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

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Abstract
To determine the biological effect of Carica papaya leaves extracts 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 intra-peritoneal injection of P-407, groups induced and treated with methanol, ethanol, ethyl acetate, n-butanol and n-hexane leaves 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 leaves extracts (methanol, ethanol, ethyl acetate, n-butanol and n-hexane) in P-407 induced hyperlipidemia.
Keywords: *Biological effect, Carica papaya, Poloxamer 407, Hyperlipidemia.*

Introduction

Papaya is native to tropical America, from Southern Mexico through the Andes of South America. It was spread to the south by Indians, and throughout the Caribbean with Spanish exploration. *Carica papaya Linnaeus*, (pawpaw), belongs to the family of *Caricaceae*. Papaya is not a tree but an herbaceous succulent plant that possess self-supporting stem. (Dick G., 2003). Papaya is a large perennial herb with a rapid growth rate. The plants are usually short-lived, but can produce fruit for more than 20 years. The papaya has a rather complicated means of reproduction. The plants are male, hermaphrodite, or female (Bruce and Peter, 2008). The male trees are uncommon, but sometimes occur when homeowners collect their own seeds. Hermaphrodite trees (flowers with male and female parts) are the commercial standard, producing a pear shaped fruit. These plants are self-pollinated (Jayasri, 2009). The plant produces natural substance (Annonaceous acetogenins) in

leaf barks and twig tissues that possess both highly anti-tumour and pesticidal properties (McLanghlin, 1992). The high level of natural self-defense compounds in the plants makes it highly resistant to insect and disease infestation (Peter, 1991).

Hyperlipidemia ia a condition characterised by an elevation of any or all lipid profile and/or lipoproteins in blood. Although elevated low density lipoprotein (LDL) is thought to be the best indicator of atherosclerosis risk, dyslipidemia (abnormal amount of lipids in the blood) can also describe elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL). Hyperlipidemia is the major precursor of lipid related ailments such atherosclerosis, coronary artery disease and also involved in sudden death syndrome (Grundy 1986). The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular disease or cerebrovascular disease (Davey and Pekkanen 1992)

Poloxamer 407 (P-407), a non-ionic surfactant is a block copolymer comprising of polyoxyethylene and polyoxypropylene units. It is known for its bio compatibility and potential to deliver different drugs for a variety of disease states (Johnston *et. al.*, 1992) and as a barrier in preventing postsurgical adhesions (Steinleitner *et. al.*, 1991). It has an unusual thermo-reversible properties, it is liquid at room temperature while it self-assembles into micelles then aggregate into a gel at body temperature. These temperature-dependent micellization and gelation properties have led to the widespread use of P407 in personal care products such as mouthwashes, deodorants, and skin care products and also as an excipient in a variety of pharmaceutical preparations (Dumortier *et. al.*, 2006). Johnston *et al.* 1992 showed that one intramuscular or intraperitoneal injection of Poloxamer 407 causes dose-dependent hyperlipidemia in rats, increasing plasma triacylglycerol (TG) more than 60 fold and cholesterol 8 fold and since then has been a growing model in different hyperlipidemic studies.

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 pawpaw (*Carica papaya*) were obtained from the premises of kogi state university, Anyigba, Kogi State. The leaves was 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, Ayingba. 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. Antrm 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 (2 days) 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^{o}C until a brownish black residue was obtained. These were kept in sealed containers and refrigerated at 2-4^{o}C until required.

Acute Toxicity Studies

The mean lethal dose (LD_{50}) 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 (1983).

Induction of hyperlipidemia

Poloxamer 407 (Lutrol F127; BASF, Ludwigshafen, Germany) was used as the inducing agent. Prior to the administration, Poloxamer 407 was dissolved in distilled water and refrigerated overnight to facilitate its dissolution. Needles and syringes to be used for administration were also cooled to prevent gelation within the syringe during injection (Megalli, 2005).

Animal Grouping and treatment

A total of 35 rats were used. The rats were randomly divided into 7 groups of 5 rats each.

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

Sample collection

At the end of the 14-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 2000 r/m for 10 minutes and serum collected into plain sample bottles for lipid analysis.

Serum lipid analysis

Total cholesterol (TC), high-density lipoprotein-cholesterol (HDL) and Triacylglycerol (TG) were determined by enzymatic method as described by Stein (1987), low-density lipoprotein cholesterol (LDL) was determined by the method of Friedewald *et al.*, (1972)

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).

Table 1 Effect of different extracts of *Carica papaya* leaves 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 different letter subscripts are significantly different $p<0.05$

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 2 Effect of different extracts of *Carica papaya* leaves 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, carbonhydrate, 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 (Chukwuka et. al., 2011).

Poloxamer 407, a non-ionic surfactant is well known to induce dose dependent hyperlipidemia (Johnston 2004) by inhibiting capillary (heparin releasable) lipoprotein lipase (LPL), the major enzyme responsible for the hydrolysis of plasma lipoprotein triglycerides (TG) and indirectly stimulating the activity of 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase, the rate limiting enzyme in cholesterol synthesis, thereby leading to hypertriglyceridemia and hypercholesterolemia respectively. Abnormal elevations 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 (Poss *et. al.*, 2011), a major risk factor in the development of coronary heart diseases (Vaziri and Morris 2011). *Carica papaya* leave 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* leave 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* leave 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 (Messina 1999). The results obtained in these studies conform to earlier report by (Chukwuka et. al., 2011) 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 (Johnston 2004). *Carica papaya* leave 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 and Patil (2011) or by inhibiting lipolysis so that fatty acids do not get converted to triglyceride.

HDL (high density lipoprotein) act as cholesterol scavengers, they pick up excess cholesterol and cholesterol esters from the blood and peripheral tissues to the liver where it is broken down to bile acids. It plays an important role in reducing blood and peripheral cholesterol concentrations and inhibits formation of atherosclerotic plaque in the aorta (Kim *et. al.*, 2008, Karmarkar 2008) there foreknown as the protective cholesterol. The present studies shows significant (P<0.05) increase in HDL by *Carica papaya* leave extracts (methanol and ethanol). This could possibly be due to increasing activity of lecithin-cholesterol acyl transferase (LCAT), an enzyme responsible for incorporating free cholesterol into HDL as suggested by Geetha *et al.* (2011), there by promoting reverse cholesterol transport and competitively inhibiting the uptake of LDL by endothelial cells and preventing the generation of oxidized LDL (Yokozawa *et. al.*, 2006).

LDL (low density lipoprotein) is responsible for transporting cholesterol to the body cells. It transports about 60-70% of total cholesterol. Therefore, an increase in TC level consequently increases 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 phagocytized 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 (Beckmann *et. al.*, 2009). 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 leave*. This result is in accordance with the work of Baum *et al,* (1998), 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 studies have demonstrated that *Carica papaya* leave has anti-hyperlipidemic effects on P-407 induced hyperlipidemia. Utilizing P-407 model, *Carica*

papaya leave 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* leave and mechanism of action.

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