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Ameliorative effect of ethanolic extract of *Carica papaya* leaves on hyper-cholesterolemic rats: The egg yolk induced model

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ABSTRACT

Cardiovascular disease is one of the leading killers in the world today, and hyperlipidemia is one of the main risk factors. The prevalence of hyperlipidemia is rising dramatically worldwide and is mostly felt in poorer nations. The majority of communities and individuals in Africa are known to turn to ethnomedicine for their medical requirements. The tropical plant *Carica papaya*, which is grown for its edible, ripe fruit in Africa, was used in folk medicine for treatment of cardiovascular issues as well as a number of serious illnesses. This study assessed the anticholesterolemic property of the ethanolic extracts of *Carica papaya* leaves, adapting the egg yolk-induced hyperlipidaemia model in Wistar albino rats. This study prepared egg yolk to induce hyperlipidaemia in the Wistar rats, then treated some groups with the extract of *Carica papaya* leaves, and other groups with the standard drug Fenofibrate. The Wistar rats in the control group were given 2% acacia instead of egg yolk. The total cholesterol, triglycerides, as well as biological and haematological parameters, were determined. The *Carica papaya* leaves extracts significantly ($p < 0.05$) decreased the total cholesterol and LDL cholesterol levels at all doses administered, but the extract and the standard drug had no significant effect on HDL cholesterol. An inverse relationship between the *Carica papaya* leaves extract doses and the cholesterol levels was observed placing the efficacy in the order of 100 mg/kg > 250 mg/kg > Fenofibrate (2.29 mg/kg) > 500 mg/kg. With the potential efficacy of *Carica papaya* leaves extract in the treatment of hypercholesterolemia and, as a result, cardiovascular diseases, more research on bioactive molecule isolation/characterisation for pharmaceutical use or incorporation into functional food products for CVD management is required.

1. Introduction

The leading cause of death in the world is cardiovascular disease (CVD). According to the World Health Organization (WHO), over 17.5 million deaths worldwide from cardiovascular disease occurred in 2012, making up nearly 31% of all fatalities. Coronary heart disease and stroke were each thought to be responsible for 7.4 million deaths [1]. Global CVD-related mortality and morbidity are expected to rise as economic growth accelerates [2]. A key risk factor for cardiovascular disease, atherosclerosis, which is connected to

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coronary heart disease, is hyperlipidaemia (of which hypercholesterolemia is a form). A high amount of lipids, particularly low-density lipoprotein cholesterol (LDL-C), cholesterol, and triglycerides in the blood that is outside of normal ranges is referred to as hyperlipidemia [3]. According to theories, hyperlipidaemia may be the root of processes such as protein glycation, glucose-auto-oxidation, and oxidative modification of LDL-C, which produce free radicals and other by-products of lipid peroxidation, which are significant risk factors for ischemic heart disorders [4]. The use of medicinal plants for treating hyperlipidemia and CVDs has grown, and it appears to have fewer negative side effects than synthetic medications, which are used to lower plasma LDL-C levels [5]. This is because synthetic medications can cause unpleasant side effects like diarrhoea, nausea, myositis, and liver dysfunction. Furthermore, the majority of people in underdeveloped nations rely on herbal remedies for their essential medical requirements, yet there is a dearth of knowledge on the effectiveness of these traditional treatments [1], thus there is need to investigate and document these claims.

The herbaceous plant known as pawpaw, or *Carica papaya* L., is found across the tropics and subtropics. It is widely grown for its culinary and nutritional worth and is accessible all year long. Recent studies have shown that the plant has a wide range of medicinal advantages, including actions against malaria [6], cancer [7], and diabetes [8,9]. The leaves of the plants include a number of active ingredients that have anti-tumor and immunomodulatory actions, in addition to latex, ripe fruit, unripe fruit, roots, stem bark, seed, and flowers, all of which have historically been used as medicine [10]. A wide spectrum of pharmacological activities, including antioxidant, antibacterial, antihypertensive, *anti*-plasmodial, anti-fungal, and anti-inflammatory effects, have also been discovered in extracts and pure chemical compounds from *C. papaya* [11]. *Carica papaya* has been reported to lower cholesterol level [2]. Since research has geared towards the use of plants in the treatment of several diseases, this study, therefore focused on the *anti*--cholesterolemic effect of ethanolic extract of *Carica papaya* leaves. The authors hypothesise that *Carica papaya* leaf extracts will reduce cholesterol level in Wistar rat relatively to the commercially sold medication (Fenofibrate).

2. Materials and methods

Reagents used for the study were procured from B.D.H Chemicals Limited, Poole England, and Aldrich Chemical Company, Dorset, England. All reagents were of analytical grades.

2.1. Collection of sample

Fresh leaves from *Carica papaya* plant were collected from the wild in Ota, Ogun State, Nigeria. The leaves were identified by certified botanists, and a voucher specimen (LUH 6034) was left in the herbarium of Department of Botany, University of Lagos. The leaves were shade-dried for one week and pulverised using an electric blender (Binatone blg, 600s).

2.2. Extraction procedures

The *Carica papaya* leaves were extracted using ethanol, based on the report of Asghar et al. [3] that ethanol had the highest extraction of phenolic compounds from pawpaw leaves. The extraction was done using a cold maceration method. About 350 g of the leaves were shaken in 3000 mL of 90% ethanol in a transparent glass container at room temperature for one week. The extract obtained was filtered, and the filtrate was concentrated to a thick solution using a rotary evaporator set at 50 °*C* (Barloworld Scientific Limited, Stone, Staffordshire UK) and under reduced pressure. The thick solution was freeze-dried (Yarog Model RE-52, Japan) for biological investigations, for a final extract yield of 6.85% w/w.

The hydro-ethanolic extract of the *Carica papaya* leaves was screened qualitatively for the presence of phytochemicalssuch as saponins, alkaloids, tannins, anthraquinones, cardiac glycosides and flavonoids [4].

2.3. GC-MS determination of phytochemical characterisation

Before the analysis, the mass spectrophotometer (MS) was auto-tuned to perfluorotributylamine (PFTBA) using criteria that had already been established to evaluate the abundance of *m/z* 69, 219, and 502 as well as other instrument optimum & sensitivity characteristics. To ensure that all levels of detection of the target components were attained, the quantities of phytochemicals in the sample were assessed using GC-MS and MSD in Scan mode. A 7820 A gas chromatograph, 5975C inert mass spectrometer (with three axis detector), and electron impact source were used from Agilent Technologies. An HP-5 capillary column (30 m in length, 0.32 mm in diameter, and 0.25 m in film thickness) covered with 5% phenyl methyl siloxane as the stationary phase (Agilent Technologies) was used to separate the compounds [12]. The carrier gas used was helium, which had a nominal pressure of 1.4902 psi, a constant flow rate of 1.4871 mL/min, and an average velocity of 44.22 cm/s. One litre of the samples was splitlessly injected at a temperature of 300 °C. With gas saver mode off, the purge flow to the spilled vent was 15 mL/min for 0.75 min, for a total flow of 16.654 mL/min. After 1 min at 40 °C, the oven's temperature was raised to 300 °C at a rate of 12 °C/min (10 min). The run took 32.667 min, with a solvent delay of 5 min. With a 70eV electron-impact ionization setting, a 230 °C ion source, a 150 °C quadrupole, and a 280 °C transfer line temperature, the mass spectrometer was operated. Scan mode (scanning from *m/z* 45–550 amu at 2.0s/scan rate) was used to collect the ions.

2.4. Experimental subjects, design and treatment and management

Wistar rats (adult male) weighing between 100 and 180 g served as the study's test subjects. They were kept in rat cages at the

Experimental Animal House of the Faculty of Pharmacy at the University of Lagos and fed regular rat food. The animals had unlimited access to clean, fresh water in bottles. All experimental methods was approved and adhered to the requirements of Health Research and Ethics Committee (HREC) of the College of Medicine of the University of Lagos (CM/HREC/PHM/09/16/014) as well as generally accepted practices for the use and care of laboratory animals.

For 14 days, rats were given oral administration of 50% egg yolk prepared in vegetable oil at a dosage of 5 g/kg body weight twice daily. A total of six groups of five Wistar rats were formed. Instead of receiving egg-yolk as an inducer, Wistar rats in Group 1 received 2% acacia, which has been shown by Dauqan and Abdullah [13] to have no effect on lipid metrics. These Wistar rats are control samples. Wistar rats in group 2 were given egg yolk to induce but were not given any treatment, whereas those in group 3 were given egg yolk to induce but were also given the usual *anti*-cholesterolemic medication Fenofibrate (Colestrim®, Inventia Healthcare, India) at a dose of 2.29 mg/kg. Group 4 Wistar rats were given egg yolk induction and received 500 mg/kg of Carica papaya leaf extract, whereas group 5 was given egg yolk induction and received 250 mg/kg of the extract and group 6 was given egg-yolk induction and received 100 mg/kg of the extract.

The basal cholesterol and triglycerides levels of all the rats were determined before and after induction with egg yolk. Blood samples were collected thrice from the rats (before induction, after induction/before treatment and after treatment) via ocular puncture. Biochemical (Chemistry Analyzer, Roche/Hitachi 902, Japan) and haematological parameters (Mind Ray Haematological Analyzer, Model 3200, Chengen, China) were also assayed for after treatment. All analysis was replicated taking five samples each per group. The Wistar rats were treated in their various groups for 14 days, after which their results were compared.

2.5. Statistical analysis

Graphpad Prism 5 was used for the statistical analysis of the results obtained. The information was presented as Mean ± Standard Error of Means (SEM). When appropriate, the difference between baseline and after induction/before treatment cholesterol and triglyceride readings were evaluated using a paired *t*-test. The difference between pre- and post-treatment cholesterol and triglyceride readings was also assessed. Using one-way ANOVA, post-treatment lipid profile, biochemical and haematological data were analyzed. Dunnet's test was used for post hoc, and $p < 0.05$ was considered significant.

3. Results and discussion

The phytochemical screening of the *Carica papaya* leaves showed that the hydroethanolic extract of *Carica papaya* leaves contains alkaloids, flavonoids, saponins, tannins, cardiac glycosides and terpenoids with the absence of anthraquinones. This observation corresponds to the work of Ekaiko et al. [5]. The chemical constituents of plants are responsible for their medicinal activities. Alkaloids in *Carica papaya* leaf extract have been implicated in the antimicrobial, antimalarial and anticancer activities [6,7,14]. Also, cardiac glycosides may be responsible for its antispasmodic effect [8], while the presence of flavonoids (a strong antioxidant) have been implicated in its antidiabetic, hypocholesterolemic/hypolipidemic effects [9].

3.1. GC-MS analysis of hydroethanolic extract of C. Papaya leaves

The GC-MS analysis of the hydroethanolic extract of *Carica papaya* leaves revealed a number of compounds contained in the leaves (Supplementary file). These includes phenolics, diterpenes, anthraquinones, and fatty acid classes, with neophytadiene (12.94%) having the largest peak area, followed by palmitic acid (10.53%), phytol (7.41%), linolenic acid (6.22%), ethyl palmitate (5.69%). Other substances that are present in significant amounts include 4,4-(1-methylethylidene)bisphenol (4.96%), phytol acetate (3.64%), *p*-isopropenylphenol (2.58%), pentadecafluorooctanoic acid dodecyl ester (2.54%), 2-chloroethyl linoleate (2.37%), 1-dodecyne (2.22%), 7; hexadecyne (2.17%), 3,5-bis(1-methylethyl)phenol (1.91%), Ethyl tridecanoate (1.36%), cyclohexanone (1.05%), D-limonene (1.02%) and tetradecamethylcycloheptasiloxane (1.01%). This outcome is analogous to that reported in literature [10,11], which likewise contained significant amounts of D-limonene, squalene, neophytadiene, phytol, palmitic acid, and phytol acetate.

These substances have been linked to the biological activity of *Carica papaya* leaves. It has been shown that indole derivatives like (2-(3-methoxyphenyl)methylidene)-1H-indol-3-one has anticancer, antioxidant, anti-diabetic, and anticholineesterase properties [15]. Another component is pentafluorooctadecanoic dodecyl ester, a compound with antibacterial potential. The presence of components such neophytadiene, phytol, squalene, phenolics, linolenic acid, and ethyl linolenate, all of which are present in the extract in substantial quantities, can be linked to the hypolipidemic impact of the extract that was observed.

Adipocytes' lipid metabolism has been shown to be controlled by the diterpene alcohol phytol via activating the Peroxisome Proliferator-activated receptor alpha (PPARα). It is also discovered that squalene, a triterpene, is present in the extract in significant amounts. Squalene is a crucial intermediary in the synthesis of phytosterol in plants and cholesterol in animals. Due to its ability to inhibit β-Hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase (the rate-limiting step in the synthesis of cholesterol through negative feedback control), it was suggested that this compound plays a role in the regulation of endogenous cholesterol synthesis. Additionally, it has reportedly been shown to be effective in delaying the onset of cardiovascular illnesses. It has also been claimed to have antioxidant properties, preventing oxidative damage to artery endothelial cells and lipoproteins [16]. Another component of the extract called neophytadiene has been linked to the cardioprotective properties of the mixture by reports of its antioxidant and anti-inflammatory properties [17]. Ethyl linolenate and linolenic acid are two important components with known hypocholesterolemic action [12]. Another study found that eating a diet high in linolenic acid increases levels of cytoprotective antioxidants like glutathione reductase and superoxide dismutase, which protects β-cell redox homeostasis and prevents lipoprotein oxidation, which

enhances lipid transport and lowers hypercholesterolemia [13]. Additionally, several of the constituents are in the class of phenolics and flavonoids, which have been linked to the antioxidant action of the extract. These compounds function as free radical trappers, singlet and triplet oxygen extinguishers, and peroxide decomposers, preventing the oxidation of biological molecules like lipoproteins. These phenolics interfere with the emulsification of lipids, hydrolysis, micellar solubilization, and absorption to produce their hypolidemic/anticholesterolemic activity.

3.2. Basal cholesterol levels versus post induction cholesterol levels and the post-treatment analysis of the cholesterol levels

The basal versus post-induction cholesterol levels of each sample groups were analyzed and the result described in Table 1. An increase in the cholesterol level was observed in all the egg-yolk induced Wistar rat groups (30.24%–40.57%) when compared with the control group (27.98%).

Thus it can be said that egg yolk was effective in inducing hypercholesterolemia in Wistar rats as there was a significant ($p < 0.05$) increase in the cholesterol levels. This corresponds with observations in literature where the egg yolk diet was used to induce hypercholesterolemia in rabbits [18] and hyperlipidaemia in Sprague-Dawley rats [19]. Despite the probability that there could be increased cholesterol production by the animals in the control group, no significance was observed in the result and animals induced with egg-yolk had higher cholesterol levels post-induction than those in the control group.

The result in Table 1 revealed that the after 14 days of treatments, the cholesterol levels of the induced Wistar rats significantly reduced, ranging from 2.56 to 2.90 mg/dL for all the treated rats (groups 3–6) as against the non-treated group 2 (3.59 mg/dL). No significant ($p > 0.05$) difference between the control group and the treated groups. The *Carica papaya* leaves extract seems to be effective in controlling the level of cholesterol at the different dosage levels used, more so in the group 6 treated with 100 mg/kg bodyweight of the extract. The significant reduction in the cholesterol level of the rats after treating with *Carica papaya* leaf extract could be due the presence of sterols in the leaves [20] which displaces cholesterol from bile salts micelles thus leading to decreased absorption of cholesterol from the intestine [20,21]. Also, some components present in *Carica papaya* leaves such as phenolics, squalene and flavonoids have been reported by several studies to have antihyperlipidemic effect [22,23]. It is suspected that flavonoids and squalene operate by a similar mechanism as "statins" by inhibiting HMG Co-A reductase, an enzyme which catalyses the conversion of HMG Co-A to mevalonate in cholesterol synthesis in the liver [24]. This finding corresponds with the results from several other studies where *Carica papaya* leaves extract significantly reduced serum cholesterol in animals induced with hyperlipidaemia using various methods of induction [25–27].

The LDL-cholesterol levels after treatment were significantly lowered in group 3 treated with Fenofibrate (0.38 mg/dL) and group 6 treated with 100mgkg of the extract (0.18 mg/dL). The values obtained for these two groups (3 and 6) are significantly different from the control, induced not-treated and other treated groups. This corresponds with literature where *Carica papaya* leaf extracts reduced LDL-cholesterol levels [27,28]. Flavonoids which are present in the extract help regulate blood lipids by enhancing the activity of Lecithin acyltransferase (LCAT). LCAT plays a major role in the assimilation of free cholesterol into HDL-cholesterol and transferring it back to LDL-cholesterol which are later taken back in liver cells [29]. This study showed that the ethanolic leaf extracts of *Carica papaya* significantly ($p < 0.05$) decreased the cholesterol and LDL levels at all doses. The group treated with 250 mg/kg extract had LDL-cholesterol level comparable to that of the standard drug, Fenofibrate. The result in Table 1 also revealed that the HDL-cholesterol values in all the group tested were not significantly different, but the values are lower in treated groups than in the induced not-treated group. The extract of *Carica papaya* leaves, as well as the standard drug used, did not significantly affect the HDL-cholesterol levels. This is in correlation with the work of Sinha et al. [30] which showed that *Carica papaya* did not affect HDL-cholesterol but reduced all other lipid parameters.

Table 1
Basal cholesterol levels versus post induction cholesterol levels, and the post-treatment cholesterol levels.

Groups	Basal Cholesterol (mg/dL)	Post Induction Cholesterol (mg/dL)	Increase in cholesterol levels (%)	(After 14 days treatment)		
				Cholesterol (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
1 (control)	2.43 ± 0.18^{b}	3.11 ± 0.14^{b}	27.98	2.85 ± 0.13^{a}	0.47 ± 0.02^{a}	1.89 ± 0.14^{a}
2(Ind)	2.44 ± 0.20^{b}	3.43 ± 0.13^{a}	40.57	3.59 ± 0.12^{b}	0.75 ± 0.15^{a}	2.27 ± 0.15^{a}
3(Ffb)	2.50 ± 0.12^{b}	3.41 ± 0.16^{a}	36.40	2.69 ± 0.13^{a}	0.38 ± 0.04^{b}	1.98 ± 0.05^{a}
4 (500 mg/kg)	2.48 ± 0.26^{b}	3.46 ± 0.12^{a}	39.52	2.90 ± 0.13^{a}	0.62 ± 0.13^{a}	1.93 ± 0.13^{a}
5 (250 mg/kg)	2.48 ± 0.26^{b}	3.23 ± 0.27^{a}	30.24	2.56 ± 0.15^{a}	0.42 ± 0.06^{a}	1.99 ± 0.06^{a}
6 (100 mg/kg)	2.47 ± 0.22^{b}	3.46 ± 0.12^{a}	40.08	2.57 ± 0.13^{a}	0.18 ± 0.03^{c}	1.99 ± 0.08^{a}

Values are means ± SEM of three independent experiment. Values within the same column followed by different letters are significantly different at $p < 00.5$.
Groups: 1(control) given 2% acacia; 2 (Ind) Induced, not treated; 3 (Ffb) Induced, treated with Fenofibrate (2.29 mg/kg body weight); 4 (500 mg/kg) Induced, treated with 500 mg/kg body weight of extract; 5 (250 mg/kg) Induced, treated with 250 mg/kg body weight of extract; 6 (100 mg/kg) Induced, treated with100 mg/kg body weight of extract. LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol.

3.3. Basal triglycerides levels versus post induction triglycerides levels and the post-treatment analysis of the triglycerides

An inverse relationship has been observed between egg yolk consumption and triglycerides levels, all the induced groups had decreased triglycerides level (26.15–55.56%) as described in Table 2. There was also a decrease of 56.99% in the triglycerides level of the control group. Although egg yolk has been implicated in hypercholesterolemia [31], however the report of Yu et al. [32] showed that consumption of egg-yolk helped to alter and attenuate hyperlipidaemia especially hypertriglyceridemia in mice fed a high-fat diet. Yu et al. [33] also reported that high-density lipoprotein from egg-yolk improved dyslipidaemia through the mediation of fatty acid metabolism in high-fat diet-induced obese mice. This corresponds with our findings where induction with egg yolk diet gave rise to high levels of total cholesterol with reduced triglycerides levels. The reduced triglycerides level in the control is similar to the work of Mohamed et al. [34] where the supplementation of gum Arabic reduced cholesterol, triglycerides and LDL-cholesterol levels.

The result from Table 2 showed the triglycerides levels of the Wistar rats after 14 days of treatment. The induced not-treated group had the lowest triglycerides level compared to the control and the treated groups. The triglycerides level of all the treated groups were higher than the induced not-treated group. The continued administration of egg-yolk in group 2 could be the reason for the reduced triglycerides level in that group, as egg-yolk has been suggested to reduce triglycerides levels and modulate the immune system in BALB/c mice [35]. This means that more of the lipids are cholesterol hence the increased cholesterol levels and reduced triglycerides in that group. Triglycerides levels in all the groups are within the normal range of >140 mg/dL.

Comparing the post-treatment cholesterol values of the groups treated with the *Carica papaya* leaves extract and the group treated with the standard drug, Fenofibrate, the leaf extract compared admirably with the standard drug, as there was no significant difference between the post-treatment cholesterol values of the groups. *Carica papaya* extract has been suggested to have a mode of action similar to the "Statins" in that it inhibits the activity of HMG-CoA reductase which catalyses an important step in cholesterol synthesis [24]. It has also been suggested that the phenolics in *Carica papaya* decrease intestinal absorption of cholesterol [36,37] while Fenofibrate works by promoting the action of lipoprotein lipase, modulating the interaction between LDL receptors and ligand, and stimulating reverse cholesterol transport.

Generally, an inverse relationship between the extract doses and the lipid profile components was observed placing the efficacy in the order of 100 mg/kg > 250 mg/kg > Fenonibrate (2.29 mg/kg) > 500 mg/kg. This suggests the potent antilipidemic and anti-cholesteremic potential of *Carica papaya* leaves extracts at minute doses, thus justifying its extensive use in traditional medicine.

3.4. Lipid profile after treatment

The post-treatment lipid profile is displayed in Table 3. When compared to the control and treated groups, the cholesterol, triglycerides, and LDL-cholesterol of the induced and untreated groups significantly increased. This was also seen in the research of Ukpabi et al. [38], who found that an aqueous papaya leaf extract was efficient in lowering triglycerides, LDL cholesterol, and total cholesterol in rats that had been given alloxan to induce diabetes. Although the induced not treated group had a little higher HDL-cholesterol level than all the other groups, there was no discernible difference in the HDL-cholesterol levels between the groups.

3.5. Haematological parameters of experimental rats after treatment

The result of the haematological parameters showed in Table 4 revealed that the white blood cells (WBC) of the groups treated with Fenofibrate, 500 mg/kg and 100 mg/kg *Carica papaya* leaves extract increased significantly, compared to the other groups. This is as a result of the extract and the drug which are seen as foreign to the body and the body produces leucocytes to combat them. Also, there is a slight increase in the white blood cell count in the induced group compared to the control, but it is not significant. Increase in white blood cell count could be as a result of stress, inflammation or infection but in this case, the increased levels of white blood cells are just normal response to xenobiotics as the increase noticed did not exceed the recommended range (4.5 10^9/L-13.8 10^9/L).

Table 2
Basal triglycerides versus post induction triglycerides, and the post treatment triglyceride levels.

Groups	Basal Triglycerides (mg/dL)	Post Induction Triglycerides (mg/dL)	Decrease in triglyceride levels (%)	(After 14 days treatment) Triglycerides (mg/dL)
1 (control)	0.93 ± 0.13^a	0.40 ± 0.08^b	56.99	1.06 ± 0.26^b
2(Ind)	0.81 ± 0.04^a	0.36 ± 0.08^b	55.56	0.63 ± 0.13^a
3(Ffb)	0.65 ± 0.15^a	0.48 ± 0.12^a	26.15	0.88 ± 0.17^b
4 (500 mg/kg)	0.76 ± 0.15^a	0.45 ± 0.06^a	40.79	1.03 ± 0.40^b
5 (250 mg/kg)	1.29 ± 0.53^a	0.58 ± 0.07^a	55.04	0.82 ± 0.05^b
6 (100 mg/kg)	1.36 ± 0.37^a	0.90 ± 0.35^a	33.82	1.14 ± 0.14^b

Values are means ± SEM of three independent experiment. Values within the same column followed by different letters are significantly different at $p < 00.5$.
Groups: 1(control) given 2% acacia; 2 (Ind) Induced, not treated; 3 (Ffb) Induced, treated with Fenofibrate (2.29 mg/kg body weight); 4 (500 mg/kg) Induced, treated with 500 mg/kg body weight of extract; 5 (250 mg/kg) Induced, treated with 250 mg/kg body weight of extract; 6 (100 mg/kg) Induced, treated with100 mg/kg body weight of extract.

Table 3
Post-Treatment Lipid profile.

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
1 (control)	2.85 ± 0.13^{a}	0.47 ± 0.06^{a}	0.47 ± 0.02^{a}	1.89 ± 0.14^{a}
2(Incd)	3.59 ± 0.12^{b}	1.06 ± 0.12^{b}	0.75 ± 0.15^{b}	2.27 ± 0.15^{a}
3(Ffb)	2.69 ± 0.13^{a}	0.53 ± 0.13^{a}	0.38 ± 0.04^{a}	1.98 ± 0.05^{a}
4 (E500)	2.90 ± 0.13^{a}	0.48 ± 0.07^{a}	0.62 ± 0.13^{a}	1.93 ± 0.13^{a}
5 (E250)	2.56 ± 0.15^{a}	0.61 ± 0.05^{a}	0.42 ± 0.06^{a}	1.99 ± 0.06^{a}
6 (E100)	2.57 ± 0.13^{a}	0.78 ± 0.23^{a}	0.18 ± 0.03^{a}	1.99 ± 0.08^{a}

Values are means ± SEM of three independent experiment. Values within the same column followed by different letters are significantly different at $p < 00.5$.
Groups: 1(control) given 2% acacia; 2 (Ind) Induced, not treated; 3 (Ffb) Induced, treated with Fenofibrate (2.29 mg/kg body weight); 4 (500 mg/kg) Induced, treated with 500 mg/kg body weight of extract; 5 (250 mg/kg) Induced, treated with 250 mg/kg body weight of extract; 6 (100 mg/kg) Induced, treated with100 mg/kg body weight of extract. LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol.

Table 4
Haematological parameters of the Wistar rats post treatment.

Groups	WBC (10^{9}/L)	RBC (10^{12}/L)	HGB (g/dL)	HCT (Pcv %)	PLT (10^{9}/L)	MCV(fl)	MCH (pg)	MCHC (g/dL)
1 (control)	6.70 ± 0.22^{a}	6.48 ± 0.43^{a}	12.78 ± 0.83^{a}	45.02 ± 3.27^{a}	474.4 ± 63.66^{a}	71.66 ± 1.44^{a}	18.52 ± 0.31^{a}	26.00 ± 0.60^{a}
2(Ind)	7.64 ± 0.21^{a}	7.65 ± 0.33^{b}	13.38 ± 0.40^{a}	51.02 ± 1.58^{a}	624.2 ± 13.75^{a}	66.28 ± 1.15^{a}	17.86 ± 0.34^{a}	25.78 ± 0.49^{a}
3(Ffb)	8.86 ± 0.96^{b}	6.51 ± 0.25^{a}	12.28 ± 0.37^{a}	48.40 ± 1.30^{a}	705.8 ± 7.55^{b}	69.32 ± 2.42^{a}	18.50 ± 0.46^{a}	26.32 ± 0.38^{a}
4 (500 mg/kg)	9.72 ± 0.27^{c}	6.66 ± 0.22^{a}	11.82 ± 0.44^{a}	45.42 ± 1.49^{a}	791.6 ± 22.24^{c}	68.90 ± 1.76^{a}	17.50 ± 0.43^{a}	26.18 ± 1.02^{a}
5 (250 mg/kg)	7.00 ± 0.47^{a}	7.04 ± 0.10^{a}	13.00 ± 0.23^{a}	49.12 ± 1.87^{a}	624.4 ± 85.56^{a}	70.24 ± 2.76^{a}	18.74 ± 0.38^{a}	26.66 ± 0.84^{a}
6 (100 mg/kg)	12.20 ± 0.55^{d}	7.41 ± 0.22^{b}	13.70 ± 0.14^{a}	51.92 ± 0.71^{a}	647.4 ± 31.78^{a}	69.68 ± 0.61^{a}	18.38 ± 0.27^{a}	26.22 ± 0.34^{a}

Values are means ± SEM of three independent experiment.
Values within the same column followed by different letters are significantly different at $p < 00.5$.
Groups: 1(control) given 2% acacia; 2 (Ind) Induced, not treated; 3 (Ffb) Induced, treated with Fenofibrate (2.29 mg/kg body weight); 4 (500 mg/kg) Induced, treated with 500 mg/kg body weight of extract; 5 (250 mg/kg) Induced, treated with 250 mg/kg body weight of extract; 6 (100 mg/kg) Induced, treated with100 mg/kg body weight of extract. WBC = White blood cells, RBC = Red blood cells, HGB = Haemoglobin, HCT = Haematocrit, PLT = Platelets, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin and MCHC = Mean Corpuscular Haemoglobin Concentration.

For the red blood cells (RBC) count, there was a significant ($p > 0.05$) increase in groups 2 (induced not-treated) and 6 (induced and treated with 100 mg/kg bodyweight of extract). But since there was no significant change in haemoglobin (HGB) concentration and haematocrit (HCT) compared with the control, then one cannot conclude that the increase in red blood cell count is as a result of polycythaemia which is a disease of the red blood cell. The red blood cell count, the haemoglobin concentration and the haematocrit result in all groups are still within the recommended ranges reported by Dharmarathna et al. [39]. Also, there is a significant increase in the platelet count of groups 3 (induced and treated with Fenofibrate) and 4 (induced and treated with 500 mg/kg bodyweight of extract) which could be as a result of injuries to the rats. However, all other haematological parameters are not significantly affected by the treatments, and are within normal ranges.

3.6. Biochemical parameters of the treated experimental rats

For the biochemical parameters in Table 5, apart from the alkaline phosphatase (ALP) which was significantly reduced in group 2 (induced, not treated) and 500 mg/kg extract treated group, the extract did not affect all the biochemical parameters assayed for. From

Table 5
Biochemical Parameters of the Wistar rats post treatment.

Groups	Protein (g/L)	Albumin (g/L)	Urea (mg/L)	Creatinine (umol/L)	ALP(U/L)	AST (U/L)	ALT (U/L)
1 (control)	82.7 ± 1.51	43.7 ± 0.47	4.10 ± 0.44	82.4 ± 1.93	38.0 ± 1.70^{a}	33.8 ± 0.97	13.6 ± 1.50
2(Ind)	81.3 ± 0.69	42.5 ± 0.19	4.03 ± 0.02	79.9 ± 0.83	29.4 ± 1.03^{b}	38.4 ± 3.37	16.6 ± 0.40
3(Ffb)	79.3 ± 1.24	41.3 ± 0.75	3.73 ± 0.32	76.3 ± 1.92	33.4 ± 2.80^{a}	31.2 ± 2.65	14.0 ± 1.30
4 (500 mg/kg)	79.7 ± 1.34	41.9 ± 0.43	3.99 ± 0.45	79.8 ± 3.02	36.4 ± 1.97^{a}	35.2 ± 6.67	14.4 ± 0.93
5 (250 mg/kg)	82.8 ± 1.33	41.3 ± 1.40	4.27 ± 0.36	74.0 ± 4.54	37.6 ± 1.63^{a}	36.2 ± 2.85	15.2 ± 1.86
6 (100 mg/kg)	82.9 ± 2.45	42.2 ± 0.59	4.34 ± 0.07	79.1 ± 3.02	24.6 ± 0.93^{c}	38.8 ± 3.40	14.4 ± 0.51

Values are means ± SEM of three independent experiment.
Values within the same column followed by different letters are significantly different at $p < 00.5$.
Groups: 1(control) given 2% acacia; 2 (Ind) Induced, not treated; 3 (Ffb) Induced, treated with Fenofibrate (2.29 mg/kg body weight); 4 (500 mg/kg) Induced, treated with 500 mg/kg body weight of extract; 5 (250 mg/kg) Induced, treated with 250 mg/kg body weight of extract; 6 (100 mg/kg) Induced, treated with100 mg/kg body weight of extract. ALP= Alkaline Phosphatase; AST = Aspartate Amino transferase; and ALT = Alanine Amino transferase.

the result, one can deduce that ethanolic extract of *Carica papaya* is not toxic to organs such as liver and kidney at the doses administered. Since the liver and kidney function tests showed that the various values gotten for the different analysis are within the recommended ranges (20–140 IU/L for ALP, 10–55 U/L for ALT, 14–59 U/L for AST, 60–85 g/L for total protein, 29–55 g/L for albumin, 3.5–20 mg/dL for urea, and 17.68–88.4 μmol/L for creatinine) reported by ARUP laboratories.

4. Conclusion

From this study, ethanolic extract of the leaves of *Carica papaya* has anticholesterolemic activities even at minute doses, but its effectiveness in treating hypertriglyceridemia cannot be ascertained. This study also showed that overconsumption of egg yolk could cause hypercholesterolemia. Ethanolic extract of *Carica papaya* leaves is effective in treating hypercholesterolemia and high LDL-cholesterol level in Wistar rat due to the presence of components such as phytol, phenolic, squalene, linolenic acid and other active constituents which are present in minute quantities all working in tandem. However, further investigations are worthwhile concerning isolation, identification, purification and characterisation of the *Carica papaya* leaves extract to boost their therapeutic use.

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Author contribution statement

Oluwaseun Hannah Ademuyiwa: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Beatrice Mofoluwaso Fasogbon, Oluwaseun Peter Bamidele: Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Grace Eigbibhalu Ukpo: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Supervised the project; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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