

Possible inflammatory responses in hypercholesterolemia patients receiving treatment using raw liquid extract of young cashew leaves in herbal homes in Nigeria

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ABSTRACT

Objectives: The objective of this study was to determine the possible inflammatory responses in hypercholesterolemia patients receiving treatment using raw liquid extract of young cashew leaves in some herbal homes in Nigeria.

Methods: Hypercholesterolemia patients (27: Aged 31–53 years; female - 10 and male - 17) 20 herbal homes in Saki-West, Saki-East, and ATISBO local governments constituting a Federal constituency in Nigeria, and non-hypercholesterolemia apparently healthy participants (50: Aged 20–64 years; female - 25 and male - 25) were recruited from the same federal constituency as test and control subjects, respectively. About 60 ml of this extract were given to the patients for an average of 14 days when there was a significant decrease in their plasma cholesterol. Plasma cholesterol was determined in the subjects using COBAS C111 auto chemistry analyzer; plasma tumor necrosis factor-alpha (TNF- α), interleukin (IL)-6, 8, and 29, intrahepatic chemokines such as fibrinogen and C-reactive protein (CRP), IL-6, CRP, hepatitis B surface antigen, anti-hepatitis C virus, and anti-human immunodeficiency virus were immunochemically determined by enzyme-linked immunosorbent assay, and identification of *Plasmodium* spp. was determined by microscopic examination of thick film-stained Giemsa stain.

Results: There was a significant reduction in the plasma values of cholesterol, TNF- α , IL-6, and CRP with a significant increase in plasma aspartate transaminase (AST) and alanine transaminase (ALT). In the hypercholesterolemia patients who were supplemented with raw liquid extract of young cashew leaves compared with the control and their basal samples ($P < 0.05$).

Discussion: Supplementation of raw liquid extract of young cashew leaves can cause a significant reduction in the plasma values of cholesterol TNF- α , IL-6, and CRP with an increase in plasma AST and ALT. In the hypercholesterolemia patients due to the phytochemicals in young cashew leaves.

Keywords: Herbal homes, herbal treatment, hypercholesterolemia patients, inflammatory responses, Nigeria, young cashew leaves

Introduction

Cashew leaves (*Anacardium occidentale*) is a medicinal plant used in the traditional treatment of some of the common diseases in South-Western Nigeria such as hypertension.^[1] The plant increases high-density lipoproteins cholesterol and decreases triglycerides and low-density lipoproteins cholesterol in human.^[2,3,4] It contains phytochemicals such as phenolic, flavonoids, steroids, triterpenes, and 33.52–46.26% of dietary fiber.^[1] Olaniyan^[1] demonstrated the cholesterol-lowering effect of the raw extract of cashew young leaves in rabbits who found decrease in cholesterol following the administration of cashew young leaf extracts. Herbal treatment in Nigeria is

gaining popularity due to its accessibility, low cost, and the effectiveness in the treatment of diseases such as malaria and hypertension.^[5,6] High cholesterol predisposes cardiovascular disease risk such as stroke which may lead to death.^[5,6] Many people are well educated on this which make some Nigerians who could not avoid fatty food possibly due to social, personal, and economic reasons check for their cholesterol plasma levels in accredited laboratories for the purpose of secondary prevention.^[5]

Interleukin (IL)-6, tumor necrosis factor-alpha (TNF- α), and C-reactive protein (CRP) are inflammatory biomarkers. IL-6 is an anti-inflammatory biomarker that acts against inflammatory

process to improve healing process while TNF- α acts to bring about inflammation and worsen the disease process.^[6-13] CRP plasma level increases in inflammation.^[14]

Study rationale/justification

Metabolism of herbal preparations is the responsibility of the liver which helps in the detoxification of poisonous content of the herbal extract to prevent cellular, tissue, and organ toxicity that may bring about damage and inflammatory process.^[1] This work was designed to determine the possible inflammatory responses in hypercholesterolemia patients receiving herbal treatment young cashew leaves in some herbal homes in Nigeria as little is known about this. This work will also provide useful information for appropriate directions on the application of herbs in the treatment of human ailment with respect to toxicity and immune responses.

Materials and Methods

Materials

Study area

Hypercholesterolemia and non-hypercholesterolemia subjects were recruited from Saki-West, Saki-East, and ATISBO local governments constituting a federal constituency in Nigeria located at the Northern part of Oyo state. It shares border with Burkina Faso and Kwara state.

Time of samples collections

It was fasting blood sample in the morning.

Study population, study design, and process of recruitment of the participants

Study design

This was a case-control-observational study.

Test participants

A total of 27 hypercholesterolemia patients aged 31–53 years (female - 10 and male - 17) were recruited from 20 herbal homes of Saki-West, Saki-East, and ATISBO local Governments area of Oyo state in Nigeria. Six were found to be infected with human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), or Plasmodium and were excluded from the study. 21 hypercholesterolemia of the 27 patients aged 31–53 years initially recruited (female – 10 and male - 17) were finally studied.

Control participants

A total of 50 non-hypercholesterolemia apparently healthy subjects not infected with HIV, HBV, HCV, or Plasmodium (aged 20–64 years; female - 25 and male - 25) were recruited from Saki-West, Saki-East, and ATISBO local governments constituting a federal constituency in Nigeria and studied as control subjects.

Methods

Preparation and administration of raw liquid extract of young cashew leaf

Young cashew leaves were plucked on daily basis and the raw liquid extract was extracted by the herbal home practitioners by squeezing. About 60 ml of this extract were given to the patients for an average of 14 days when there was a significant decrease in their plasma cholesterol.

Method of data analysis

The results obtained were subjected to statistical analysis to determine *t*-value, probability at 0.05 level of statistical significance using the Statistical Package for the Social Sciences 18.0.

Ethical consideration

The proposal was approved by ethical and research committee of Baptist Medical Centre, Saki-Nigeria, and the consent of each of the subjects was obtained before the commencement of the work.

Inclusion criteria

Hypercholesterolemia subjects free of HIV, HBV, HCV, and Plasmodium infections were recruited as test subjects while non-hypercholesterolemia subjects free of HIV, HBV, HCV, and Plasmodium infections were recruited as control subjects.

Exclusion criteria

Hypercholesterolemia subjects with HIV, HBV, HCV, and Plasmodium infections were excluded from this work. Those on cholesterol-lowering medications were also excluded from the study.

Biochemical and immunochemical assay methods

Samples: Amount of blood taken, method of separation, and storage

5 ml of venous blood sample collected from each subject was centrifuged at 1000 rpm for 5 min for the extraction of plasma. The plasma was stored at 6°C in the refrigerator.

Plasma concentration of total cholesterol was determined by CABAS C111 auto chemistry analyzer using Roche reagent.

TNF- α enzyme-linked immunosorbent assay (ELISA)

Plasma TNF- α was analyzed using Abcam's kit. The analysis was carried out according to manufacturer's instruction (Abcam's kit).

IL-6 ELISA

The analysis was carried out according to manufacturer’s instruction (Abcam’s kit).

Determination of anti-HCV, anti-HIV, HB surface antigen (HBsAg), HB e antigen (HBeAg) and HBe antibody (Ab). These were carried out using the reagent kits of BIORAD (for anti-HIV, HB surface antigen (HBsAg), HB e antigen (HBeAg) and HBe antibody (Ab).) and DIA-PRO(for anti-HCV)

Results

There was a significant decrease in the plasma value of total cholesterol when the hypercholesterolemia subjects were given raw liquid extract of young cashew leaves ($P < 0.05$) [Table 1 and Figure 1]. The plasma value of total cholesterol obtained in the non-hypercholesterolemia control subjects was significantly lower than the value of the parameter in the hypercholesterolemia subjects before and after the administration of cashew leaves raw extract ($P < 0.05$) [Table 1 and Figure 1].

There was a significant decrease in the plasma TNF- α , IL-6, and CRP when the hypercholesterolemia subjects were given raw liquid extract of young cashew leaves ($P < 0.05$) [Tables 1 and 2 and Figure 2]. The plasma value of plasma TNF- α , IL-6, and CRP obtained in the non-hypercholesterolemia control subjects was significantly lower than the value of the parameters in the hypercholesterolemia subjects before the administration of cashew leaves raw liquid extract

($P < 0.05$) [Tables 1 and 2 and Figure 2]. However, there was no significant difference in the plasma value of TNF- α , IL-6, and CRP in the hypercholesterolemia subjects after the administration of cashew leaves raw liquid extract compared with the results obtained in non-hypercholesterolemia control subjects ($P > 0.05$) [Tables 1 and 2 and Figure 2].

There was a significant increase in the plasma level of aspartate transaminase (AST) in hypercholesterolemia subjects after they were given cashew leaves raw liquid extract compared to when they were not supplemented with the extract and also compared with the value of AST in the control subjects ($P < 0.05$) [Tables 1 and 2 and Figure 2]. A significantly higher mean value of alanine transaminase (ALT) was obtained in the hypercholesterolemia subjects after they were given cashew leaves raw liquid extract than in non-hypercholesterolemia control subjects ($P < 0.05$) [Tables 1 and 2 and Figure 2].

The results also showed no significant difference in the plasma value of ALT in hypercholesterolemia subjects before and after they were given cashew leaves raw liquid extract and also when their basal plasma values of ALT and AST were compared with the results obtained from non-hypercholesterolemia control subjects ($P > 0.05$) [Tables 1 and 2 and Figure 2].

Discussion

The results showed evidence of Plasmodium, HCV, or HBV infection in 22.2% (6) of the 27 hypercholesterolemia

Table 1: Comparative analysis of the mean and standard deviation of plasma value of total cholesterol, cytokines, and liver enzymes obtained in the subjects

Biochemical parameters	Before treatment versus After treatment		Before treatment versus Non-hypercholesterolemia normal control subjects		After treatment versus Non-hypercholesterolemia normal control subjects	
	t-value	P value	t-value	P value	t-value	P value
Total cholesterol (mg/dl)	15.5651	0.0021*	16.26346	0.002*	3.28	0.041*
IL-6 (pg/ml)	5.3033	0.017*	8.49706	0.007*	0.6868	0.28
TNF- α (pg/ml)	3.0	0.048*	6.64078	0.011*	1.4552	0.14
CRP (mg/L)	4.02492	0.028*	4.47214	0.023*	0.7071	0.28
ALT (U/L)	-2.7735	0.055	0.89443	0.233	3.7947	0.032*
AST (U/L)	-5.00879	0.019*	0.68599	0.282	5.7469	0.0145*

AST: Aspartate transaminase, ALT: Alanine transaminase, TNF- α : Tumor necrosis factor-alpha, CRP: C-reactive protein

Table 2: The mean and standard deviation of plasma value of total cholesterol, cytokines, and liver enzymes obtained in the subjects

Biochemical parameters	Before treatment (n=21)	After treatment (n=21)	Non-hypercholesterolemia normal control subjects (n=50)
Total cholesterol (mg/dl)	372±10.0	182±7.0	142±10.0
IL-6 (pg/ml)	3.2±0.2	1.7±0.2	1.3±0.1
TNF- α (pg/ml)	4.5±0.3	3.0±0.4	2.4±0.1
CRP (mg/L)	3.4±0.4	1.6±0.2	1.4±0.2
ALT (U/L)	7.0±1.0	12±1.5	6.0±0.5
AST (U/L)	5.4±0.5	11±1.0	5.0±0.3

AST: Aspartate transaminase, ALT: Alanine transaminase, TNF- α : Tumor necrosis factor-alpha, CRP: C-reactive protein

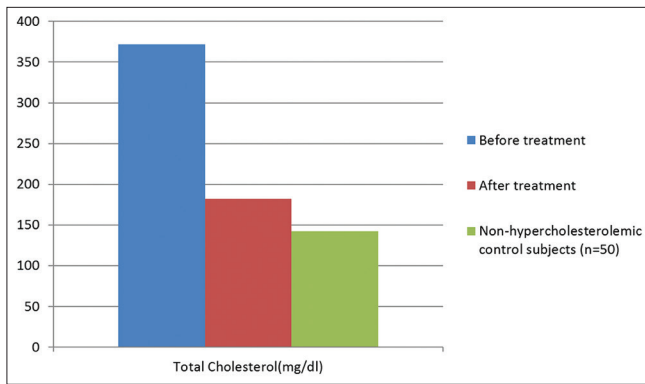


Figure 1: Comparative description of the plasma values of total cholesterol obtained in the subjects

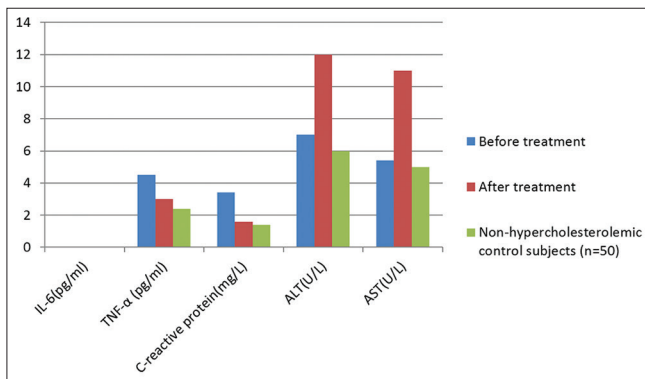


Figure 2: Comparative description of the plasma values of IL-6, aspartate transaminase, alanine transaminase, and tumor necrosis factor-alpha obtained in the subjects

subjects initially recruited. The frequency of the biomarkers of infectious agents found among the 27 hypercholesterolemia subjects initially recruited include 7.4% (2) HBsAg, 3.7% (1) anti-HCV and HBeAg, 3.7% (1) HBeAb, and 7.4% (2) Plasmodium infection, 77.8% (21) were not infected by HCV, HBV, and Plasmodium. There was no evidence of coinfection in hypercholesterolemic subjects. The frequencies of the occurrence of biomarkers of the infectious agents in hypercholesterolemic subjects was lower than 2.4% (2) anti-HIV, 4.9% (4) anti-HCV, 8.5% (7) HBsAg, 3.7% (3) HBeAg, 11% (4) HBeAb, 4.9% (11) Plasmodium spp., 1.2% (1) anti-HIV + HBsAg + HBeAg, and 39.0% (32) with either anti-HIV, anti-HCV, HBsAg, HBeAg, HBeAb, Plasmodium spp., or coinfection.

These significant differences could be associated with the fact that high cholesterol protects against infectious diseases which may be responsible for the higher frequency of the occurrence of biomarkers of the infectious agents in non-hypercholesterolemia subjects than the hypercholesterolemia subjects.^[15,16]

There was a significant decrease in the plasma value of total cholesterol when the hypercholesterolemia subjects were given raw liquid extract of young cashew leaves. This further

affirms the report of Olaniyan^[1] that the cashew leaf extract has a cholesterol-lowering effect. The plasma value of total cholesterol obtained in the non-hypercholesterolemia control subjects was significantly lower than the value of the parameter in the hypercholesterolemia subjects before and after the administration of cashew leaves raw extract.

There was a significant decrease in the plasma TNF- α , IL-6, and CRP when the hypercholesterolemia subjects were given raw liquid extract of young cashew. The plasma value of TNF- α , IL-6, and CRP obtained in the non-hypercholesterolemia control subjects was significantly lower than the value of the parameters in the hypercholesterolemia subjects before the administration of cashew leaves raw liquid extract. This could be explained as hypercholesterolemia could induce immune defense against infectious agents such as pro- and anti-inflammatory responses, leading to increase in the plasma inflammatory biomarkers such as TNF- α , IL-6, and CRP. IL-6 due to inflammation are released in excess to reduce the release and the effect of TNF- α . Decreased plasma value of IL-6 and TNF- α in the hypercholesterolemia subjects in this study when they were supplemented with raw liquid extract of young cashew leaves could be due to over-utilization of IL-6 as anti-inflammatory cytokines to reduce the effects of TNF- α . In addition, raw liquid extract of young cashew leaves has anti-inflammatory activities.^[1,14] In addition, it has been suggested that the blood lipids play a key role in the immune defense system. Again, inflammatory response of the arterial intima to injury is a crucial step in the genesis of atherosclerosis and that infections may be one type of such injury.^[17,18]

It has also been reported that the abdominal obesity is the main source of pro-inflammatory cytokines, and vascular systemic inflammation can be produced by adipose tissue which can also be linked with the findings of this work.^[19] However, there was no significant difference in the plasma value of TNF- α , IL-6, and CRP in the hypercholesterolemia subjects after the administration of cashew leaves raw liquid extract compared with the results obtained in non-hypercholesterolemia control subjects. This indicates that cashew leave extract may have anti-inflammatory activities or may be as a result of its cholesterol-lowering properties which reduce inflammatory responses specifically the plasma level of the indices of inflammation.

There was a significant increase in the plasma level of AST in hypercholesterolemia subjects after they were given cashew leaves raw liquid extract compared to when they were not supplemented with the extract and also compared with the value of AST in the control subjects. A significantly higher mean value of ALT was obtained in the hypercholesterolemia subjects after they were given cashew leaves raw liquid extract than in non-hypercholesterolemia control subjects. AST and ALT are liver enzymes, their elevated level on the administration of cashew leaves raw liquid extract could indicate that the extract may have hazardous effect on the liver

which may include induced hepatitis, liver damage, and liver dysfunction. AST is found in other cells other than the liver cells, the extract may have caused destruction of cells other than the hepatocytes for an appreciable increase in plasma AST. In addition, AST is similar to ALT in that they are found in liver parenchymal cells. The difference is that ALT is found majorly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, while AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells.^[20-22]

Study Limitations

This study was limited by sample size as only those hypercholesterolemia patients who chose to be treated in the 20 herbal homes were recruited. In addition, six of them infected with HIV, HBV, HCV, or Plasmodium were excluded from the study.

Conclusion

There was a significant reduction in the plasma values of cholesterol, TNF- α , IL-6, and CRP when the hypercholesterolemia subjects were given the raw liquid extract of cashew young leaves. Administration of the raw liquid extract of cashew young leaves in the hypercholesterolemia subjects induced elevated AST and ALT.

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