Egg Yolk increases Lipid Profile in Wistar Rats; Protective Effect of *Carica papaya* seed extract

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Abstract
Background: Egg yolk has been reported to contain a significant amount of cholesterol. There have been controversies with repect to the impact of dietary egg yolk on plasma cholesterol. This study was carried out to investigate the effect of egg yolk on the lipid profile and the hypolipidemic potential of *Carica papaya* seed extract in Wistar rats.
Method: Thirty five (35) Adult Wistar rats weighing between 150-200g were assigned into seven groups with five (5) animals per group. Rats in group A served as control while group B1 animals were administered with 2ml of egg yolk for two weeks. Group B2 animals were administered with 2ml of egg yolk for four weeks; group C1 and C2 were administered with 400mg/kg body weight (bwt) of aqueous seed extract of *Carica papaya* for two weeks and four weeks respectively; while group D1 and D2 were co-administered with 2ml of egg yolk and 400mg/kg bwt of aqueous seed extract of *Carica papaya* for two weeks and four weeks respectively. At the end of the experimental period, the rats were sacrificed and blood samples were collected into plain sample bottles. Lipid profile (Total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), Very low density lipoprotein (VLDL-C) were analyzed using spectrophotometric methods
Results: There was a significant increase in total cholesterol, triglycerides, low density lipoproteins (LDL), very low density lipoproteins (VLDL) and a significant decrease in high density lipoproteins (HDL) in rats administered with egg yolk for two weeks and four weeks when compared with those of the control. In rats that were co-treated with egg yolk and *Carica papaya* there was a significant decrease in total cholesterol, triglycerides, low density lipoproteins (LDL), very low density lipoproteins (VLDL) and a significant improvement in high density lipoproteins (HDL) when compared to those that were treated with egg yolk only for two weeks and four weeks respectively. **Conclusion:** This study has shown egg yolk increases serum lipids and *Carica papaya* seed extract possesses hypolipidemic effect and could be of therapeutic benefit in the management of dyslipidemia and other lipid associated disorders.

Keywords: Egg yolk, Lipid profile, *Carica papaya*

Received: *11th March, 2023* ***Accepted:*** *2nd May, 2023*

Introduction

Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum cholesterol and low density lipoprotein (1). Hypercholesterolemia is risk factor for the development and progression

of atherosclerosis and other cardiovascular diseases (2). Several studies have linked dietary cholesterol to the risk of developing cardiac diseases (3, 4). Plasma cholesterol level, particularly the low-density lipoprotein (LDL) cholesterol has been reported to be directly associated with cardiovascular diseases (5). This relationship between blood cholesterol and heart disease was first established by the Framingham Heart Study (6). Egg yolk has been reported to contain a significant amount of cholesterol (7, 8). A single egg yolk contains approximately 215 to 275mg of cholesterol (depending on the size) which is more than the 200mg/day limit recommended by the National Cholesterol Education Program (NCEP) for patients with cardiovascular disease (9). The daily recommended limit for cholesterol intake is 300mg/day (10).

There have been conflicting reports over the years on the impact of dietary egg yolk on blood cholesterol. Some studies have reported egg yolk does not significantly alter plasma total cholesterol and other lipids (11, 12, 13, 14) while others have reported egg yolk increases blood cholesterol levels (15, 16, 17). Several drugs help to reduce plasma LDL-C levels, but these synthetic medications also produce adverse effects such as diarrhoea, nausea, myositis and abnormal liver function (18). Hence the search for alternative compounds with hypolipidemic potential.

Carica Papaya is a fast growing tropical perennial tree that is mainly cultivated for its nutritional, medicinal and industrial properties (19). It is popularly known as pawpaw or *papaya* and belongs to the family *Caricaceae*. Its fruits are edible, sweet and juicy and contain more than 1000 seeds which are embedded in the fruit pulp (20). The seeds have been reported to contain oleic acid, Vitamins A, B, C and E (21), flavonoids, saponins, tannins (22). The seeds are black in color and are often considered as a by-product. However, *Carica papaya* seed extracts have been reported to have several medicinal properties notably antibacterial activity (23), anti-helminthic (24), nephro-protective activity (25), anti-fertility effect (26), antioxidant effect (27) and relatively few reports on its hypolipidemic effect. With conflicting reports on the impact of egg yolk on plasma lipids, this study was carried to investigate the effect of dietary egg yolk on lipid profile and the hypolipidaemic effect of *Carica papaya* seed extract.

Materials and Method

Experimental animal

Thirty five (35) adult Wistar rats weighing 150-200g were purchased from the animal house of the Department of Anatomy, School of Basic Medical Sciences, University of Benin. These animals were kept in well cleaned plastic cage with wire mesh cover and had free access to clean water and mash *ad libitum* in accordance with the guidelines of National Research Council Guide for the care of laboratory animals (28), They were allowed to acclimatize for two weeks with free access to food and water before the commencement of the experiment.

Experimental Design

Rats were assigned into seven groups with five (5) rats per group. Group A served as control while group B1 rats were administered with 2ml of raw egg yolk for two weeks. Group B2 animals were administered with 2ml of raw egg yolk for four weeks. Group C1 and C2 were administered with 400mg/kg bwt of aqueous seed extract of *Carica papaya* for two weeks and four weeks respectively. Group D1 and D2 animals were co-administered with 2ml of raw egg yolk and 400mg/kg bwt of aqueous seed extract of *Carica Papaya* for two weeks and four weeks respectively.

Collection of Plant Material

Nine mature, ripe fruits of *Carica papaya* were collected from the farm of Faculty of Agriculture, University of Benin, Benin City, Edo – State, Nigeria.

Preparation of Extract

The *Carica Papaya* seed extraction was carried out according to the methods previously described by Adeneye et al. (29). The fruits were first cut into pieces and the seeds were separated out from the fruit. The seeds were gently but thoroughly rinsed in tap water and completely air-dried at room temperature for 3 weeks (29). The dried seeds were pulverized into fine powder using a new domestic mixer grinder (Kanchan Tycoon®, Kanchan International Limited Unit III, Daman, India). 60 g of the powdered *Carica Papaya* seeds was boiled in 500 mL of distilled water for 40 minutes after which it was filtered using a piece of clean white cotton gauze. The filtrate was evaporated to complete dryness at 40 ºC, producing a fine chocolate color solid residue (29). The extraction process was repeated 4 times and the solid residue was weighed after extraction and pooled together in an air- and water-proof container kept in a refrigerator. Fresh preparations were made from the stock whenever required. The various dose calculations were done based on the average mg/kg body weight of the animals.

Collection of blood samples

At the end of the experimental period, the final weights of the rats were recorded before being subjected to an overnight fast. The rats were anesthetized under chloroform sedation and a midline cut was made through the anterior abdominal wall of the rats with a dissecting scissors. Blood samples were collected by cardiac puncture and then kept in plain bottles. The blood samples were allowed to clot, and then serum was obtained by centrifugation which was utilized for biochemical analysis.

Assays for the Serum Lipid Profiles

The serum levels of cholesterol, triglyceride, and HDLC were measured spectrophotometrically, using enzymatic colorimetric assay kits (Randox, Northern Ireland). Total Serum Cholesterol was analyzed using the method of Allain (30) while HDL-C was analyzed using the method of Lopes (30). Serum triglyceride was determined by the enzymatic method of Stein, (32) low-density lipoprotein cholesterol (LDLC) and very low-density lipoprotein cholesterol (VLDLC) was extrapolated from total cholesterol, triglyceride and HDL by the method of Friedwald (33).

Statistical analysis

Statistical analysis was carried out using the Graphpad Prism 6 software, version 6.1. One-way Analysis of variance (ANOVA) was used to assess the significant differences among multiple groups under various treatments. Results were expressed as Mean ± SEM and P values ≤ 0.05 or $p \leq 0.01$were considered statistically significant.

Results

Effect of egg yolk and *Carica papaya* on total cholesterol

The total cholesterol (TC) was significantly higher in rats feed with 2ml of egg yolk for 2 weeks (group B1) and 4 weeks respectively (group B2) when compared to those of the control (group A) (figure 4.1 and figure 4.2). However, in rats co-administered with aqueous extract of *Carica papaya* seeds and 2ml of egg yolk for 2 weeks (group D1) and 4 weeks (group D2), there was a significant reduction ($P< 0.01$) in the serum total cholesterol when compared with those treated with egg yolk only for 2 weeks (group B1) and 4 weeks (group B2) respectively (see figure 4.1 and figure 4.2).

Effect of egg yolk and *Carica papaya* on triglycerides

Triglycerides(TG) concentration was significantly higher in rats fed with 2ml of egg yolk for 2 weeks (group B1) and 4 weeks (group B2) when compared to those of the control (group A) respectively. In rats co-administration of aqueous extract of *Carica papaya* seeds and 2ml of egg yolk for 2 weeks (group D1) and 4 weeks (group D2) there was a significantly reduction ($P < 0.01$) the serum triglyceride concentration when compared with those treated with egg

yolk only for 2 weeks (B1) and 4 weeks (B2) respectively (see figure 4.3 and figure 4.4).

Effect of egg yolk and *Carica papaya* on Low density lipoprotein cholesterol (LDL-C)

There was a significant increase in the LDL-C in rats fed with 2ml of egg yolk for 2 weeks (group B1) and 4 weeks (group B2) when compared to those of the control (group A) respectively. However, in rats co-administered with aqueous extract of *Carica papaya* seeds and 2ml of egg yolk for 2 weeks (group D1) and 4 weeks (group D2), *Carica papaya* was able to significantly reduce ($P < 0.01$) the LDL-C when compared with those treated with egg yolk only for 2 weeks (group B1) and 4 weeks (group B2) respectively (See figure 4.7 and figure 4.8).

Effect of egg yolk and *Carica papaya* on Very Low density lipoprotein cholesterol (VLDL-C)

VLDL was significantly higher in rats fed with 2ml of egg yolk for 2 weeks (B1) and 4 weeks (B2) when compared to those control (group A) respectively. In rats co-administered of aqueous extract of *Carica papaya* seeds and 2ml of egg yolk for 2 weeks (group D1) and 4 weeks (group D2), there was a significantly reduction ($P <0.01$) of VLDL (group D1) when compared with those treated with egg yolk only for 2 weeks (B1) and 4 weeks (B2) respectively (See figure 4.9 and figure 4.10).

Effect of egg yolk and *Carica papaya* on High density lipoprotein cholesterol (HDL-C)

There was a significant increase in the HDL-C in rats fed with 2ml of egg yolk for 2 weeks (group B1) and 4 weeks (group B2) when compared to those of the control (group A) respectively. In rats co-administered of aqueous extract of *Carica papaya* seeds and 2ml of egg yolk for 2 weeks (group D1) and 4 weeks (group D2),, there was a significantly improvement ($P < 0.01$) in HDL (group D1) when compared with those treated with egg yolk only for 2 weeks (B1) and 4 weeks (B2) respectively.

Discussion

High dietary fat intake is a major risk factor for the development of obesity and cardiovascular diseases (34). Lipid profile measurement is a valuable tool for the assessment of cardiovascular health as well as risk of liver, cardiovascular and metabolic diseases (35). Atherosclerosis, coronary artery disease and other cardiovascular diseases are often associated with increased serum lipids except high density lipoproteins (36). There has been conflicting reports on the association of dietary egg yolk (which contains a significant amount of cholesterol) on plasma total cholesterol over the years with some studies reporting egg yolk has no effect on plasma total cholesterol (11,12) and others reporting a significant effect of dietary egg yolk on total cholesterol (37,15). This necessitated this study which was carried out to investigate the effect of egg yolk on the lipid profile of Wistar rats and the hypolipidemic effect of *Carica papaya* seed extract.

In this study, the total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein were significantly ($P<0.01$) increased in rats administered with 2ml of egg yolk exclusively for the two weeks and four weeks respectively. This finding is in tandem with the findings of Chakrabarty *et al*., (38); Weggemans *et al.* (15); Sacks *et al.* (39) and Djousse *et al.* (17) who have similarly reported that egg yolk to increases serum lipids. The increase in total cholesterol, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) can be attributed to the significant amount of cholesterol contained in egg yolk. Egg yolk contains 275mg of cholesterol (9). Dietary cholesterol has been shown to increase LDL, VLDL in numerous studies (40, 41). Egg yolk has also been reported to

contain significant amount of saturated and trans-fats, which can increase triglyceride levels considerably (42). High density lipoprotein (HDL) was significantly reduced in rats administered with egg yolk for 2 weeks and 4 weeks. HDL is termed the good cholesterol and plays an important role in removing cellular cholesterol and transporting them to the liver where it is converted to bile acids and eventually excreted from the body (43). Thus they are very beneficial and reduce the risk of cardiovascular diseases. The significant reduction in HDL and increase in cholesterol, LDL, VLDL and triglycerides in rats treated with egg yolk thus further highlights the negative impact of excessive consumption of egg yolk and their increase risk in inducing dyslipidemia

However, there was a significant reduction ($P < 0.01$) in the mean level of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and a significant improvement in high density lipoprotein (HDL) in rats that were co-administered with 2ml of egg yolk and 400mg/kg body weight of aqueous seed extract of *Carica papaya* for two and four weeks respectively when compared with rats treated with 2ml of egg yolk only. The result indicates that *Carica papaya* seed extract possess hypolipidemic effect. The hypolipidemic effect of *Carica papaya* seed extract has been previously reported by (44, 45). The hypolipidemic effect of *Carica papaya* can be attributed to its chemical composition, which shows the presence of alkaloids, flavonoids, tannins, saponin and cardiac glycosides (46, 22). Flavonoids, saponins and tannins have been reported to play significant roles in the metabolism of lipids (47) and reduce serum lipid level in animals (48). Inhibition of hepatic cholesterol biosynthesis, enhanced plasma lecithin: Cholesterol acyl transferase activity or reduction of lipid absorption in the intestine has also been reported as mechanisms by which *Carica papaya* induce hypolipidaemia (49). The reduction in the serum triglyceride level by *Carica papaya* seed extract has been attributed to its ability to inhibit lipolysis (50, 45). In the co-treatment protocol between egg yolk and *Carica papaya* there was a significant improvement in HDL in these rats when compared to rats that received egg yolk only for two and four weeks. This significant improvement in HDL by *Carica papaya* thus indicates that it could be beneficial in the management of hypercholesterolemia and other cardiovascular disorders.

Conclusion

This study has shown egg yolk increases serum lipids and *Carica papaya* seed extract possesses hypolipidemic effect and could be of therapeutic benefit in the management of dyslipidemia and other lipid associated disorders

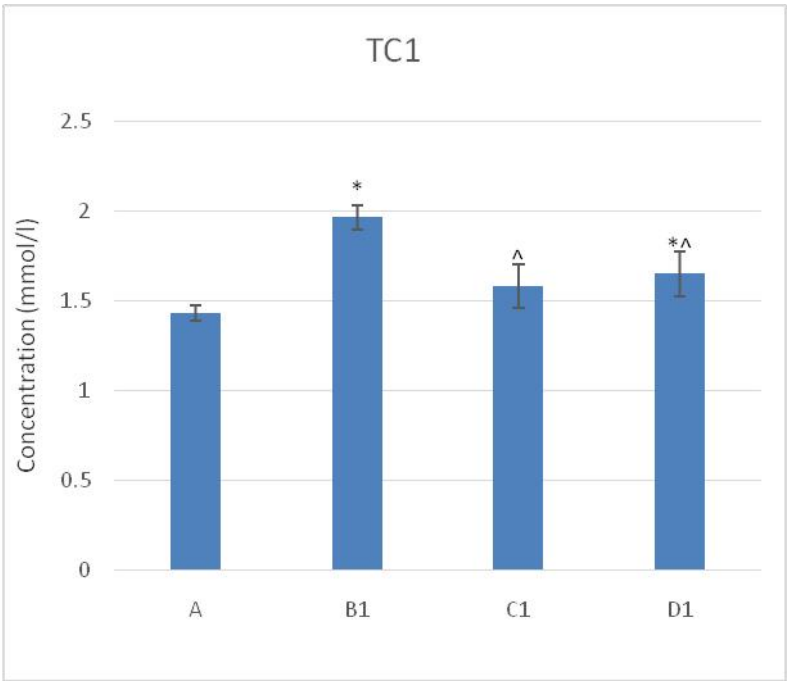

Figure 4.1: Serum Total cholesterol (TC) in rats for the first two weeks. The data are represented as Mean ± SEM. n=5; * represents significant (P<0.01) difference compared with group A; ^ represents significant (P<0.01) difference compared with group B1.

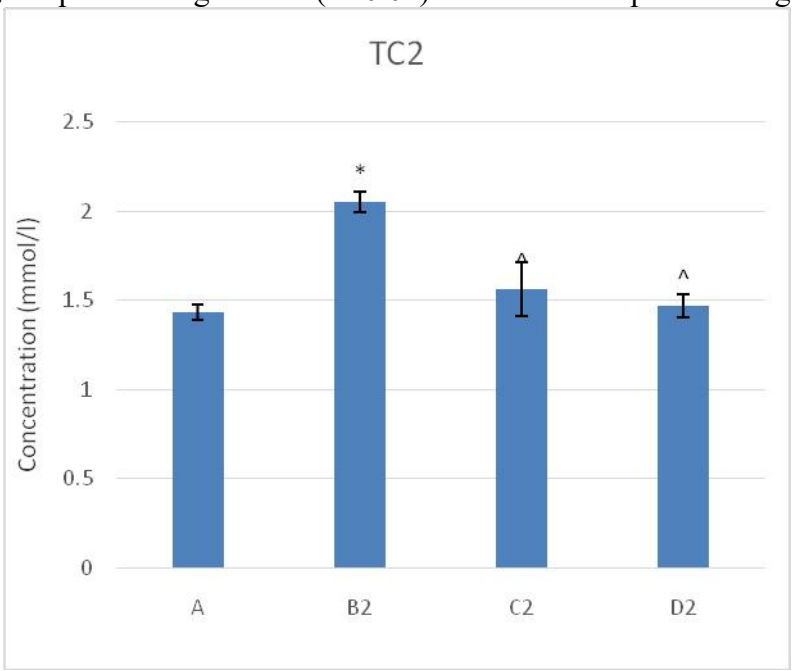

Figure 4.2: Serum Total cholesterol (TC) in rats for the four weeks. The data are represented as Mean ± SEM. n=5; * represents significant (P<0.01) difference compared with group A; ^ represents significant (P<0.01) difference compared with group B2.

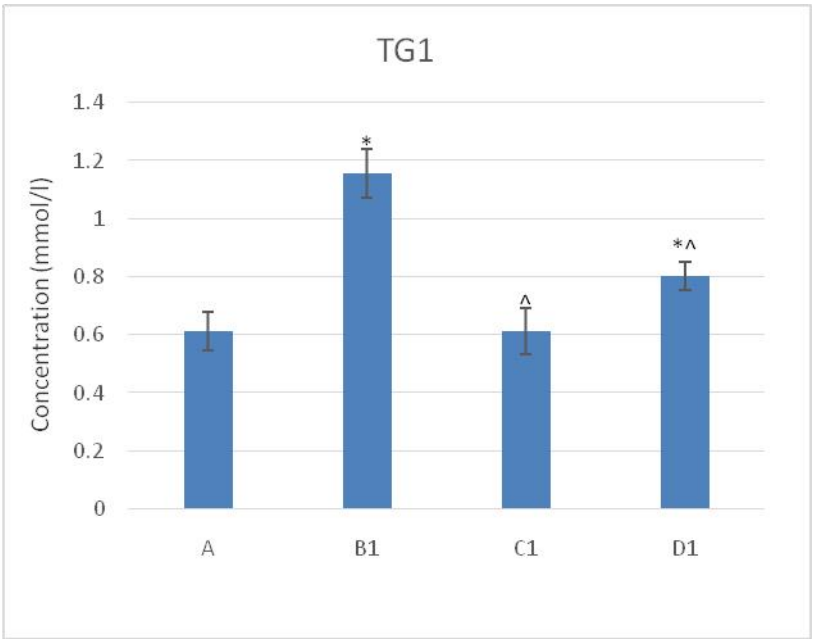

Figure 4.3: Serum Triglyceride concentration (TG) for the first two weeks. The data are represented as Mean ± SEM in the Bar Charts, n=5; * represents significant (P<0.01) difference compared with group A; ^represents significant (P<0.01) difference compared with group B1.

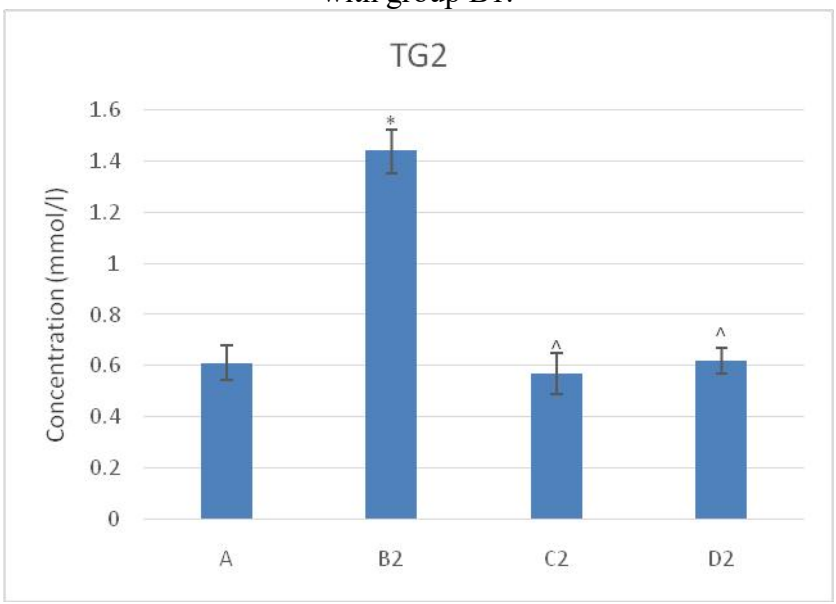

Figure 4.4: Serum Triglyceride concentration (TG) for four weeks. The data are represented as Mean ± SEM in the Bar Charts, n=5; * represents significant (P<0.01) difference compared with group A; ^represents significant (P<0.01) difference compared with group B2.

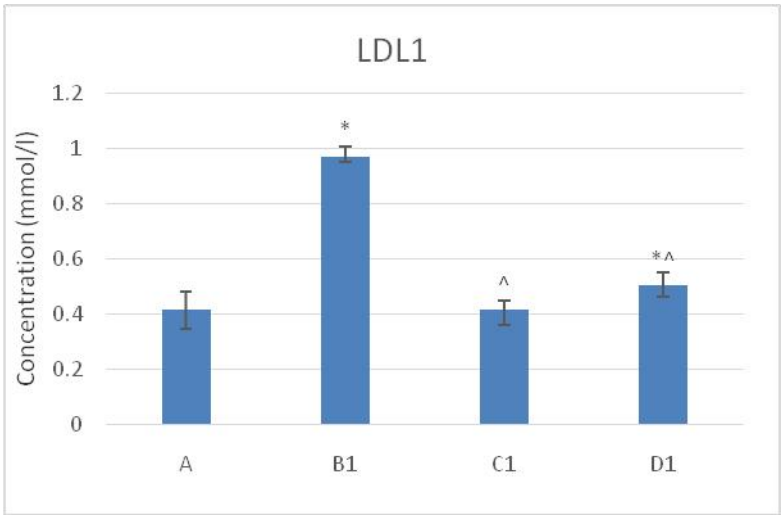

Figure 4.7: Serum Low Density Lipoprotein Cholesterol (LDL-C) in rats for the first two weeks. The data are represented as Mean ± SEM in the Bar Charts, n=5; * represents significant (P<0.01) difference compared with group A; ^represents significant (P<0.01) difference compared with group B1.

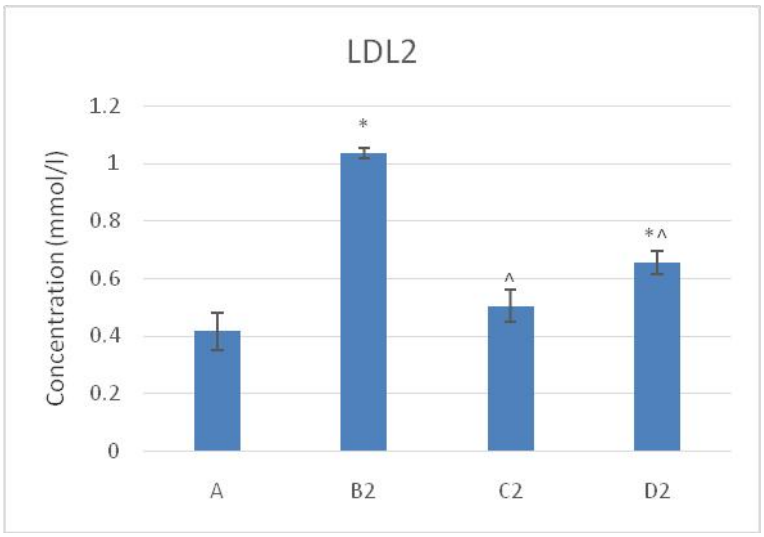

Figure 4.8: Serum Low Density Lipoprotein Cholesterol (LDL-C) in rats for four weeks. The data are represented as Mean ± SEM in the Bar Charts, n=5; * represents significant (P<0.01) difference compared with group A; ^represents significant (P<0.01) difference compared with group B2.

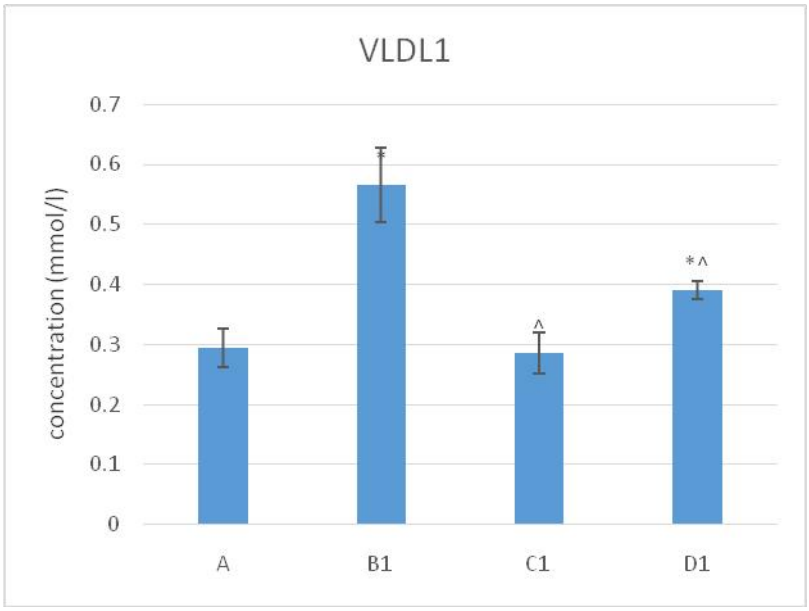

Figure 4.9: Serum Very Low Density Lipoprotein Cholesterol (VLDL-C) in rats for the first two weeks. The data are represented as Mean ± SEM in the Bar Charts, n=5; * represents significant (P<0.01) difference compared with group A; ^represents significant (P<0.01) difference compared with group B1

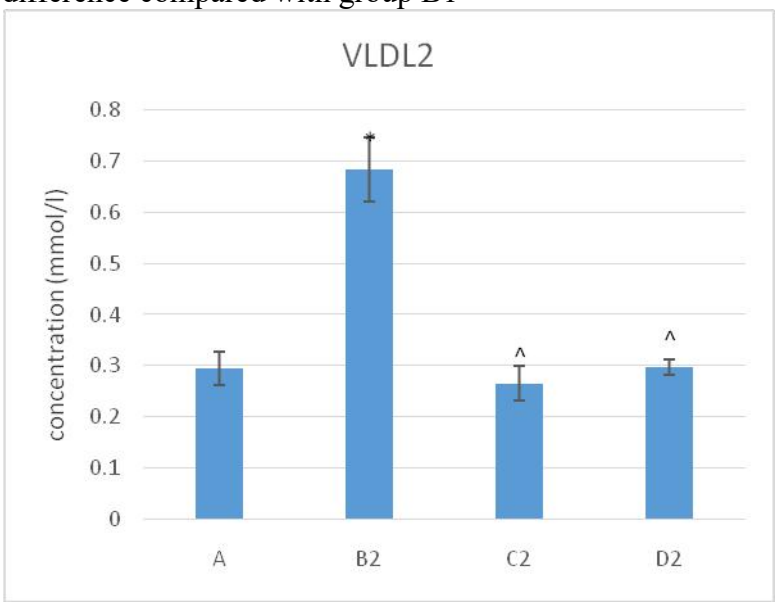

Figure 4.10: Serum Very Low Density Lipoprotein Cholesterol (VLDL-C) in rats for four weeks.. The data are represented as Mean ± SEM in the Bar Charts, n=5; * represents significant (P<0.01) difference compared with group A; ^represents significant (P<0.01) difference compared with group B2.

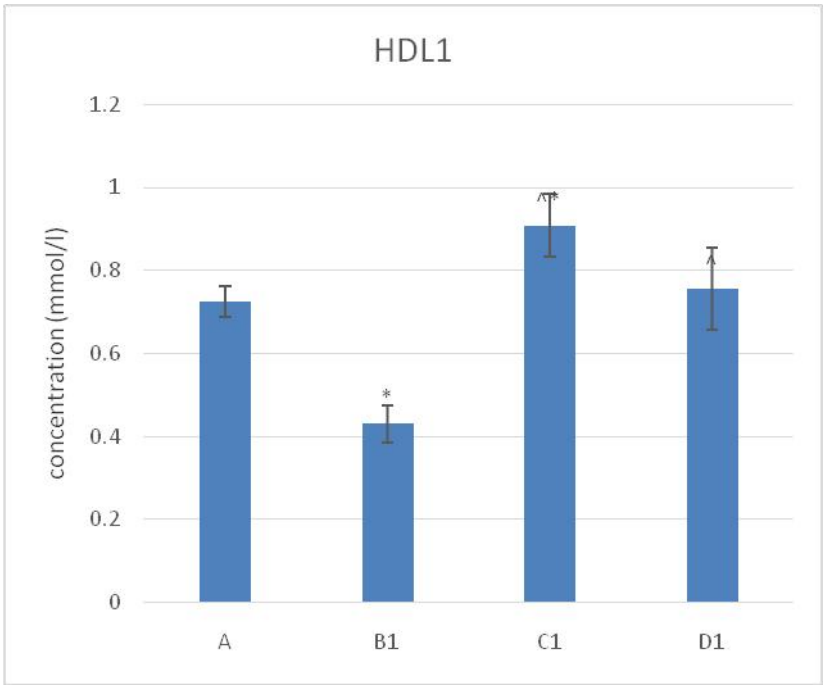

Figure 4.9: Serum High Density Lipoprotein Cholesterol (HDL-C) in rats for the first two weeks. The data are represented as Mean ± SEM in the Bar Charts, n=5; * represents significant (P<0.01) difference compared with group A; ^ represents significant (P<0.01) difference compared with group B1.

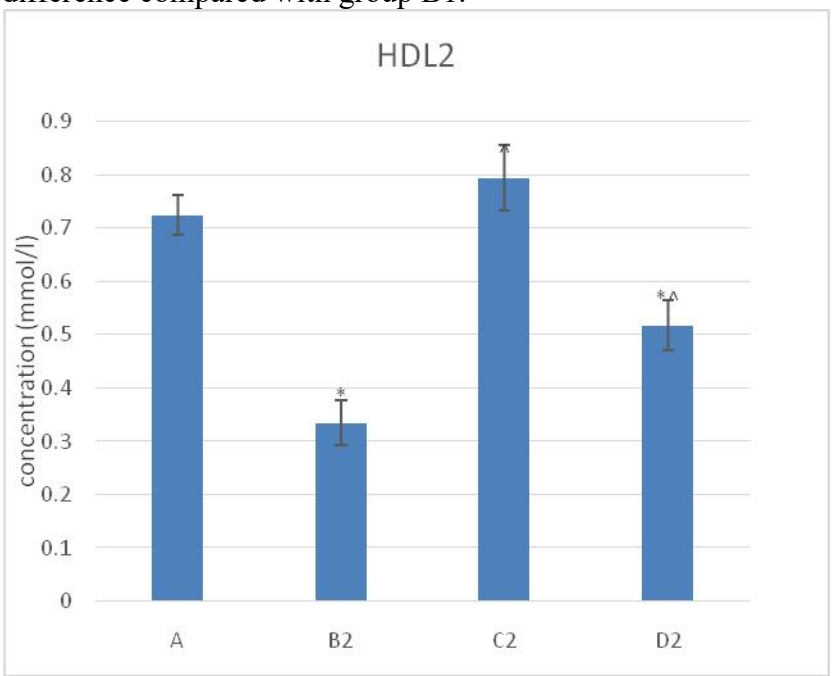

Figure 4.10: Serum High Density Lipoprotein Cholesterol (HDL-C) in rats for four weeks.The data are represented as Mean ± SEM in the Bar Charts, n=5; * represents significant (P<0.01) difference compared with group A; ^ represents significant (P<0.01) difference compared with group B2.

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How to Cite this article: Inneh, C. A., & Momodu, O. I. (2023). Egg Yolk increases Lipid Profile in Wistar Rats; Protective Effect of Carica papaya seed extract. *Journal of Basic and Applied Medical Sciences*, 3(1), 65-78.