BAJOPMAS JOURNAL 16(1): 1 – 7
Printed in Bayero University, Kano - Nigeria.

ASSESSMENT OF EFFECT OF *OLEA EUROPAEA* AQUEOUS LEAF EXTRACT ON LIPID PROFILE PARAMETERS IN HIGH-FAT DIET FED MALE WISTAR RATS.

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ABSTRACT
Olive leaf extract (OLE) is a natural product derived from the leaves of the olive tree (*Olea europaea*). This study was carried out in order to understand the effect of its aqueous leaf extract on cholesterol, triglycerides, high-density lipoproteins (HDL), and low-density lipoprotein (LDL) levels in male Wistar rats fed with high-fat diet. Fifteen male Wistar rats were divided into three groups: control (normal diet), high-fat diet (HFD) only, and HFD + Aqueous *Olea europaea* (olive) leaf extract groups. The rats in the HFD + Aqueous *Olea europaea* (olive) leaf extract group received a daily oral administration of Aqueous *Olea europaea* (olive) leaf extract at a dose of 200 mg/kg body weight for three weeks after being fed a high-fat diet for five weeks. The results showed that the high-fat diet group exhibited elevated levels of total cholesterol (TCHOL) and Low-density lipoprotein (LDL) level compared to the control group (normal diet). However, there was a statistically significant reduction ($p < 0.05$) in total cholesterol and LDL levels in group receiving Aqueous Olea europaea (olive) leaf extract alongside the high-fat diet when compared to those receiving high-fat diet alone (HFD group). Triglyceride (TG) and High-density Lipoprotein (HDL) levels did not show significant differences among the groups. This study highlights the adverse effects of a high-fat diet on cholesterol levels in male Wistar rats and demonstrates the beneficial impact of Aqueous *Olea europaea* (olive) leaf extract in mitigating these effects.

Keywords: Olea europaea, hypercholesterolemia, high fat diet, lipid profile, Wistar rats.

1.0 Introduction
Olive leaf extract (OLE) is a natural product derived from the leaves of the olive tree (Zaitun). This product has been used traditionally as a remedy for a variety of ailments, including fever, hypertension, and infections [1]; [2]; [3]. More recently, it has been shown to have a range of potential health benefits, including antioxidant, anti-inflammatory, and hypolipidemic effects [4]. OLE has been shown to reduce serum cholesterol levels in both animal models and human clinical trials [5].

Hypercholesterolemia, or elevated levels of cholesterol in the blood, is a major risk factor for cardiovascular disease (CVD), and is the leading cause of death globally [6]. CVD is a term used to describe a range of conditions that affect the heart and blood vessels, including coronary artery disease, heart failure, and stroke [7]. Elevated cholesterol levels can contribute to the development of CVD by increasing the risk of plaque formation in the arteries, which can lead to blockages and reduce blood flow to the heart and other organs [8].

The management of hypercholesterolemia typically involves lifestyle changes, such as a low-cholesterol diet and regular physical activity, as well as pharmacological therapies, including statins, bile acid sequestrants, and cholesterol absorption inhibitors [8]. However,

these interventions may not be effective or well-tolerated in all individuals, highlighting the need for alternative approaches.

Several studies have demonstrated the hypolipidemic effects of OLE in both animal models and human clinical trials. For example, a study in rats fed a high-cholesterol diet found that supplementation with OLE significantly reduced serum cholesterol levels and improved liver function [9]; [10]; [11]. Another study in humans with hypercholesterolemia found that OLE supplementation significantly reduced total cholesterol, Low density lipo-protein (LDL) cholesterol, and triglyceride levels, while increasing High density lipo-protein (HDL) cholesterol levels [12].

Despite these findings, the mechanisms by which OLE exerts its hypolipidemic effects are not fully understood, and further research is needed to clarify its potential as a cholesterol-lowering agent. Thus, this study is focusing to understand how this natural product can mitigate the effect of hyperlipidemia.

2.0 MATERIALS AND METHODS

2.1 Plant substance, feed, reagents and equipments

Aqueous *Olea europaea* (olive) leaf extract. 1kg of standard rat chow mixed with 250g of butter. Body weight scale and cholesterol assay kit, standard rat cages and bedding, syringes, needles, centrifuging machine and refrigerator.

2.2 Plant material and extraction

Olive leaves were collected from agriculturists at Kabuga market along Gwarzo road, Kano state. A specimen voucher was deposited at the herbarium of plant biology, Bayero University, Kano. The *Olea europaea* leaves were dried under shade and then grounded to powder form. A 100g of ground olive leaves were soaked in 500 ml distilled water. The mixture was stirred at room temperature overnight, then paper filtered, and the aqueous phase was extracted.

2.3 Ethical clearance

Animal care, handing and use were carried out in accordance with National institute of Health Guide for the care and use of laboratory animals, 8th edition, and ethical approval was obtained from Animal care and use research ethics committee, Bayero University Kano, Nigeria.

2.4 Animals and conditions

Male Wistar rats aged 8-10 weeks with body weight ranging from 180-200g was used. The rats were purchased from Bayero University Kano, Department of Human Physiology's Animal House. The control groups were fed standard rat chow, the high-fat diet group (group 2) were fed high-fat diet only. The High-fat diet + Aqueous Olive Leaf extract group were fed high-fat diet for 5 weeks then after they received a daily oral administration of Aqueous *Olea europaea* (olive) leaf extract at a dose of 200 mg/kg body weight for 3 weeks [13]. The rats were housed in standard rat cages and had access to food and water ad libitum. The body weight of the rats was measured weekly with a weighing scale.

2.5 Experimental design

2.5.1 Animal Grouping

Fifteen (15) male Wistar rats were randomly divided into three groups of 5 rats each: Group 1: control group. Group 2: High-fat diet group, which were fed high-fat diet only. Group 3: High-fat diet + Aqueous *Olea europaea* (olive) Leaf extract group, which were fed high-fat diet only for 5 weeks then after that they received a daily oral administration of Aqueous *Olea europaea* (olive) leaf extract at a dose of 200 mg/kg body weight for 3 weeks [13].

Sample collection

At the end of the 8-week study period, the rats were anesthetized with an intraperitoneal injection of ketamine (40-70 mg/kg) and xylazine (10 mg/kg). Blood sample was collected for the measurement of Total cholesterol, Low-Density Lipoproteins (LDL), High-Density Lipoproteins (HDL) and Triglycerides.

Laboratory analysis

The laboratory analysis was carried out at Northwest University Laboratory Kwanar Dawaki. For all Laboratory analysis, Mindray BA 88A Biochemistry auto analyzer was used and Reagents were obtained from Randox laboratory. Total cholesterol is quantified using an enzymatic reaction that involves cholesterol esterase, cholesterol oxidase, and peroxidase. Cholesterol esters in the serum were first hydrolyzed to free cholesterol and fatty acids by cholesterol esterase. The free cholesterol was then oxidized by cholesterol oxidase, producing hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacted with a chromogenic system (4-aminoantipyrine and phenol) to produce a quinoneimine dye, which was measured spectrophotometrically at 500 nm [14]. Triglycerides were hydrolyzed by lipase to glycerol and free fatty acids. The glycerol was then phosphorylated by glycerol kinase and oxidized by glycerol-3-phosphate oxidase, generating hydrogen peroxide. This hydrogen peroxide reacted with 4-aminoantipyrine and 4-chlorophenol in the presence of peroxidase, forming a quinoneimine dye that was measured at 500nm. HDL was selectively separated from other lipoproteins using phosphotungstic acid and magnesium chloride, which precipitated LDL and VLDL. The supernatant, containing HDL, was then analyzed using the enzymatic cholesterol method [15]. Low-density Lipoprotein (LDL) concentration was estimated according to the formula LDL-C = TC- (TG/5)-HDL-C [16].

Statistical analysis

Data collected was analyzed using Statistical Package for Social Sciences (IBM SPSS) software, version 25.0. Also the Data was expressed as Mean±SEM and One-way Analysis of Variance (ANOVA) was used to analyze differences across groups. A Tukey's post hoc test for multiple comparison between groups, P values less than 0.05 were considered to be statistically significant.

3.0 RESULTS

The mentioned tables show the effect of the aqueous *Olea europaea* leaf extract (OLE) on serum lipid parameters in male Wistar rats fed High-fat diet.

The results of the study revealed significant changes in the lipid profile parameters among group 2 and group 3. In Table 1, the Total Cholesterol (TCHOL) levels were significantly increased ($p < 0.05$) in the High-Fat Diet (HFD) group compared to the control group, indicating the successful induction of hypercholesterolemia. However, there was significant decrease ($p < 0.05$) in TCHOL levels when High-fat diet + of Aqueous *Olea europaea* (olive) leaf extract (HFD + OLE) group was compared with HFD group ($p = 0.006$). Although it remained higher than the control group. This suggests that Aqueous *Olea europaea* (olive) leaf extract (OLE) supplementation may have a modest cholesterol-lowering effect.

Table 1: Comparison of Total Cholesterol levels in all the groups (MEAN ± SEM)

GROUPS	T/CHOL	F-value	P-value
CONTROL	61.56 ± 1.55		
HFD + OLE	57.24 ± 1.05^{c}	8.261	0.006
HFD	71.64 ± 4.04^{a}		

KEY: TCHOL = Total Cholesterol, HFD = High-Fat diet, OLE = Aqueous *Olea europaea* (olive) Leaf extract, [a] = significantly higher as compared to Control ($p < 0.05$), [c] = significantly lower as compared to HFD group ($p < 0.05$).

In terms of Triglycerides (TG) levels, table 2 shows no significant difference between the control, High-Fat Diet + Aqueous *Olea europaea* (Olive) Leaf extract (HFD + OLE) group and HFD groups ($p = 0.103$).

Although there was no significant difference in triglyceride (TG) and high-density lipoprotein (HDL) levels between the three groups, the High-Fat Diet + Aqueous *Olea europaea* (olive) Leaf extract (HFD + OLE) group had numerically lower Triglycerides (TG) levels compared to the HFD group.

Table 2: Comparison of Triglycerides levels in all the groups (MEAN ± SEM)

GROUPS	TG (mg/dl)	F-value	P-value
CONTROL	11.88 ± 1.94		
HFD + OLE	7.92 ± 0.92	2.758	0.103
HFD	14.76 ± 2.87		

KEY: TG = Triglycerides, HFD = High-Fat diet, OLE = Aqueous *Olea europaea* (olive) Leaf extract.

In table 3, there was no significant difference between the control, HFD + OLE, and HFD groups in terms of HDL ($p = 0.296$). While there was a numerical increase in HDL levels in the High-Fat Diet + Aqueous *Olea europaea* (olive) Leaf extract (HFD + OLE) group compared to the other groups, the difference was not statistically significant. However, table 4 shows a significant difference among the experimental groups in terms of Low-density Lipoproteins (LDL) levels. The High-fat Diet (HFD) group exhibited significantly higher Low-density Lipoproteins (LDL) levels compared to the control group, indicating the adverse effect of the high-fat diet on Low-density Lipoproteins (LDL) cholesterol. However, the High-Fat Diet + Aqueous *Olea europaea* (olive) Leaf extract (HFD + OLE) group demonstrated lower Low-density Lipoproteins (LDL) levels than both the High-fat Diet (HFD) and control groups, suggesting a potential protective effect of OLE against LDL elevation.

Table 3: Comparison of High density lipo-protein levels in all the groups (MEAN ± SEM)

GROUPS	HDL / Cholesterol (mg/dl)	F-value	P-value
CONTROL	14.68 ± 2.25		
HFD + OLE	17.28 ± 1.10	1.351	0.296
HFD	12.84 ± 2.18		

KEY: HDL = High density lipo-protein, HFD = High-Fat diet, OLE = Aqueous *Olea europaea* (olive) Leaf extract

Table 4: Comparison of Low density lipo-protein levels in all the groups (MEAN ± SEM)

GROUPS	LDL - Cholesterol (mg/dl)	F-value	P-value
CONTROL	44.28 ± 2.02		
HFD + OLE	36.36 ± 1.32[b]	7.603	0.007
HFD	48.96 ± 3.19[a]		

KEY: LDL = Low density lipo-protein, HFD = High-Fat diet, OLE = Aqueous *Olea europaea* (olive) Leaf extract, [a] = significantly higher as compared to Control ($p < 0.05$), [c] = significantly lower as compared to HFD group ($p < 0.05$).

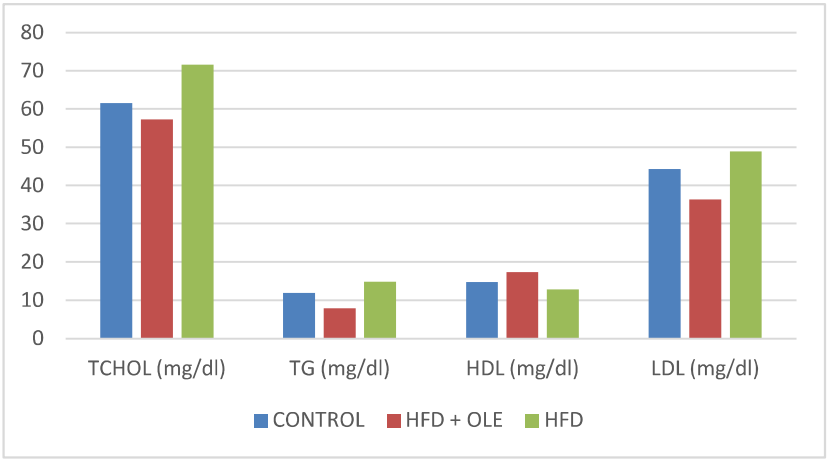

Fig 1: Comparison of lipid profile levels among control, high-fat diet + aqueous *Olea europaea* (olive) leaf extract group and high fat diet groups.

4.0 DISCUSSION

The Total Cholesterol (TCHOL) and Low-density Lipoprotein (LDL) increased observed in this study after feeding the Wistar rats with high fat diet is in close alignment with a previous study conducted by [17]. This demonstrates the impact of high-fat diet on inducing hypercholesterolemia and dyslipidemia. Elevated levels of Total Cholesterol (TCHOL) and Low-density Lipoprotein (LDL) are associated with an increased risk of atherosclerosis and cardiovascular diseases [18]. These findings underscore the reliability of the experimental model in inducing hypercholesterolemia.

Interestingly, the administration of Aqueous *Olea europaea* (olive) leaf extract alongside the HFD resulted in significantly reduced Total Cholesterol (TCHOL) and Low-density Lipoprotein (LDL) levels compared to the High-fat Diet (HFD) group alone. This finding suggests that the Aqueous *Olea europaea* (olive) leaf extract may possess lipid-lowering properties. Olive leaf extract has been reported to contain bioactive compounds, including oleuropein, hydroxytyrosol, and tyrosol, which have demonstrated anti-hypercholesterolemic effects [19]. These compounds have been shown to inhibit cholesterol biosynthesis, enhance Low-density Lipoprotein (LDL) receptor activity, and reduce Low-density Lipoprotein (LDL) oxidation, thereby contributing to the regulation of lipid metabolism and cholesterol homeostasis.

Although Triglyceride (TG) levels did not reach statistical significance, a trend towards elevated Triglyceride (TG) levels was observed in the High-fat Diet (HFD) group compared to the control group. Triglycerides (TGs) are another important lipid component associated with cardiovascular risk, and their reduction is desirable for overall cardiovascular health. Further investigations with a larger sample size and longer duration of Aqueous *Olea europaea* (olive) leaf extract (OLE) administration may help elucidate the effects on Triglyceride (TG) levels.

In contrast, High-density Lipoprotein (HDL) levels did not show a statistically significant difference between the High-fat Diet (HFD) and control groups. High-density Lipoprotein (HDL) acts as a protective factor against atherosclerosis by promoting reverse cholesterol transport, and its reduction is associated with an increased risk of cardiovascular diseases. The non-significant alteration in High-density Lipoprotein (HDL) levels in this study could be

attributed to various factors, including the duration of the intervention or individual variations in response to the High-fat Diet (HFD).

5.0 CONCLUSION

This study demonstrates that a high-fat diet induces hypercholesterolemia in male Wistar rats, as evidenced by elevated Total Cholesterol (TCHOL) and Low-density Lipoprotein (LDL) levels. The administration of Aqueous *Olea europaea* (olive) leaf extract alongside the high-fat diet effectively mitigated the adverse effects by reducing Total Cholesterol (TCHOL) and Low-density Lipoprotein (LDL) levels. These findings suggested that Aqueous *Olea europaea* (olive) leaf extract (OLE) may hold a promising impact as a potential therapeutic agent for managing hypercholesterolemia. Therefore, investigations are warranted to explore the underlying mechanisms and long-term effects of Aqueous *Olea europaea* (olive) leaf extract OLE supplementation on lipid profiles.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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