

In-vivo biological effect of *Carica papaya* leaf extracts on P-407 induced hyperlipidemic Wistar rats

Abstract

To determine the biological effect of *Carica papaya* leaf extracts on Poloxamer induced hyperlipidemia. Thirty-five healthy albino rats of the same sexes weighing 150-200g were divided into seven; group given feed and water only, group induced by an intraperitoneal injection of P-407, groups induced and treated with methanol, ethanol, ethyl acetate, n-butanol and n-hexane leaf extracts. In all the groups, P-407 and the extracts were administered at a dose of 1000mg/kg and 30mg/kg body weight respectively. At the end of the 14 day, the animals were sacrificed and blood sample were collected for determination of serum levels of: Total cholesterol (TC), Triacylglycerides (TG), Low-density lipoprotein (LDL) and High-density lipoprotein (HDL). The studies showed that animals in induced treated groups significantly ($p < 0.05$) lower serum levels of TC, TG, LDL and significantly ($p < 0.05$) increased HDL when compared to the P-407 induced hyperlipidemia control. The studies show the phytotherapeutic effect of *Carica papaya* leaf extracts (methanol, ethanol, ethyl acetate, n-butanol and n-hexane) in P-407 induced hyperlipidemia.

Keywords: biological effect, *Carica papaya*, Poloxamer 407, hyperlipidemia

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Introduction

Tropical America is the home of Papaya, which was spread to the south by the Indians, and to the Caribbean by the Spanish. Caricaceae is the family *Carica papaya* Linnaeus, (paw paw), belongs to, and it is an herbaceous perennial plant with rapid proliferation rate.¹ Papaya has a short life span, but has a fruitage period of 20 years. Papaya has a complex type of reproduction as they are hermaphrodite i.e. with both male and female part.² The male trees are rare, but can be obtained when homeowners collect their own seeds. The commercially available papayas are the hermaphrodite trees that produce fruits that are pear shaped. These plants are self-fertilizing.³ The plant yields natural substance (*Annonaceous acetogenins*) in leaf barks and tissues of twig that has potent antitumor and pesticidal activities.⁴ Papayas are rich in self-defense compounds that confer a high level of immunity to attack by insects and to diseases.⁵

Hyperlipidemia is a biomedical condition characterized by a marked increase in any or all of the serum lipid profile and/or lipoproteins. Although high serum concentration of low density lipoprotein (LDL) was thought to be a strong indicator of risk of atherosclerosis, dyslipidemia (abnormal concentration of serum lipids), it can also describe high concentration of total cholesterol (TC) or total glyceride (TG), or low concentrations of high density lipoproteins cholesterol (HDL). Hyperlipidemia is the major predecessor of ailments such as atherosclerosis sudden death syndrome and coronary artery diseases which are lipid related.⁶ The major purpose of treatment of hyperlipidemic patients is to lower the possible risk of future development of ischemic heart disease or the occurrence of further cardiovascular disease or cerebrovascular disease.⁷

Poloxamer-407 (P-407), a non-ionic surfactant is a block copolymer which consists of polyoxyethylene and polyoxypropylene units. It is popular for its biocompatibility and its ability to deliver drugs for different disease states⁸ and acts as an obstacle in preventing post-surgical adhesions.⁹ P-407 has unusual thermo-reversible

properties, which at room temperature is liquid, while at body temperature it aggregates and forms a gel, prior to forming micelles. This property of temperature dependent micelle and gel formation makes them commercially useful in personal care products such as mouth washes, deodorants and skin care products and serves as an inactive substance that serves as the vehicle or medium for a variety of pharmaceutical preparations.¹⁰

Intramuscular or intraperitoneal injection of poloxamer 407 causes hyperlipidemia in rats depending on the administered dosage as was shown by Johnston et al., 1992, as elevating plasma triacylglycerol (TG) beyond 60 fold, and cholesterol beyond 8 fold backed up by this fact. However, subsequent hyperlipidemic studies have brought about new emerging models prior to Johnston's findings.

Materials and methods

Materials

Collection of plant samples: The present study was conducted between May and July, 2017 in Biochemistry Department, Kogi State University, Anyigba, in Kogi state, Nigeria. Fresh green leaves of papaw (*Carica papaya*) were obtained from the premises of Kogi State University, Anyigba, Kogi State. The leaves were cut into smaller portion, sun dried for three weeks at room temperature and reduced to coarse powder using hand blender. The sample was packed into an air tight container before storage until required for further analysis.

Experimental animals: A total of thirty-five (35) healthy albino rats of the same sexes weighing between 150–200g were obtained from the Department of Biochemistry Animal house, Faculty of Natural Sciences, Kogi State University, Anyigba. The rats were kept in well aerated cages and allowed to acclimatize for a week before the commencement of the in-vivo study.

Chemicals and reagents: All assay kits were from Randox laboratories Ltd. Ardmore, Co. Antrim UK. Chemicals and reagents

used were all of analytical grade.

Extraction: Ten (10) gram of the grounded leaves sample was weighed into different conical flasks containing 100 ml of the extractants (methanol, ethanol, ethyl acetate, n-butanol and n-hexane). The contents of the different flasks were shaken and the tops was covered with aluminium foil and kept at room temperature for 48 hours (2days) and filtered off using Whatman filter paper (Cat no 1001 125) of pore size 125mm. The filtrate was concentrated by drying in a water bath maintained at a temperature of 45°C until a brownish black residue was obtained. These were kept in sealed containers and refrigerated at 2-4°C until required.

Acute toxicity studies: The mean lethal dose (LD₅₀) of the extracts (methanol, ethanol, ethyl acetate, n-butanol and n-hexane) was conducted to determine the suitable dose for the evaluation of the effect of the extracts. This was done using the method described by Lorke.¹¹

Induction of hyperlipidemia: The inducing agent was poloxamer 407. Before administration, P-407 was completely dissolved in water and refrigerated overnight to aid its complete dissolution. The syringe and needle to be used for the induction was cooled to avoid gelation within the syringe during injection.¹²

Animal Grouping and treatment: A total of 35 rats were used. The rats were randomly divided into 7 groups of 5 rats each:

- i. Group I: Normal Control rats fed with normal chow and distilled water only for 14 days (NC).
- ii. Group II: Hyperlipidemic Control rats induced without treatment (HC).
- iii. Group III: Hyperlipidemic rats treated with methanol extract at 50mg/kg body weight/day for 14days (H+Met).
- iv. Group IV: Hyperlipidemic rats treated with ethanol extract at 50mg/kg body weight/day for 14days (H+Eth).
- v. Group V: Hyperlipidemic rats treated with ethyl acetate extract at 50mg/kg body weight/day for 14days (H+E.Ace).
- vi. Group VI: Hyperlipidemic rats treated with n-butanol extract at 50mg/kg body weight/day for 14days (H+n-But).
- vii. Group VII: Hyperlipidemic rats treated with n-hexane extract at 50mg/kg body weight/day for 14days (H+n-Hex).

Sample collection: At the end of the 14th day, chloroform-inhalation anesthesia was performed on the experimental animals. The anesthetized animals were bled by cardiac puncture. The blood samples were collected and centrifuged at a speed of 2000r/m for 10minutes and serum collected into plain sample bottles for lipid analysis.

Serm lipid analysis: Total cholesterol (TC), high-density lipoprotein-cholesterol (HDL) and Triacylglycerol (TG) were determined by enzymatic method as described by Stein,¹³ low-density lipoprotein cholesterol (LDL) was determined by the method of Friedewald *et al.*¹⁴

Statistical analysis

The results are presented as means±Standard deviations. Differences between means were assessed using Analysis of variance (ANOVA) and post test using Dunnett multiple comparison test. *P* value less than 0.05 was considered significant (*p*<0.05).

Results

Changes in total cholesterol and Triacylglycerol

The result shows that animals in the group induced without treatment shows a significant (*p*<0.05) increase in TC and TG when compared with all other groups. The animals induced and treated shows that ethanol extract significantly (*p*<0.05) decreased the TC and TG when compared to other induced treated groups. The ethanol extract had the highest percentage reduction in TC (48.14) and TG (44.21) when compared with other induced treated groups (Table 1).

Changes in high density lipoprotein (HDL) and low density lipoprotein (LDL)

The result shows that animals in the group hyperlipidemia group (HC) shows a significant (*p*<0.05) decrease in HDL and increase in LDL when compared with all other groups. The animals induced and treated shows that ethanol extract significantly (*p*<0.05) increase the HDL and decreased the LDL when compared to other induced treated groups. Again, the ethanol extract had the highest percentage increase in HDL (48.71) and reduction in LDL (40.04) when compared with other induced treated groups (Table 2).

Table 1 Effect of different extracts of *Carica papaya* leaf on total cholesterol and triacylglycerol

Groups	Total cholesterol (TC)		Triacylglycerol (TG)	
	Concentration (mg/dl)	Percentage reduction (%)	Concentration (mg/dl)	Percentage reduction (%)
Group one (NC)	202.35±0.01 ^a	59.84 ^e	126.72±0.10 ^a	85.87 ^e
Group two (HC)	503.97±7.54 ^f	0.00 ^a	897.36±6.30 ^f	0.00 ^a
Group three (H+Met)	305.30±22.61 ^c	39.42 ^c	572.54±0.01 ^c	36.19 ^c
Group four (H+Eth)	261.32±22.60 ^b	48.14 ^d	500.63±31.49 ^b	44.21 ^d
Group five (H+E.Ace)	346.63±22.63 ^d	31.22 ^c	677.54±18.89 ^d	24.49 ^c
Group six (H+n-But)	421.96±6.14 ^e	16.27 ^b	755.23±21.22 ^e	15.83 ^b
Group seven (H+n-Hex)	431.96±7.54 ^e	14.28 ^b	767.12±22.12 ^e	14.51 ^b

Values are expressed as mean±SD of triplicate determination. Values in the same column with 5 different letter subscripts are significantly different *p*<0.05.

Table 2 Effect of different extracts of *Carica papaya* leaf on high density lipoprotein and low density lipoprotein

Groups	High density lipoprotein (HDL)		Low density lipoprotein (LDL)	
	Concentration (mg/dl)	Percentage increase (%)	Concentration (mg/dl)	Percentage reduction (%)
Group one (NC)	73.35±0.16 ^f	62.42 ^f	50.15±1.22 ^a	49.90 ^f
Group two (HC)	45.16±1.09 ^a	0.00 ^a	100.10±2.12 ^f	0.00 ^a
Group three (H+Met)	60.65±1.32 ^d	34.30 ^d	68.11±1.11 ^c	31.95 ^d
Group four (H+Eth)	67.16±2.09 ^e	48.71 ^e	60.01±1.10 ^b	40.04 ^e
Group five (H+E.Ace)	57.16±1.09 ^c	26.57 ^c	82.12±1.12 ^d	17.96 ^c
Group six (H+n-But)	50.16±1.22 ^b	11.07 ^b	90.11±2.22 ^e	9.98 ^b
Group seven (H+n-Hex)	49.11±2.02 ^b	8.74 ^b	92.17±4.62 ^e	7.92 ^b

Values are expressed as mean±SD of triplicate determination. Values in the same column with different letter subscripts are significantly different $p < 0.05$.

Discussion

The medicinal effect of plants in the management of diseases is attributed to the presence of the bioactive substances in them. These bioactive substances include flavonoids, saponins, tannins, glycosides, steroids, carbohydrate, anthraquinone and alkaloids. Bioactive substances in medicinal plants are known for their anti-inflammatory, anti-lipidemic, anti-diabetic, anti-microbial, anti-atherosclerotic and anti-carcinogenic properties.¹⁵

Poloxamer 407, a non-ionic surfactant is popularly known to cause dose dependent hyperlipidemia¹⁶ by inhibiting the activity of capillary (heparin releasable) lipoprotein lipase (LPL), the enzyme majorly responsible for the breakdown of plasma lipoprotein triacylglyceride, and indirectly induce the activity of the enzyme 3-hydroxy-3-methylglutaryl COA (HMG COA) reductase, which is the rate determining enzyme for the synthesis of cholesterol, which inherently leads to hypertriglyceridemia and hypercholesterolemia respectively. Abnormal elevation in concentration of lipids such as total cholesterol (TC) and triglyceride (TG) results in a condition known as "Hyperlipidemia".

Hyperlipidemia is responsible for the onset and progression of atherosclerosis,¹⁷ a major risk factor in the development of coronary heart diseases.¹⁸ *Carica papaya* leaf extracts (methanol and ethanol) significantly ($p < 0.05$) reduced TC and TG concentrations. These reductions in TC and TG suggest the ameliorative potential of *Carica papaya* leaf extracts (methanol and ethanol) in hyperlipidemia. The elevation of TC concentration in this study was achieved by the indirect stimulation of HMG CoA reductase following an intraperitoneal (i.p) injection of P407 (Johnston 2004). Hence the possible TC lowering effects of *Carica papaya* leaf extracts (methanol and ethanol) could be attributed to decreased activity of hepatic HMG CoA reductase and/or stimulation of cholesterol-7-alpha-hydroxylase, which converts cholesterol into bile acids. It could also be due to the presence of saponins, a phytochemical which forms insoluble complexes with cholesterol or their bile salt precursor, thus making them unavailable for absorption.¹⁹ The results obtained in these studies conform to earlier report by Chukwuka *et al.*,¹⁵ that bioactive substances in plants possess anti-lipidemic activity.

Increase in TG concentration following P407 i.p. injection results primarily from an inhibition of TG degradation, P-407 directly inhibits capillary lipoprotein lipase (LPL) responsible for plasma

TG hydrolysis.¹⁶ *Carica papaya* leaf extracts (methanol and ethanol) reduction in TG levels may have been either due to the activation of endothelium bound lipoprotein lipase which hydrolyses the triglyceride into fatty acid hence decreasing triglyceride levels as seen in a report by Sikarwar & Patil²⁰ or by inhibiting lipolysis so that fatty acids do not get converted to triglyceride.

High density lipoproteins (HDL) scavenges cholesterol, and mops up excess cholesterol and cholesterol ester from the blood and peripheral tissues where they are they are transported from and broken down to bile acids by the liver. HDL plays a crucial role in depleting plasma and peripheral concentrations of cholesterol and prevents atherosclerotic plaque formation in the aorta,^{20,21} and are therefore known as protective cholesterol. The present studies shows significant ($p < 0.05$) increase in HDL by *Carica papaya* leaf extracts (methanol and ethanol). This could most likely be because of the increasing activity of the enzyme Lecithin-cholesterol acyl transferase (LCAT), responsible for incorporating free cholesterol into HDL,²³ which reversibly stimulates the transport of cholesterol and inhibits competitively, the uptake of LDL by endothelial cells and inhibiting the production of oxidized LDL.²⁴ Cholesterol transport to the cells of the body is mediated by LDL, which transports 60-70% of the total cholesterol. Therefore, an elevation in concentration of total cholesterol (TC) consequently leads to increase in LDL.

The increased LDL which was not removed in the process of lipid metabolism is likely to flow into the sub-endothelial space, as well as to undergo oxidation. The oxidized LDL is phagocytised by the scavengers of macrophages and the fat-laden macrophage is left with the lipid core filled with cholesterol after necrocytosis and then arteriosclerosis is initiated.²⁵ It was reported that some isoflavones (a type of flavonoid) increase resistance to LDL oxidation, like soybean isoflavones and genistein derivatives. This work also shows significant ($p < 0.05$) reduction in LDL levels by methanol and ethanol extracts of *Carica papaya* leaf. This result is in accordance with the work of Baum *et al.*,²⁶ who reported that plants secondary metabolites may work by increasing LDL receptors densities in the liver binding to apolipoprotein B thereby making liver cells more efficient to remove LDL from blood.

Conclusion

In conclusion, the present study have demonstrated that *Carica*

papaya leaf has anti-hyperlipidemic effects on P-407 induced hyperlipidemia. Utilizing P-407 model, *Carica papaya* leaf was shown to be effective in significantly lowering total cholesterol, triglycerides and low density lipoprotein levels; thus it can be used in the treatment and/or prevention of cardiovascular diseases. However, more work is needed to investigate the anti-hyperlipidemic component(s) in *Carica papaya* leaf and mechanism of action.

Acknowledgements

None.

Conflicts of interest

Author declares that there is none of the conflicts.

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