

Original Research Article

## Cholesterol Lowering Effect of Cashew Leaf (*Anacardium occidentale*) Extract on Egg Yolk Induced Hypercholesterolaemic Rabbits

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**Abstract:** Cashew leaves (*Anacardium occidentale*) is a medicinal plant applied in the treatment of some non-scientific claims of common diseases in South-Western Nigeria such as hypertension. It contains phytochemicals such as Phenolic, flavonoids, steroids, triterpenes and 33.52% to 46.26% of dietary fiber. This work was therefore designed to determine the Cholesterol Lowering Effect of Cashew leaf (*Anacardium occidentale*) extract on Egg Yolk Induced Hypercholesterolaemic Rabbits. Fifteen rabbits classified into control group A of five rabbits; experimental group B induced with hypercholesterolemia using 20% egg yolk(B1) and thereafter given cashew leaf ethanolic extract(B2) and group C of five rabbits induced with hypercholesterolemia using 20% egg yolk(C<sub>1</sub>) thereafter treated with the aqueous extract of cashew leaf(C<sub>2</sub>). Outcomes of biochemical evaluation of experimental rabbits were compared with the control rabbits. Plasma Total cholesterol(CHOL-T), Low Density Lipoprotein-cholesterol(LDL-C), Total Triglycerides(TG-T), High Density Lipoprotein-cholesterol (HDL-C) were evaluated in the rabbits by autoanalysis using ROCHE reagent on COBAS C111 autoChemistry analyzer. There was a significant increase in plasma total cholesterol, LDL-C, total triglycerides and HDL-C when the rabbits were fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days compared with control rabbits fed on normal diet and water for 7 days with  $p < 0.05$ . A significant decrease was also obtained in plasma Total cholesterol and Total triglycerides in the rabbits given 400mg/Kg of either ethanolic or aqueous extract of cashew leaves extract after they were being given 20% of powdered egg yolk of the total meal weight plus water for seven days which was more in ethanolic extract than the aqueous extract with  $p < 0.05$ . There was also a significantly lower LDL-C in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of ethanolic extract for another seven days. than when the rabbits were fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days and also than in the rabbits induced with hypercholesterolemia using 20% of powdered egg yolk of the total meal weight followed by aqueous cashew leaf extract ( $p < 0.05$ ). This work revealed the efficacy of 20% egg yolk at inducing hypercholesterolaemia while ethanolic and aqueous extract have been found to decrease plasma levels of CHOL-T, TG-T and LDL-C and as such could be applied in the treatment of hypertecholesterolaemia which may result into hypertension.

**Keywords:** *Anacardium occidentale*, hypertecholesterolaemia, Plasma Total cholesterol (CHOL-T), Low Density Lipoprotein-cholesterol(LDL-C)

### INTRODUCTION

Cashew leaf (*Anacardium occidentale*) possess medicinal benefits and have been used as remedy for both diarrhea and colic. Cashews leaf extract is utilized to reduce blood sugar and blood pressure levels. Oils extracted from the seeds prove effective in the preparation of insecticides. The infusion of the bark of the cashew tree has astringent properties and is used as a mouthwash for treating oral ulcers and as a remedy for sore throat and influenza. Leaves of the cashew tree, when boiled with water, serve as an anti-pyretic and are used for the treatment of aches and pains throughout the body. Phytochemicals in cashew leaf (*Anacardium*

*occidentale*) have been reported to include Phenolic, flavonoids, alkaloids, tannins steroids and triterpenes. Though, tannic is toxic but has some health benefits. The presence of flavonoids in the extract could be attributed to the antioxidative effect of the plant [1].

The extracts of the leaves and stem bark of cashew plant were screened for phytochemically for the presence of secondary metabolites and for in vitro antibacterial activity. The methanol and aqueous leaf extracts and the methanol extract of the stem bark were tested against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Candida*

*albicans* and *Escherichia coli* using the agar dilution method. The leaf methanol extracts presented a higher activity than the aqueous extracts. The antibacterial activity was greatest against *K. pneumoniae*, *Bacillus anthracis* and *Candida albicans*. Also the leaf extracts showed greater activity than the stem bark extracts. Of the six organisms tested, the stem bark extracts had activity against three. The mean diameter of the zones of inhibition exhibited by the extracts was between 13mm and 22mm. *Bacillus subtilis* showed the highest zone of inhibition (22mm) to the leaf methanol extract. The extracts compared favourably with ampicillin used as a standard control [2].

Lipid tests are used to assess the risk of heart attack or stroke. As the most important of the cardiac risk tests they provide a good, clear indication of whether someone is likely to have a coronary event caused by a blockage of the blood vessels or atherosclerosis (narrowing of the arteries caused by build-up of fatty deposits). A complete lipid profile involves routine tests such as *Total Cholesterol*, *Triglycerides*, *HDL Cholesterol* and *LDL Cholesterol* to measure the levels of triglycerides and cholesterol in the blood. In addition we also offer an *extended lipid profile* and assays measuring *emerging risk factors* of cardiovascular disease (CVD) [3-6].

Low Density Lipoproteins (LDL) are synthesised in the liver by the action of various Lipolytic enzymes on triglyceride rich Very Low Density Lipoproteins (VLDLs). Specific LDL receptors exist to facilitate the elimination of LDL from plasma by liver parenchymal cells. It has been shown that most of the cholesterol stored in atherosclerotic plaques originates from LDL. For this reason the LDL-Cholesterol concentration is considered to be the most important clinical predictor, of all single parameters, with respect to coronary atherosclerosis. Accurate measurement of LDL-Cholesterol is of vital importance in therapies which focus on lipid reduction to prevent atherosclerosis or reduce its progress and to avoid plaque rupture [3, 4].

Cholesterol measurements are used in the diagnosis and treatments of lipid lipoprotein metabolism disorders. Lipids play an important role in the body; they serve as hormones or hormone precursors, aid in digestion, provide energy, storage and metabolic fuels, act as functional and structural components in biomembranes and form insulation to allow nerve conduction and prevent heat loss.

In clinical chemistry, over the last decade however, lipids have become associated with lipoprotein metabolism and atherosclerosis [5].

High-density lipoproteins (HDL) are one of the major classes of plasma lipoproteins. They are composed of a number of heterogeneous particles,

including cholesterol and vary with respect to size and content of lipid and Apolipoprotein. HDL serves to remove cholesterol from the peripheral cells to the liver, where the cholesterol is converted to bile acids and excreted into the intestine.

Accurate measurement of HDL-C is of vital importance when assessing patient risk from CHD. Direct measurement gives improved accuracy and reproducibility when compared to precipitation methods.

HDL is usually requested with other tests, either with cholesterol or as part of a lipid profile, including LDL and triglycerides. The combination of total cholesterol and HDL is very useful for screening for the risk of heart disease [6].

This work was therefore designed to determine the Cholesterol Lowering Effect of Cashew leaf (*Anacardium occidentale*) extract on Egg Yolk Induced Hypercholesterolaemic Rabbits.

## MATERIALS AND METHODS

### Study area

Animal house of Achievers University, Owo-Nigeria equidistant between Nigeria Federal capital territory-Abuja and former Federal capital-Lagos. It has a Latitude: 6.98575 , Longitude: 5.27103 and Time Zone: UTC+1, Africa/Lagos.

### Study population

Rabbits were bought from Oja Ikoko-a major market in Owo and were identified and confirmed having same sex (male) in the Department of Biological Sciences, Achievers University, Owo-Nigeria. This include 15 rabbits with weight ranging from 0.9-1.3Kg grouped as follows:

**Group A:** Five rabbits weighing  $1.2 \pm 0.1$  Kg fed with normal meal and water were studied as control group A.

**Group B<sub>1</sub>:** Five rabbits weighing  $1.0 \pm 0.1$  Kg fed with normal meal containing 20% of powdered egg yolk of the total meal weight and water for seven days

**Group B<sub>2</sub>:** Five rabbits weighing  $1.0 \pm 0.1$  Kg fed with normal meal containing 20% of powdered egg yolk of the total meal weight and water for seven days followed by the administration of with 400mg/Kg of ethanolic extract for another seven days.

**Group C<sub>1</sub>:** Five rabbits weighing  $1.0 \pm 0.1$  Kg fed with normal meal containing 20% of powdered egg yolk of the total meal weight and water for seven days

**Group C<sub>2</sub>:** Five rabbits weighing  $1.0 \pm 0.1$  Kg fed with normal meal containing 20% of powdered egg yolk of the total meal weight and water for seven days followed by the administration of 400mg/Kg of aqueous extract for another seven days

### Preparation of the Cashew Extracts

Cashew leaves were plucked from major farms in and around Owo-Nigeria and was identified by the Department of Biological Sciences. The leaves of Cashew leaves were air dried for 14 days, Ethanolic and aqueous extraction was carried out by soaking 50g of powers of Cashew leaves into 500ml of each of ethanol and sterile distilled water for 24hours. Following the report of Das *et al.* [7] that solvent to sample ratio of 10:1 (v/w; solvent to dry weight ratio) has been used as ideal. Each extract was filtered through Whatmann filter paper No.1 and filtrates concentrated at room temperature in order to reduce the volume. Further concentration and drying by volume extraction was carried out using rotary evaporator and stored in refrigerator prior to use. Four hundred milligramme of the extract powder was dissolved in 2ml of distilled water for administration.

### Preparation of egg yolk powder

Local eggs were purchased from Oja-Ikoko, Owo-Nigeria and presented to the Biological sciences department of Achievers University, Owo-Nigeria for identification. The shell of the egg was removed and the egg yolk was extracted. The egg yolk was air dried and grinded into powder.

### Preparation of 20% egg yolk powder of normal rabbit meal

The normal meal was weighed. 20% of the weight was removed using weighing balance the 20% was replaced by egg yolk powder and this was used to induce hypercholesterolemia observed in B<sub>1</sub> and C<sub>1</sub>.

### Blood specimen

Blood samples were collected from the veins lining the ear of the rabbits after each treatment into lithium heparinized bottles for the estimation of Total cholesterol, LDL cholesterol and Total triglycerides.

### Determination of Biochemical Parameters

Plasma concentration of Total cholesterol, Low Density Lipoprotein-cholesterol (LDL<sub>CL</sub>), Total Triglycerides, High Density Lipoprotein-cholesterol (HDL<sub>CL</sub>) was determined by CABAS C111 auto-Chemistry analyzer using Roche reagent.

### Method of Data analysis

The results obtained was subjected to statistical analysis using SPSS 18.0

### Ethical Consideration

The rabbits were treated and sacrificed in line with the ethical guideline as provided by Research and Ethical Committee of the Department of Biological Sciences, Achievers University, Owo-Nigeria.

### RESULTS

The results obtained showed a significantly higher plasma value of total cholesterol and

triglycerides in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days (B<sub>1</sub> and C<sub>1</sub>) than the control rabbits A with  $p < 0.05$ . There was significant lower plasma total cholesterol in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of with 400mg/Kg of ethanolic extract of cashew leaves for another seven days. than rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of aqueous extract for another seven days ( $p < 0.05$ ). Significantly lower plasma total cholesterol was also obtained in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of ethanolic extract for another seven days. Than when the rabbits were fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days ( $p < 0.05$ ). There was also a significantly lower plasma total cholesterol in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of aqueous extract for another seven days than when the rabbits were fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days ( $p < 0.05$ ). Plasma LDL-C was significantly lower in control group A rabbits .than rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days with  $p < 0.05$ . There was also a significantly lower LDL-C in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of ethanolic extract for another seven days than when the rabbits were fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of aqueous extract for another seven days with  $p < 0.05$ . There was a significantly lower plasma total Triglycerides in rabbits studied as control group A. than rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days ( $p < 0.05$ ). There was also a significantly lower plasma total Triglycerides in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of ethanolic extract for another seven days

than when the rabbits were fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days(p<0.05). There was a significantly lower plasma total Triglycerides in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of aqueous extract for another seven days than when the rabbits were fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days(p<0.05). There was a significantly lower plasma LDL –C in control rabbits than rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days; There was also a significantly lower plasma LDL –C in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days followed by the administration of with 400mg/Kg of ethanolic extract

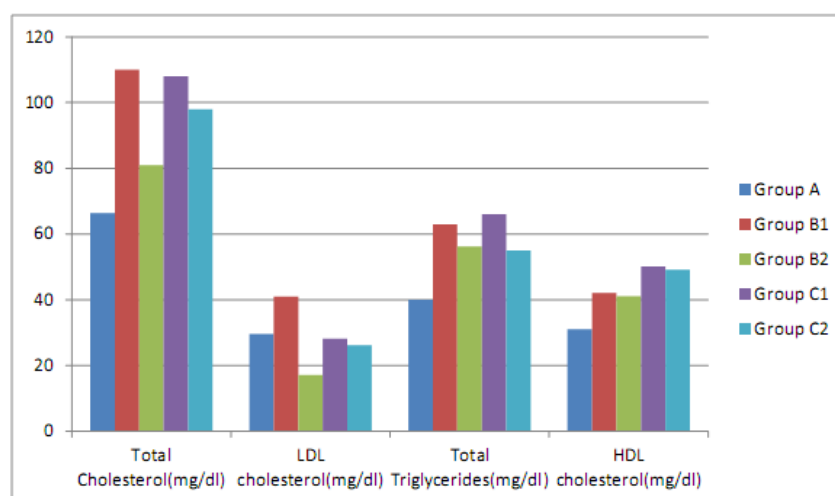
for another seven days than when the rabbits were fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days(p<0.05). There was a significantly lower plasma LDL –C in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of with 400mg/Kg of ethanolic extract for another seven days. than a group of rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of aqueous extract for another seven days with p<0.05. There was a significantly lower plasma HDL- C in control group A rabbits than rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days with p<0.05.

**Table 1: The mean and standard deviation values of Plasma concentration of Total cholesterol, Low Density Lipoprotein-cholesterol (LDL-C), Total Triglycerides, High Density Lipoprotein-cholesterol (HDL-C)**

| Groups                     | Group A   | Group B <sub>1</sub> | Group B <sub>2</sub> | Group C <sub>1</sub> | Group C <sub>2</sub> |
|----------------------------|-----------|----------------------|----------------------|----------------------|----------------------|
| Total Cholesterol(mg/dl)   | 66.40±5.2 | 110.0±2.0            | 81.0±2.0             | 108.2±2.0            | 98.0±2.1             |
| LDL cholesterol(mg/dl)     | 29.6±2.0  | 41.0±2.1             | 17.1±2.0             | 28.2±3.0             | 26.1±2.0             |
| Total Triglycerides(mg/dl) | 40.0±2.0  | 63.0±1.1             | 56.2±1.1             | 66.0±2.0             | 55.0±2.5             |
| HDL cholesterol(mg/dl)     | 31.0±4.4  | 42.1±2.0             | 41.1±2.0             | 50.1±2.0             | 49.2±2.2             |

**Table 2: Comparative analysis of the values of the Plasma concentration of Total cholesterol, Low Density Lipoprotein-cholesterol(LDL-C), Total Triglycerides, High Density Lipoprotein-cholesterol (HDL-C) after cholesterolaemia induction and administration of aqueous and ethanolic extract of cashew leaf**

|                            |     | A vsB <sub>1</sub> | AVsC <sub>1</sub> | B <sub>1</sub> Vs, B <sub>2</sub> | C <sub>1</sub> Vs C <sub>2</sub> | B <sub>2</sub> Vs C <sub>2</sub> |
|----------------------------|-----|--------------------|-------------------|-----------------------------------|----------------------------------|----------------------------------|
| Total Cholesterol(mg/dl)   | “t” | -7.43              | -7.09             | 10.25                             | 4.02                             | -8.05                            |
|                            | “p” | 0.008*             | 0.009*            | 0.005*                            | 0.03*                            | 0.007*                           |
| LDL cholesterol(mg/dl)     | “t” | -3.88              | 0.55              | 9.4                               | 0.56                             | -3.4                             |
|                            | “p” | 0.03*              | 0.3               | 0.006*                            | 0.32                             | 0.04*                            |
| Total Triglycerides(mg/dl) | “t” | -10.29             | -9.19             | 4.95                              | 3.28                             | -1.99                            |
|                            | “p” | <b>0.005*</b>      | <b>0.006*</b>     | <b>0.01*</b>                      | <b>0.04*</b>                     | <b>0.92</b>                      |
| HDL cholesterol(mg/dl)     | “t” | -2.46              | -4.25             | 0.47                              | -0.43                            | -1.99                            |
|                            | “p” | <b>0.07</b>        | <b>0.03*</b>      | <b>0.34</b>                       | <b>0.36</b>                      | <b>0.09</b>                      |



**Fig-1: Chart of the mean ± SD CHOL-T, LDL-C, TG-T, HDL-C obtained in experimental groups and control**



However there was no significant difference in LDL-C in control rabbits group A compared with rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days; also in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days compared with rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of aqueous extract for another seven days ( $p>0.05$ ). No significant difference in plasma Total-TG in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of ethanolic extract for another seven days compared with rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days followed by the administration of 400mg/Kg of aqueous extract for another seven days including plasma HDL-C in control group A compared with Five rabbits weighing  $1.0 \pm 0.1$  Kg fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days ( $p<0.05$ ). This was also obtained in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days compared with rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of with 400mg/Kg of ethanolic extract for another seven days ( $p>0.05$ ). No significant difference was also obtained in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days compared with rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of aqueous extract for another seven days and in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of with 400mg/Kg of ethanolic extract for another seven days. compared with rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of aqueous extract for another seven days with  $p>0.05$ .

## DISCUSSION

There was a significant increase in plasma total cholesterol, LDL-C, total triglycerides and HDL-C when the rabbits were fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days compared with control rabbits fed on normal diet and water for 7 days. This could be attributed to the fact that egg yolks, being mainly stored food, tend to be very concentrated, and in particular tend to be rich in nutrients such as vitamins, minerals, lipids and proteins. As food, the chicken egg

yolk is a major source of vitamins and minerals. It contains all of the egg's fat and cholesterol [8-10].

A significant decrease was also obtained in plasma Total cholesterol and Total triglycerides in the rabbits given 400mg/Kg of either ethanolic or aqueous extract of cashew leaves extract after they were being given 20% of powdered egg yolk of the total meal weight plus water for seven days which was more in ethanolic extract than the aqueous extract. There was also a significantly lower LDL-C in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight plus water for seven days followed by the administration of 400mg/Kg of ethanolic extract for another seven days. than when the rabbits were fed with normal meal containing 20% of powdered egg yolk of the total meal weight with water for seven days and also than in the rabbits induced with hypercholesterolemia using 20% of powdered egg yolk of the total meal weight followed by aqueous cashew leaf extract.

**Monounsaturated Fats for the Heart: Cashews** are a good source of healthy dietary fats, which are essential for our body to absorb the fat-soluble vitamin A, vitamin D, vitamin E, and vitamin K and produce fatty acids that are vital for the development of the brain and blood clotting. These healthy fats include Monounsaturated fats or MUFA and polyunsaturated fats or PUFA. They are good for the heart and help to reduce bad cholesterol (LDL cholesterol) if consumed in appropriate quantities, even in individuals with diabetes. LDL cholesterol can rise as a result of excessive consumption of saturated fats, posing a major threat for cardiovascular diseases like atherosclerosis or the hardening of arteries. Studies have demonstrated that choosing unsaturated fats over saturated fats enhances levels of HDL cholesterol, reduces triglyceride levels, and beneficially lowers the blood pressure as well. The inclusion of nuts such as cashews, fish, and vegetable oils including olive oil and canola oil in the diet can provide these healthy unsaturated fats to the body. Cashew leaves contain 33.52% to 46.26% of dietary fiber which reduces low-density lipoprotein (LDL), the "bad" cholesterol. Fiber can reduce the absorption of cholesterol into your bloodstream. Five to 10 grams or more of fiber a day decreases your total and LDL cholesterol. An important action of some fibers is to reduce the reabsorption of bile acids in the ileum and hence the amount and type of bile acid and fats reaching the colon. A reduction in the reabsorption of bile acid from the ileum has several direct effects. Dietary fiber may act on each phase of ingestion, digestion, absorption and excretion to affect cholesterol metabolism. An important action of some fibers is to reduce the reabsorption of bile acids in the ileum and hence the amount and type of bile acid and fats reaching the colon. A reduction in the reabsorption of bile acid from the ileum has several direct effects. The fibers that are most effective in influencing sterol metabolism (e.g.

pectin) are fermented in the colon. It is therefore unlikely that the reduction in body cholesterol is due to adsorption to this fermented fiber in the colon [11-14].

Cholesterol lowering effect of the leaf extract could be linked with the antioxidant phytoconstituents of the leaf. Antioxidants include some vitamins and minerals, but to appreciate the value of antioxidants, you first need to understand the potential dangers of free radicals, a form of oxygen that has been chemically modified into a highly unstable substance. Free radicals are unstable because they are missing electrons, which must be replaced. So they seek out other compounds in the body and steal electrons to restore stability. If the compound giving up its electrons is the fat and protein in an LDL-cholesterol molecule, the result is the formation of fatty lesions in the walls of the blood vessels [15].

### CONCLUSION

This work has been used to reveal efficacy of 20% egg yolk at inducing hypercholesterolaemia while ethanolic and aqueous extract have been found to decrease plasma levels of CHOL-T, TG-T and LDL-C and as such could be applied in the treatment of hypercholesterolaemia which may result into hypertension.

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