CHOLESTEROL LOWERING EFFECT OF *Cnidoscolous aconitifolius* IN RABBITS INDUCED WITH HYPERCHOLESTEROLEMIA USING EGG YOLK

Abstract

Study Background; *Cnidoscolous aconitifolius* leaf (Chaya; Efo Iyana ipaja in Yoruba language) is a common vegetable plant served as sauce and has many traditional health benefit claims such as treatment of alcoholism, insomnia, gout, scorpion stings, memory and vision impairment owing to its phytochemical and phytonutrient constituents **Aim and Objectives:** This work was designed to determine the cholesterol lowering effect of *Cnidoscolous aconitifolius* in rabbits induced with hypercholesterolemia using egg yolk. **Materials and Methods**: fifteen (15) rabbits grouped into 3 major classes of 5 rabbits each has A (Control rabbits given only normal meal and water), B1 (5 rabbits give 20% egg yolk of normal meal and water for 7 days), B2 (B1 given 400mg/kgBW of ethanolic extract of *Cnidoscolous aconitifolius* leaf after egg yolk containing meal), C1 5 rabbits give 20% egg yolk of normal meal and water for 7 days), C2 (C1 given 400mg/kgBW of aqueous extract of *Cnidoscolous aconitifolius* leaf after egg yolk containing meal). 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. **Result:** There was a significant increase in the plasma CHOL-T, LDL-C, TG-T, and HDL-C in the rabbits given 20% of powdered egg yolk of the total meal weight and water for seven days compared with the control rabbits ($p<0.05$). There was also a significant decrease in the plasma value of CHOL-T, LDL-C and TG-T when the hypercholesterolemic rabbits (B1 and C1) were supplemented with 400mg/kgBW of ethanolic and aqueous of extract *Cnidoscolous aconitifolius* leaf for 7days (B2 and C2) ($p<0.05$). **Conclusion:** Induction of hypercholesterolemia using 20% egg yolk of normal meal and water resulted into a significant increase in plasma value of CHOL-T, LDL-C, TG-T, and HDL-C while supplements of 400mg/kgBW of ethanolic and aqueous extract of *Cnidoscolous aconitifolius* leaf for 7days significantly reduced the plasma value of CHOL-T, LDL-C and TG-T in the hypercholesterolemic rabbits.

Keywords: Cholesterol, *Cnidoscolous Aconitifolius,* Rabbits , Hypercholesterolemia, Egg Yolk

Introduction

Cnidoscolous aconitifolius leaf (Chaya; Efo Iyana ipaja in Yoruba language) is a common vegetable South-Western part of Nigeria prepared as a sauce. It has many non-scientific traditional uses to cure alcoholism, insomnia, gout, scorpion stings, memory and vision impairment. *Cnidoscolous aconitifolius* leaf is a good source of protein, vitamins, calcium, and iron; and is also a rich source of antioxidants [1]. However, raw leaves of the vegetable are toxic as they contain a glucoside that can release toxic cyanide (toxic hydrocyanic acid) similar to cassava . Cooking for about 5 minutes or more prior to consumption could inactivate the toxic components [1-3]. The leaf contains: protein (5.7%), crude fiber (1.9%), calcium (199.4 mg/100 g), potassium (217.2 mg/100 g), iron (11.4 mg/100 g), vitamin C (164.7 mg/100 g), and carotene (0.085 mg/100 g) [4]. Omotoso *et al.,* [5] reported 20 phyto-chemotypes with different therapeutic activities, which include, 9- Octadecenoic acid (Z) and its esters, n-Hexadecanoic acid, n-Octadecanoic acid, n-Octacosane, 1,2,3-Propanetriol and its derivatives, and l-(+)-Ascorbic acid-2,6-dihexadecanoate.The Preliminary phytochemical analysis of *Cnidoscolous aconitifolius* leaf reported by Obichi et al., [6] showed that the leaf extracts contain tannins (5.72±0.00), saponins (12.49±0.021), alkaloids (17.45 ±0.65), flavonoids (23.72 ±0.02), cyanogenic glycosides (0.75 ±0.10) and phytate (1.97 ±0.06).and concentration of vitamin A (5.24mg/kg), vitamin B3 (1.40mg/kg), vitamin B6 (37.23mg/kg), vitamin B12 (15.98mg/kg), vitamin C (382.00mg/kg) and vitamin E (18.28mg/kg).

A large egg of 50 g total weight, contains 17 g of egg yolk which contains about 2.7 g protein, 210 mg cholesterol, 0.61 g carbohydrates, and 4.51 g total fat.. It contains fat-soluble vitamins (A, D, E, and K) are found in the egg yolk. Egg yolk is rich cholesterol anf fat soluble vitamins. Rabbits are known as an animal good at storing fats or lipids [7-10].

This work was designed to determine the cholesterol lowering effect of *Cnidoscolous aconitifolius* in rabbits induced with hypercholesterolemia using egg yolk.

MATERIALS AND METHODS

Study area

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

Study population Rabbits were bought from OjaIkokoamajor Owo market and were identified and confirmed having same sex in the Department of Biological Sciences, Achievers University, Owo-Nigeria. These include 25 rabbits of the same sex with weight ranging from 1.0-1.4Kg grouped as follows:

Group A: Five rabbits weighing 1.2 ±0.1 Kg fed with normal meal and water for 7 days were studied as control group A.

Group B_1: Five rabbits weighing 1.1 ±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_2: Five rabbits weighing 1.3 ±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 of *Cnidoscolus aconitifolius* for another seven days.

Group C_1: Five rabbits weighing 1.2 ±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_2: Five rabbits weighing 1.2 ±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 of *Cnidoscolus aconitifolius* for another seven days

Preparation of the *Cnidoscolus aconitifolius* Extracts

Cnidoscolus aconitifolius (iyana ipaja In Yoruba) was purchased from OjaIkoko, Owo-Nigeria and was identified by the Department of Biological Sciences. The leaves of *Cnidoscolus aconitifolius* (iyana ipaja In Yoruba)- *Cnidoscolus aconitifolius* was air dried for 14 days, Ethanolic and aqueous extraction was carried out by soaking 50g of powers of *Cnidoscolus aconitifolius* into 500ml of each of ethanol and sterile distilled water for 24hours. Following the report of Das *et al.*, [11] 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 OjaIkoko, Owo-Nigeria and presented to the Biological sciences department of Achievers University, OwoNigeria 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 B1 and C1.

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 (LDLCL), Total Triglycerides, High Density Lipoproteincholesterol (HDLCL) 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

Result

The result obtained showed a significantly higher mean value of Plasma Total Cholesterol, LDL cholesterol, Total Triglycerides and HDL cholesterol in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight and water for seven days (Group B1 and C1) compared with rabbits fed with normal meal and water for 7 days (Group A) with $p< 0.05$ (Table 1 and 2, figure 1).

There was also a significantly lower mean plasma value of Total Cholesterol, LDL cholesterol, and Total Triglycerides in rabbits rabbits (B2 and C2) given 400mg/KgBW of ethanolic and aqueous extract of *Cnidoscolus aconitifolius* for another seven days. compared with when the same set of rabbits (Group B1 and **C1)** were only fed with normal meal containing 20% of powdered egg yolk of the total meal weight and water for seven days with $p<0.05$ (Table 1 and 2, figure 1).

There was no significant difference in the Plasma HDL in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight and water for seven days (Group B1 and C1) compared to when the same set of rabbits (B2 and C2) were administered with 400mg/KgBW of ethanolic and aqueous extract of *Cnidoscolus aconitifolius* for another seven days with $p>0.05$ (Table 1 and 2, figure 1).

There was no significance difference in plasma value of Total Cholesterol, LDL cholesterol, Total Triglycerides and HDL cholesterol in rabbits hypercholesterolemic rabbits given 400mg/Kg of aqueous extract of *Cnidoscolus aconitifolius* for another seven days compared with those administered of the ethanolic extract of the same plant with $p>0.05$ (Table 1 and 2, figure 1).

Table 1: Mean and standard Deviation of The plasma value of Total Cholesterol, LDL cholesterol, Total Triglycerides and HDL cholesterol in control and Experimental Rabbits

	Group A	Group B1	Group B2	Group C1	Group C2
Total Cholesterol(mg/dl)	79.0±4.0	122.0±3.0	89.0±1.0	120.0±1.0	90.0±1.0
LDL cholesterol(mg/dl)	42.0±1.0	53.0±3.0	32.0±1.0	51.0±2.0	34.0±1.0
Total Triglycerides(mg/dl)	52.0±1.0	76.0±2.0	63.5±0.5	79.0±1.0	63.0±1
HDL cholesterol(mg/dl)	40.0±2.0	54.0 ±3.0	53.0±1.0	59.0±2.0	57.0±1.0

Table 2: Comparative analysis of the plasma value of Total Cholesterol, LDL cholesterol, Total Triglycerides and HDL cholesterol in control and Experimental Rabbits

		A vsB_1	A VsC_1	B_1 Vs, B_2	C_1Vs C_2	B_2 Vs C_2
Total Cholesterol(mg/dl)	"t"	-8.6	-9.94	10.44	21.21	-0.71
	"p"	0.007**	0.005**	0.005**	0.001**	0.28
LDL cholesterol(mg/dl)	"t"	-3.48	-3.55	6.64	6.13	-1.41
	"p"	0.04*	0.04*	0.01*	0.02*	0.15
Total Triglycerides(mg/dl)	"t"	-10.7	-19.09	5.81	11.31	0.00
	"p"	0.004**	0.001**	0.01*	0.004**	0.5
HDL cholesterol(mg/dl)	"t"	-3.88	-6.72	0.31	0.89	2.82
	"p"	0.03*	0.01*	0.39	0.23	0.05

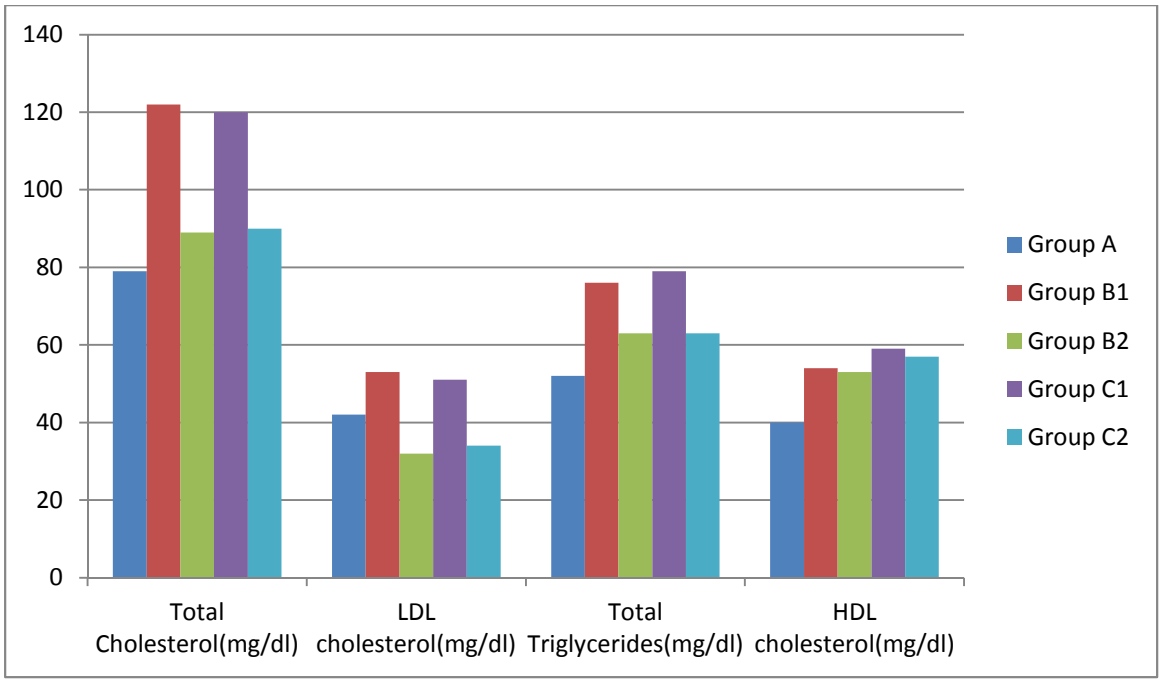

Figure 1: Descriptive analysis of the plasma value of Total Cholesterol, LDL cholesterol, Total Triglycerides and HDL cholesterol in control and Experimental Rabbits

Discussion

The result obtained showed a significantly higher mean value of Plasma Total Cholesterol, LDL cholesterol, Total Triglycerides and HDL cholesterol in rabbits fed with normal meal containing 20% of powdered egg yolk of the total meal weight and water for seven days (Group B1 and C1) compared with rabbits fed with normal meal and water for 7 days (Group A)

Significant increase in plasma value of **Total Cholesterol, LDL cholesterol, Total Triglycerides and HDL cholesterol following the administration of** 20% of powdered egg yolk of the total meal weight in the experimental rabbits could be attributed to the fact that Egg yolk is highly rich in vitamins, minerals, lipids and proteins. It contains nutrient and its primary function is to supply food for the development of the embryo.Egg yolk constitute about 33% of the liquid weight of the egg; it contains about

60 Calories, three times the energy content of the egg white. Furthermore, the weight of egg yolk in a large egg of 50 g total weight, is 17 g which contains arbout 2.7 g protein, 210 mg cholesterol, 0.61 g carbohydrates, and 4.51 g total fat.. It contains fat-soluble vitamins (A, D, E, and K) are found in the egg yolk. Egg yolk, in addition is one of the few foods naturally containing vitamin D [7-10].

There was also a significantly lower mean plasma value of Total Cholesterol, LDL cholesterol, and Total Triglycerides in rabbits rabbits (B2 and C2) given 400mg/KgBW of ethanolic and aqueous extract of *Cnidoscolus aconitifolius* for another seven days. compared with when the same set of rabbits (Group B1 and **C1)** were only fed with normal meal containing 20% of powdered egg yolk of the total meal weight and water for seven days This finding could be attributed to the report of NRC [12] that the potassium content (217.2 mg/100 g) is an important mineral nutrient in the control of hypertension and in the reduction of risks of stroke [12]. Rotimi *et al.,* [13] findings also showed that a high-fibre diet modulates plasma lipid and modifies plasma lipoprotein distribution and composition. Albrink *et al.,* [14] reported that carbohydrate-induced hyperlipemia does not occur if the high carbohydrate diet is rich in dietary fiber, and furthermore that the insulin-stimulating potential of foods in a very high-carbohydrate diet is a critical determinant of the magnitude of carbohydrate-induced lipemia. This could also be attributed to the constituent organic acid (toxic hydrocyanic acid) in the leaf extract [1].

Conclusion: Induction of hypercholesterolemia using 20% egg yolk of normal meal and water resulted into a significant increase in plasma value of CHOL-T, LDL-C, TG-T, and HDL-C while supplements of 400mg/kgBW of ethanolic and aqueous extract of *Cnidoscolous aconitifolius* leaf for 7days significantly reduced the plasma value of CHOL-T, LDL-C and TG-T in the hypercholesterolemic rabbits.

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