DOI: https://doi.org/10.54361/LJMR.20.3.108

Libyan Journal of Medical Research

www.lmjr.ly/ E.ISSN: 2413-6096

Original Article

Protective Effects of Virgin Olive Oil on Liver function and Histopathological Alterations in Diet-induced Hyperlipidaemic Rats

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***Received:**13-09-2026-**Accepted:** 30-10-2026 -**Published:** 14-11-2026*

Abstract

Background: Hyperlipidaemia may predispose to liver injury through lipid deposition, oxidative stress, and inflammatory reactions. This study aimed to investigate the protective role of virgin olive oil in the biochemical and histological changes of the liver in diet-induced hyperlipidaemic male albino rats. **Materials and Methods:** Fifty adult male albino rats were divided into five groups (10 rats each): normal diet (ND), hyperlipidaemic diet (HLD), HLD plus low dose of virgin olive oil (HLD+LVOO), HLD plus high dose of virgin olive oil (HLD+HVOO) and HLD followed by ND (HLD+ND). Liver samples were assessed using a light microscope following haematoxylin and eosin staining. Liver function tests, namely ALT, AST and ALP serum activity, were measured. **Results:** ALT, AST and ALP were progressive increases in the HLD group, accompanied by significant architectural damages of liver. The administration of virgin olive oil resulted in decreased elevations of these enzymes and partial preservation of histopathological characteristics, with the strongest effects observed at the higher dose. A dietary recovery without virgin olive oil gave only some biochemical and histological restoration. **Conclusion**: Virgin olive oil has prevented liver injuries that accompany hyperlipidaemia in this rat model, with both biochemical and histopathological benefits being correlated with dose, although more study on the mechanism is needed.

Keywords: Virgin olive oil; hyperlipidaemia; liver injury; ALT; AST; ALP; histopathology; rats.

Introduction:

Hyperlipidaemia is defined by the presence of elevated circulating lipid concentrations. Hyperlipidaemia is frequently associated with obesity, insulin resistance, metabolic syndrome, and cardiovascular disease. Since the liver is critical for lipid synthesis, oxidation, storage, transport, delivery and production that outstrip hepatic clearance leads to lipid accumulation in hepatocytes. Overwhelmingly, lipid-mediated metabolic burden can drive steatosis, mitochondrial dysfunction, oxidative stress, inflammation, and cumulative structural injury [1-3].

The primary pathway by which aberrant lipid metabolism contributes to hepatocellular injury is through oxidative stress. Aberrant increases in ROS lead to lipid peroxidation, dysfunction of cellular and mitochondrial membranes, and inflammation that may result in swelling of hepatocytes, vacuolar degeneration, inflammatory cell infiltration, and destruction of architecture [2, 3]. Routine biochemical tests (serum ALT, AST and ALP) for liver injury were assessed. An increase in predominantly ALT and AST suggests a hepatocellular process, while an elevation of ALP may reflect a cholestatic process or a biliary component. However, the limited specificity of these enzymes to tissues and their failure to reflect the tissue architecture of an organ, measuring biochemistry alongside histopathology gives an overview picture of the liver structure in an experimental setup [4].

Virgin olive oil is one of the main providers of fat in the Mediterranean diet. Virgin olive oil is mostly rich in monounsaturated fatty acids such as oleic acid, but it is also characterised by several minor bioactive compounds such as oleocanthal, tyrosol, hydroxytyrosol, oleuropein derivatives, oleosides, lignans, tocopherols and squalene [1-3]. Olive-oil phenolics have antioxidant and anti-inflammatory effects, affecting in addition lipid metabolism, signalling pathways and immune responses [4-7]. Biological basis from experimental models There are other examples in experimental models to illustrate a hepatic protective action. Supplementation with olive oil resulted in a better lipid profile and antioxidant function in hypercholesterolemic rats [10]. Virgin olive oil also lowered hepatic lipid peroxidation and the levels of serum transaminases in rat models exposed to ethanol [11], and extra virgin olive oil also had a beneficial effect on liver biochemistry and histological injury in other models of injury from toxins [12]. In models of high-fat diet, the

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components of olive oil have also demonstrated a better management of liver lipids and a reduction in steatotic damage [7, 13]. The effectiveness of protection depends on the dose and components in the oil, the experimental model, and the time of administration. Herein, we have analysed the protective effect of both low and high doses of virgin olive oil supplementation through estimation of serum ALT, AST and ALP and investigation of liver histopathology in diet-induced hyperlipidaemic male albino rats and included one group that came back from the hyperlipidaemic diet to the normal diet to find out whether dietary correction could resolve these disorders.

Materials & Methods

Induction of hyperlipidaemia:

Hyperlipidaemia was artificially achieved by feeding the specified groups of rats with a high fat diet for 3 weeks before the treatment period. The experimental diet was high in fat, comprising 20 g of fat per 100 g of diet, which included 19 g of butter oil and 1 g of soybean oil per 100 g. The development of fat formulations was designed as a response to changes in standard diets [14].

The olive oil used in this study was obtained from an olive field produced in Tarhuna, Libya, that produces the fruit Olea europaea L. (family Oleaceae) olives as identified by the Agriculture Research Center (ARC) by fatty acid profile (C16:0 (11.32 g/100 g), C16:1 (n-9) (0.11 g/100 g), C16:1 (n-7) (0.84 g/100 g), C18:0 (4.34 g/100 g), C18:1 (n-9) (74.12 g/100 g), C18:2 (n-6) (7.64 g/100 g), and C18:3 (n-3) (0.61 g/100 g)). Each group was intubated orally with olive oil. The oil was given to rats in two doses, a low dose (1 ml/100 g body weight) and a high dose (2 ml/100 g body weight).

Animals and *experimental design*: Fifty male Sprague Dawley rats, apparently healthy, initially weighing 90-110 g, were used in the current investigation. Animals were kept in the animal house of the Faculty of Veterinary Medicine at Azzytuna University**,** Libya, for two weeks to acclimatise to standard commercial cages equipped with automatic drinking and feeding systems. Animals were kept under environmental conditions of 22 ± 2°C in a 14-h light/10-h dark cycle during the experiment. The experimental manipulations conform to the guidelines of the Bioethics Committee at the Biotechnology Research Centre, Tripoli, Libya (ref number: NBC: 001.A.26.52).

Blood collection and serum separation: Samples were collected weekly in the morning before feed and water from the retro-orbital venous plexus under ether inhalation anaesthesia into a plain tube and allowed to clot at room temperature before centrifugation at 1000 g for 20 minutes to obtain serum. The non-haemolysed serum supernatant was collected as a clear liquid and stored at -20°C until the time of performing the biochemical tests.

Schematic overview of the experimental design	
50 male Sprague-Dawley rats allocated into five experimental groups (n = 10 per group).	
Group	**Experimental regimen**
Group 1	Rats were fed a standard basal diet (ND) throughout the experiment + 1 mL/100 normal saline by gastric gavage during the 30-day treatment period.
Group 2	Rats were fed a high-fat diet for 3 weeks to induce hyperlipidaemia; serum confirmation; continued high-fat diet + 1 mL/100 normal saline by gastric gavage for 30 days.
Group 3	Rats were fed a high-fat diet for 3 weeks to induce hyperlipidaemia, followed by serum confirmation; a continued high-fat diet + virgin olive oil 1 mL/100 g body weight by gastric gavage for 30 days.
Group 4	Rats were fed a high-fat diet for 3 weeks to induce hyperlipidaemia, followed by serum confirmation and continued high-fat diet. + Virgin olive oil 2 mL/100 g body weight by gastric gavage for 30 days.
Group 5	Rats were fed a high-fat diet for 3 weeks to induce hyperlipidaemia, followed by serum confirmation, and switched to the basal diet for the 30-day experimental period.
All groups: blood samples collected at weekly intervals during the 30-day treatment period.	
Experimental sequence: 3-week hyperlipidaemia induction (Groups 2-5) -> serum confirmation -> 30-day intervention -> weekly blood samplings for 4 weeks. By the end of the experiment the animals will be euthanised and the organs collected.	

Liver function biomarkers:

1. Determination of alanine aminotransferase (ALT) activity (U/L): The activities of serum alanine aminotransferase (ALT) were measured using a kit obtained from Biodiagnostic Egypt, following the method described in reference [15].

2.Aspartate aminotransferase (AST) activity (U/L): The activity of aspartate aminotransferase (AST) was measured using a kit obtained from Biodiagnostic Egypt, following the method described in reference [15].

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3. Determination of Alkaline Phosphatase (ALP) activity (U/L): The determination of alkaline phosphatase (ALP) in serum was performed using a kit obtained from Biodiagnostic Egypt, following the method described in reference [16].

Histopathological examination: By the end of the experiment, the animals were euthanised, and the liver samples were preserved in 10% formalin for histopathological examination; the samples were processed in a standard manner, embedded in paraffin, cut, and stained with haematoxylin and eosin. Light microscopy examination of sections was performed for architectural evaluation of liver hepatocytes.

Statistical analysis: Values were reported as means ± standard error (SE). Analysis of variance among groups was performed using one-way analysis of variance with the least significant difference post hoc test. The significance criterion was $p <= 0.05$.

Results:

Liver-function biomarkers: The HLD group showed progressive increases in serum ALT, AST, and ALP activities across the four weeks. At week 4, ALT, AST, and ALP were 43.42 +/- 0.39, 116.54 +/- 0.18, and 117.70 +/- 0.36 U/L, respectively, compared with 34.62 +/- 4.28, 92.60 +/- 0.23, and 106.30 +/- 3.69 U/L in the ND group. Both virgin olive oil groups showed lower week-4 enzyme activities than the untreated HLD group, with the HLD+HVOO group showing the lowest values. The HLD+ND group showed partial recovery but remained above the ND group. Table 1

Table 1. Effect of virgin olive oil supplementation on serum ALT, AST, and ALP activities in hyperlipidaemic rats.

Parameters / Groups		BD	HLD	HLD+LVOO	HLD+HVOO	HLR+BD	LSD
ALT (U/L)	I	32.92±0.93	41.20±0.54	40.64±2.47	39.91±0.17	40.84± 0.50	1.89
	II	33.48 ±0.22	42.18± 0.13	39.76±0.02	37.80±0.01	40.45± 0.01	
	III	33.96 ±0.49	43.08± 0.07	38.74±0.39	36.80±0.01	39.62± 0.01	
	IV	34.62 ±0.28	43.42± 0.39	37.28±0.14	35.25±0.02	38.90± 0.20	
AST (U/L)	I	92.94±0.25	112.88±0.14	110.65±0.13	109.90±2.59	111.20±0.10	0.66
	II	92.81 ±0.27	113.70±0.38	109.72±0.31	107.40±0.30	110.19±0.02	
	III	92.73±0.14	114.60±0.34	107.80±0.15	104.26±0.26	109.70±0.28	
	IV	92.60±0.23	116.54±0.18	105.64±0.11	101.24±0.27	108.26±0.27	
ALP (U/L)	I	104.54±1.33	113.78±1.13	113.70±2.09	112.68± 1.35	113.60±0.15	1.83
	II	105.40±1.52	114.72±1.19	112.64±2.14	112.28±1.41	113.14±0.02	
	III	105.74±1.66	115.92±1.41	112.56±1.74	110.72±1.38	112.82±0.21	
	IV	106.30±1.69	117.70±1.36	111.86±1.70	109.54±0.35	112.40±0.35	
ANOVA							P≤ 0.05

Data indicate mean ± standard error at $p \leq 0.05$, N = 10 rats, and BD = basal diet. HLD = Hyperlipidaemic diet, HLD+LVOO = Hyperlipidaemic diet + low dose of virgin olive oil, HLD+HVOO = Hyperlipidaemic diet + high dose of virgin olive oil, HLR+BD = Hyperlipidaemic rats followed by a basal diet, I = 1st week, II = 2nd week, III = 3rd week, IV = 4th week, LSD = (Least significant difference). ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, and ALP = Alkaline phosphatase.

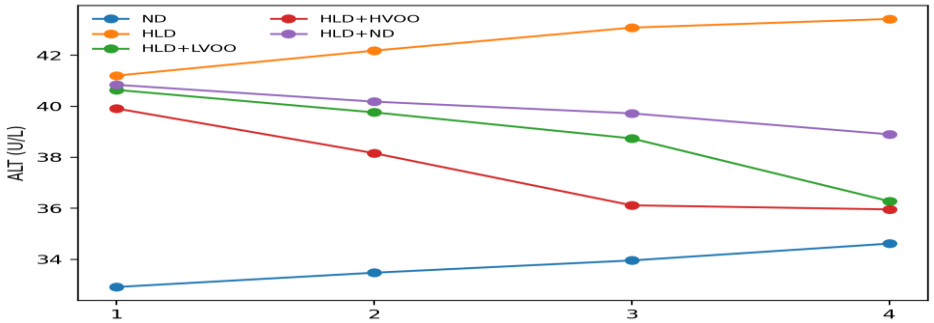


Figure 1. Effect of virgin olive oil supplementation on serum ALT levels in

DOI: https://doi.org/10.54361/LJMR.20.3.108

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BD = basal diet. HLD = Hyperlipidaemic diet, HLD+LVOO = Hyperlipidaemic diet + low dose of virgin olive oil, HLD+HVOO = Hyperlipidaemic diet + high dose of virgin olive oil, HLR+BD = Hyperlipidaemic rats followed by a basal diet, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, and ALP = Alkaline phosphatase.

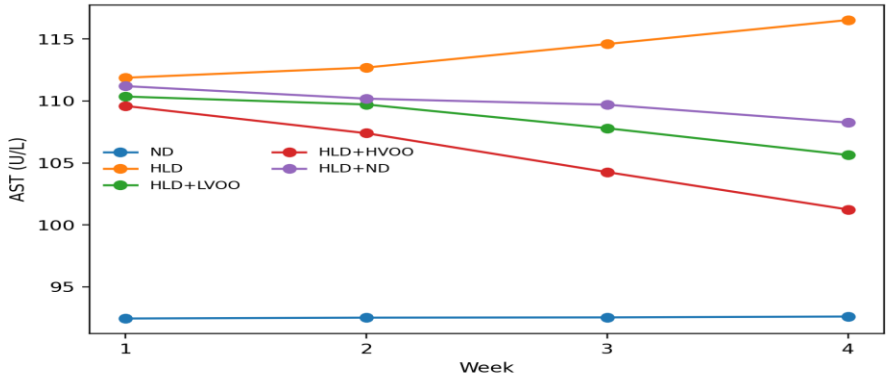


Figure 2. Effect of virgin olive oil supplementation on serum AST levels in hyperlipidaemic rats.

BD = basal diet. HLD = Hyperlipidaemic diet, HLD+LVOO = Hyperlipidaemic diet + low dose of virgin olive oil, HLD+HVOO = Hyperlipidaemic diet + high dose of virgin olive oil, HLR+BD = Hyperlipidaemic rats followed by a basal diet, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, and ALP = Alkaline phosphatase.

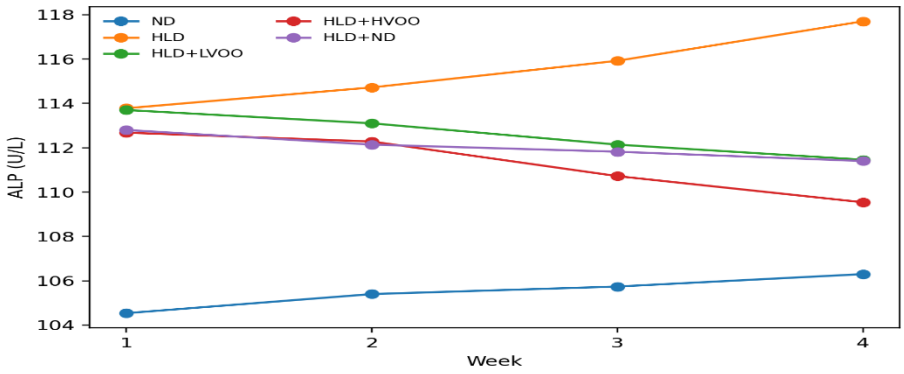


Figure 3. Effect of virgin olive oil supplementation on serum ALP levels in hyperlipidaemic rats.

BD = basal diet. HLD = Hyperlipidaemic diet, HLD+LVOO = Hyperlipidaemic diet + low dose of virgin olive oil, HLD+HVOO = Hyperlipidaemic diet + high dose of virgin olive oil, HLR+BD = Hyperlipidaemic rats followed by a basal diet, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, and ALP = Alkaline phosphatase.

Histopathological findings:

In the ND group, the hepatic architecture was well-preserved; the hepatic cords were regularly distributed around a central vein, with normal interspaced sinusoids. The HLD group is characterised by loss of normal arrangement of hepatocytes, congestion and dilatation of sinusoids, bile duct proliferation, inflammatory infiltration around vessels, hepatocyte swelling, severe vacuolation and hydropic degeneration. In the HLD+LVOO group, the hepatocyte arrangement appeared mostly preserved with few scattered foci of vacuolated hepatocytes. The HLD+HVOO group was similar to the ND group; the hepatocytes and sinusoids were near-normal and had absent or mild focal fatty change. In the HLD+ND group, there was mild persistent sinusoidal congestion, focal fat droplet accumulation, and a low number of infiltrating mononuclear cells.

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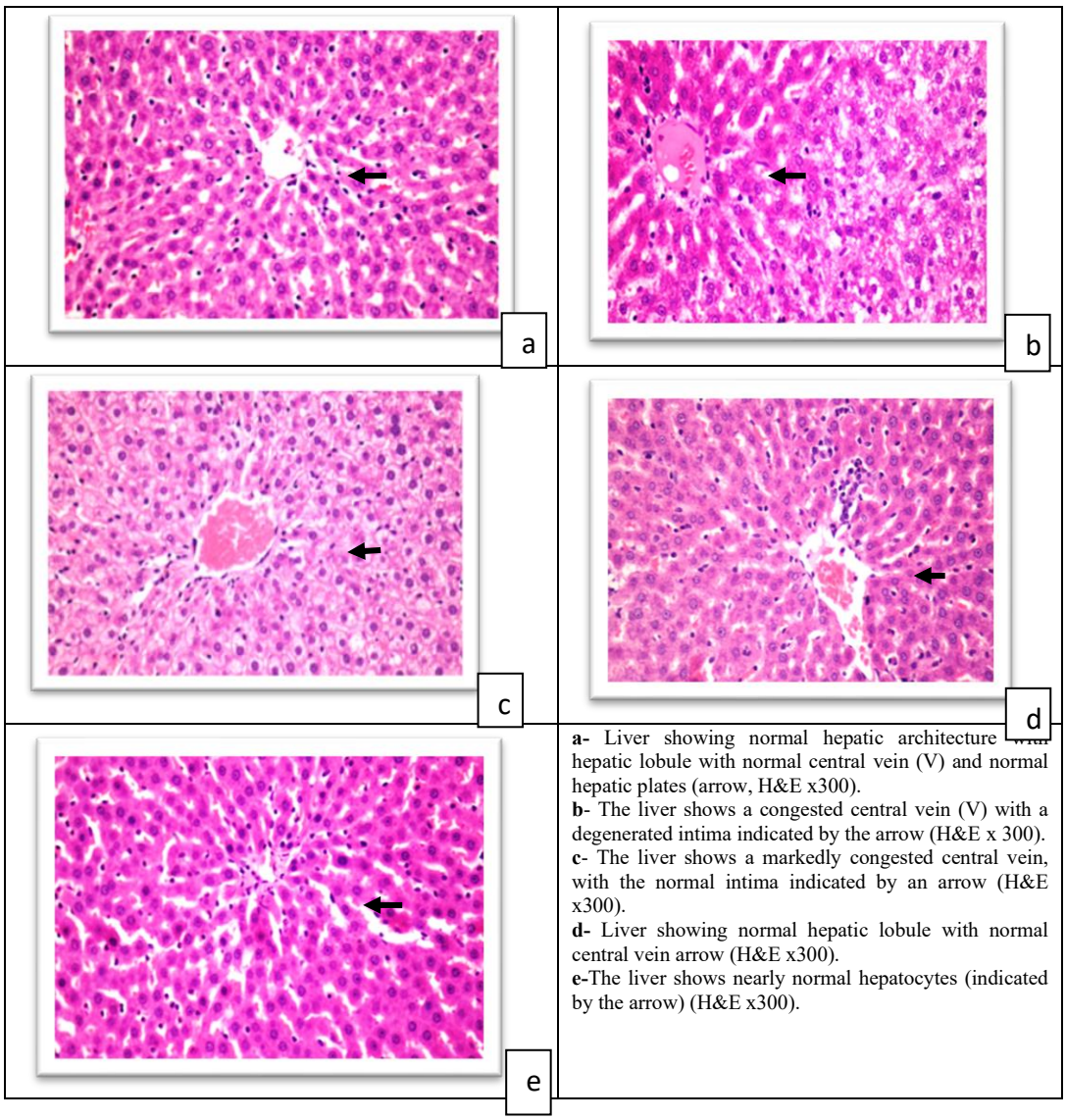


Figure. 4. Effect of virgin olive oil on liver of hyperlipidaemic rats.
a = Control, b = Hyperlipidaemic diet, c = Hyperlipidaemic diet + low dose of virgin olive oil, d = Hyperlipidaemic diet + high dose of virgin olive oil, e = Hyperlipidaemic rats followed by a basal diet.

Discussion

Our study indicates that the HLD diet-induced hyperlipidaemia is associated with progressive increases in serum ALT, AST, and ALP and dramatic changes in liver morphology. Our findings confirm that the overconsumption of lipids leading to hyperlipidaemia induces hepatic oxidative stress, mitochondrial injury, and inflammatory injury [1–3]. Together, our biochemical and histopathological data indicate that the HLD diet induces the hepatocellular injury rather than simply changing the serum levels.

The rise in AST and ALT is indicative of damage to hepatocyte membranes, cellular swelling, and the intracellular enzymes spilling over into the circulating blood, and elevated ALPs may be a consequence of co-existing cholestasis and biliary insults [4]. The structural alterations, which include swollen cells, vacuoles within the cells, vascular engorgement, inflammation, and bile duct hypertrophy, align with the biochemical data.

Virgin-olive-oil supplements attenuated increased serum enzymes and hepatic histologic architecture in the two- to nine-fold virgin-olive-oil dose group in the manner in

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which the amount was added (high > low > none). This effect appears reasonable since virgin olive oil contains both oleic acid and several other phenolic components with antioxidant, anti-inflammatory and cholesterol-modulating mechanisms of action [5-9]. In experimental model systems, olive-oil phenolic extracts have reduced reactive oxygen species and inhibited both the NF-kappa B and MAPK signalling pathways [8], while in a randomised diet study, rich virgin olive oil improved the gene expression of several inflammatory- and oxidative-response molecules [9].

Our results are also consistent with studies in cholesterol-fed rats that observed beneficial changes in lipid metabolism and improved antioxidant response following supplementation with olive oil [10]. Virgin olive oil also showed a beneficial effect in other experimental models of injury, limiting hepatic lipid peroxidation and reducing serum transaminase levels [11], whereas extra virgin olive oil was shown to ameliorate hepatic biochemical and histopathological abnormalities [12]. Studies of high-fat diet-induced steatosis also highlight the influence of the compounds present in olive oil on hepatic lipid regulation and antioxidant properties [7, 13]. These experimental observations were made using different models of the disease and different preparations of olive oil; therefore, a direct quantitative comparison should be undertaken with caution, but the direction is the same. After withdrawal of the hyperlipidaemic diet (with removal of olive oil), animals on a normal diet underwent only partial recovery, which indicates that withdrawal of the hyperlipidaemic diet during the available recovery time did not cause full reversibility of the established biochemical and histological injuries. Accumulation of lipid, oxidative damage or inflammation (but these were not determined) might have resulted in the partial recovery. There were several study limitations. We did not assess hepatic lipids, markers of oxidative stress, inflammatory mediators or signalling pathways. We did not know the phenolic content or oxidative stability of virgin olive oil, which might affect its biological activities.

Furthermore, this is a study on rats; the implications are not necessarily applicable to humans.

Additional research would be warranted to determine the test oil, evaluate hepatic lipids and markers of oxidative stress and inflammation and to include prolonged-term follow-up.

Conclusion:

Supplementation with virgin olive oil ameliorated hyperlipidaemia-induced liver injury in male albino rats. Changes in the serum levels of AST, ALT, and ALP, as well as liver histopathology, reflected the liver damage induced by hyperlipidaemia. This benefit was dose-dependent: high-dose virgin olive oil administration offered greater protection to liver tissues against hyperlipidaemia-induced injury and indicates the need for further studies on the mechanisms of virgin olive oil's benefits in metabolic liver injury.

Conflict of Interest and Funding:

The author declares no conflict of interest and received no specific funding for this work.

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