corrêa, R. C. G., Gonçalves, J. L., Granato, D., Pereira, J. A. M., Barros, L., & Ferreira, I. C. F. R. (2020).** Food Bioactive Compounds and Emerging Techniques for Their Extraction: Polyphenols as a Case Study. Foods (Basel, Switzerland), 10(1), 37. https://doi.org/10.3390/foods10010037

46. **Cárdenas-Castro, A.P., Fernández-Ochoa, Á., Cádiz-Gurrea, M.d.l.L., Segura Carretero, A., Sáyago-Ayerdi, S.G. (2023).** Bioactive Phytochemicals from Avocado Oil Processing By-Products. In: Ramadan Hassanien, M.F. (eds) Bioactive Phytochemicals from Vegetable Oil and Oilseed Processing By-products. Reference Series in Phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-030-91381-6_18

47. **Cheng, F. W., Rodríguez-Ramírez, S., Shamah-Levy, T., Pérez-Tepayo, S., & Ford, N. A. (2024).** Association Between Avocado Consumption and Diabetes in Mexican Adults: Results From the 2012, 2016, and 2018 Mexican National Health and Nutrition Surveys. Journal of the Academy of Nutrition and Dietetics, S2212-2672(24)00192-8. Advance online publication. https://doi.org/10.1016/j.jand.2024.04.012.

48. **Márquez-Ramírez, C. A., Olmos-Orizaba, B. E., García-Berumen, C. I., Calderón-Cortés, E., Montoya-Pérez, R., Saavedra-Molina, A., Rodríguez-Orozco, A. R., & Cortés-Rojo, C. (2021).** Avocado Oil Prevents Kidney Injury and Normalizes Renal Vasodilation after Adrenergic Stimulation in Hypertensive Rats: Probable Role of Improvement in Mitochondrial Dysfunction and Oxidative Stress. Life (Basel, Switzerland), 11(11), 1122.

https://doi.org/10.3390/life11111122.

49. **Okobi, O. E., Odoma, V. A., Okunromade, O., Louise-Oluwasanmi, O., Itua, B., Ndubuisi, C., Ogbeifun, O. E., Nwatamole, B. C., Elimihele, T. A., Adekunle, J. O., Adekunle, A. A., Obi, C. B., & Evbayekha, E. O. (2023).** Effect of Avocado Consumption on Risk Factors of Cardiovascular Diseases: A Systematic Review and Meta-Analysis. Cureus, 15(6), e41189. https://doi.org/10.7759/cureus.41189.

50. **Mohamed, S. M., Abdel-Rahim, E. A., Aly, T. A., Naguib, A. M., & Khattab, M. S. (2022).** Barley microgreen incorporation in diet-controlled diabetes and counteracted aflatoxicosis in rats. Experimental biology and medicine (Maywood, N.J.), 247(5), 385–394. https://doi.org/10.1177/15353702211059765.

51. **Dankyi, B. O., Kukuia, K. K. E., Koomson, A. E., & Amponsah, S. K. (2024).** Antidiabetic Activity of Food Phytochemicals. In Plant Food Phytochemicals and Bioactive Compounds in Nutrition and Health (pp. 298-330). CRC Press.

52. **Elmoslemany, A. M., El-Magd, M. A., Ghamry, H. I., Alshahrani, M. Y., Zidan, N. S., & Zedan, A. M. G. (2021).** Avocado Seeds Relieve Oxidative Stress-Dependent Nephrotoxicity but Enhance Immunosuppression Induced by Cyclosporine in Rats. Antioxidants (Basel, Switzerland), 10(8), 1194. https://doi.org/10.3390/antiox10081194

53. **García-Berumen, C. I., Vargas-Vargas, M. A., Ortiz-Avila, O., Piña- Zentella, R. M., Ramos-Gómez, M., Figueroa-García, M. D. C., Mejía- Zepeda, R., Rodríguez-Orozco, A. R., Saavedra-Molina, A., & Cortés- Rojo, C. (2022)**. Avocado oil alleviates non-alcoholic fatty liver disease by improving mitochondrial function, oxidative stress and inflammation in rats fed a high fat-High fructose diet. Frontiers in pharmacology, 13, 1089130. https://doi.org/10.3389/fphar.2022.1089130

54. **Ogunmoyole, T., Dada, I., & Adebamigbe, O. A. (2021).** Ameliorative potentials of Persea americana leaf extract on toxicants-induced oxidative assault in multiple organs of wistar albino rat. Clinical Phytoscience, 7, 1-15. https://doi.org/10.1186/s40816-020-00237-1

55. **de Oliveira Marques, S., Muller, A. P., Luciano, T. F., Dos Santos Tramontin, N., da Silva Caetano, M., Luis da Silva Pieri, B., Amorim, T. L., de Oliveira, M. A. L., & de Souza, C. T. (2022).** Effects of Avocado Oil Supplementation on Insulin Sensitivity, Cognition, and Inflammatory and Oxidative Stress Markers in Different Tissues of Diet-Induced Obese Mice. Nutrients, 14(14), 2906. https://doi.org/10.3390/nu14142906

56. **Cinelli, M. A., Do, H. T., Miley, G. P., & Silverman, R. B. (2020).** Inducible nitric oxide synthase: Regulation, structure, and inhibition. Medicinal research reviews, 40(1), 158-189
. https://doi.org/10.1002/med.21599.

57. **Wang, L., Tao, L., Hao, L., Stanley, T. H., Huang, K. H., Lambert, J. D., & Kris-Etherton, P. M. (2020).** A Moderate-Fat Diet with One Avocado per Day Increases Plasma Antioxidants and Decreases the Oxidation of Small, Dense LDL in Adults with Overweight and Obesity: A Randomized Controlled Trial. The Journal of Nutrition, 150(2), 276–284. https://doi.org/10.1093/jn/nxz231.