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Full Length Research Paper

Diet and alkaloid extract of *Garcinia Kola* induce reduction in serum levels of selected indices of coronary heart disease and liver functions

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This study was designed to evaluate the effect of *Garcinia kola* diet and alkaloid extract on selected serum biochemical indices of coronary heart and liver functions with the view of ameliorating such conditions. A total of 30 male albino rats assigned into three groups of 10 rats each were used. Group I (control) received basal feed (vital growers mash) at 54g/kg body weight of rats. Group II and III received alkaloid extract and diet of *Garcinia kola* at doses of 100mg/kg and 54g/kg body weight respectively. The treatment was terminated after 28 days and the rats sacrificed. Blood was collected by cardiac puncture, spun at 5000 rpm for 5 minutes and serum collected was analyzed in the laboratory for the selected biochemical indices of coronary heart disease and liver function. The results obtained indicated that whereas crude alkaloid extract caused a significant (P<0.05) decrease in serum total cholesterol, low density lipoprotein cholesterol and triglyceride (2.00 ±0.17, 0.30± 0.08, 0.87±0.06, mmol/L) relative to control (2.90± 0.30, 0.69± 0.29 and 0.57±0.06 mmol/L) There was however no significant (P>0.05) changes in serum levels of HDL,LDL and triglyceride produced by Garcinia kola seed diet. Both alkaloid extract and diet produced a significant (p<0.05) reduction in Alanine Aminotransferase (8.00±2.00; 9.66±3.51 IU/L) relative to control (16.00± 1.41 IU/L). While alkaloid extract produced significant (P<0.05) increase in AST, the diet caused non-significant (P>0.05) changes in AST & ALP. Therefore alkaloid extract may have a positive effect on the selected biochemical indices of coronary heart disease whereas the diet may not.

Keywords: Diet, alkaloid extract, *Garcinia Kola,* biochemical indices, coronary heart diseases, Liver function.

INTRODUCTION

Coronary artery disease (CAD) otherwise known as coronary heart disease (CHD) is a disease of coronary artery arising from excess fat depositions at the intima of the arterial vascular wall (Martin, 2000). It may also arise from atherosclerotic plaques that are formed at the wall of coronary artery (FAO/WHO, 1993).

Cholesterol fractionation tests isolate and measure total cholesterol in serum, low density lipoproteins (LDL) and high density lipoproteins (HDL) by ultracentrifugation or electrophoresis (Stanley *et al.,2005).* Framingham's heart study has shown that cholesterol in HDL is inversely related to the incidence of coronary artery disease. The higher the serum HDL level, the lower the incidence of (CAD); conversely, the higher the LDL, the higher the incidence of CAD.

Since the 1940s and 1950s studies within populations as well as cross cultural comparison produced abundant evidence that high serum cholesterol levels are associated with increase risk of coronary heart disease (Levy *et al.,* 1979, Anderson *et al.,* 1987). Associations between diet, serum cholesterol levels and risk of CHD have been well documented in cross country comparison

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(Keys *et al, 1986; Lewis et al* 1978). It has recently been suggested that oxidized LDL is the major cause of atherosclerosis (FAO/WHO, 1993). Atherosclerosis as one of the complications of imbalance between fat intake and its oxdiation (Zurlo *et al.* 1990) can therefore be balanced when the oxidation of fat equals the intake. Oxidised LDL, is more readily taken up by the monocyte which leads to the formation of atherosclerotic plaques. Some studies therefore suggest that various antioxidants limit the development of atherosclerosis in animals and humans (Steinberg *et al.*,, 1989, Frei *et al.,* 1989) High intakes of vitamin E have been associated with reduced risk of coronary heart disease in both men (Rimm *et al;.* 1993*)* and women (Stampfer effective as well (Bjorkkern *et al*., *1991).* Theobromine rich cocoa powder induced significant weight loss in obese wister rats (Eteng *et al*., 2006). Iranloye *et al*., (2006) reported the antioxidatent effect of Kolaviron; a bioflavonoid of *Garcinia kola*.

This study therefore is designed to find out the effect of diet and alkaloid extract of *Garcinia kola* on serum levels of selected biochemical indices of coronary heart disease and liver function in rats with the aim of ameliorating such condition.

MATERIALS METHODS

Preparation of stock solution of crude alkaloid extracts of *Garcinia kola* seeds

This was obtained by methanolic extraction using a soxhlet extractor and partitioned in equal volumes of chloroform water phase for 25 hours in a separating funnel. The water soluble phase (which contains the alkaloid) confirmed by detecting its' presence by the method of Sofowora (1982) was evaporated to dryness using a rotary evaporator. The extract was ground into powder and stored in a refrigerator at a temperature of 4^0C for future use. One gram of crude alkaloid extract powder was dissolved in one milliliter (m1) of distilled water and suspended in 9.0m1 of normal saline (0.9% NaC1). The stock solution was administered to the rats for 28 days at a dose of 1000mg/kg body weight via a gastric intubation.

Animal Treatments

A total of 30 male albino rats weighing between 150-200 grams at the inception of the experiment were used. The rats were assigned into three treatment groups of 10 rats per group. Group 1 received basal feed (vital grower's mash) containing 14.50% crude protein, 7.0% fat, 7.2% crude fibre, 0.8% calcium, 0.4% phosphorus and 2,500Kcal metabolizable energy as basic ingredients at a dose of 54g per kg body weight. Group II and III apart from basal feed, received crude alkaloid extract and *Garcinia kola* seed diet at doses of 1000mg/kg and 54g/kg body weights respectively. The experiment was terminated at the end of 28 days and the rats sacrificed by euthanasia and blood sample collected via cardiac puncture, spun for 5000 rpm and analyzed in the laboratory for lipid and enzyme of liver function.

Assay of Lipid Profile

Component lipids were estimated using enzymatic colorimetric diagnostic kits obtained from Randox laboratories, antrium, United Kingdom. The GPO=PAP method of Trinder (1969) was used for the determination of serum triglycerides. The total cholesterol in serum was estimated using the CHOP-PAP method of Richmond (1973) and Flegg (1973) while high density lipoprotein (HDL) cholesterol was determined using phosphotungstate precipitation method of Richmond (1973). The low density lipoproteion (LDL) cholesterol was estimated as the difference between total cholesterol and high density lipoprotein with triglycerides divided by a factor of five.

Assay of Selected Serum Enzymes

The method of Thomas (1995) was used for the determination of Alkaline phosphates activity in serum. This method is based on the photo colorimetric determination of inorganic phosphate splits off from glycerophosphate with phosphates of the blood serum in an alkaline medium.

The estimation of Aspartate and Alanine aminotransferases (AST & ALT) were carried out according to the method of Reitman and Frankel, 1994.

Statistical Analysis

Data collected were computed and analyzed using group comparison according to (Nwabuoike, 1986).The student's t-test was used to compare the significance of differences between treatments while analysis of variance was used to compare significance of difference among and within group of treatments.

RESULT

The effect of diet and crude alkaloid extract of *Garcinia kola* seed on serum lipid and enzyme of liver function levels of male albino rats are presented in table 1. The crude alkaloid extract caused a significant ($P<0.05$) reduction in serum total cholesterol (2.00±0.17 mmol/L) low density lipoprotein (0.3±0.08 mmol/L), relative to controls (2.90±0.30; 0.69±0.29 mmol/L) respectively with

Table 1. Effect of diet of *Garcinia kola* Seeds and crude Alkaloid Extract on Serum Lipid Levels Albino Rat

	Experimental group	Number of rats	Total Cholesterol mmol/L	HDL mmol/L	Mmol/L LDLmmol/L	Triglyceride mmol/L
I	**Control 54g/kg**	10	2.90±0.30[a]	2.10±0.01[a]	0.69±0.29[b]	0.57±0.06[a]
II.	**Crude Alkaloid Extract(1000mg/kg.**	10	2.00±0.17[b]	1.80±0.24[a]	0.3±0.08[a]	0.87±0.06[b]
III	**Garcinia kola seeds diet 54g/kg**	10	2.40±0.17[c]	0.56±0.04[b]	0.56±0.04[b]	0.55±0.07[a]

Values are means ± SD and n = 10. Mean values in the same column with different superscripts are significantly (P < 0.05) different

Tables 2. Effect of Garcinia kola Seeds Diet on Selected Serum Enzymes of Liver Function

	Experimental group	Number of rats	Aspartate Amino transferase (AST) IU/L	Alanine Amino Transferase (ALT) IU/L	Alkaline Phosphatase (ALP) IU/L
I	**Control 54g/kg**	10	27.00±0.01[a]	16.0±1.14[a]	36.50±3.54[a]
II.	**Crude Alkaloid Extract(1000mg/kg.**	10	39.67±6.35[b]	8.00±2.00[b]	40.00±1.41[b]
III	**Garcinia kola seeds diet 54g/kg**	10	31.33±4.50[c]	9.66±3.52[bc]	32.57±7.09[ac]

Values are means ± SD and n = 10. Mean values in the same column with different and combined superscripts are significantly (P < 0.05) different.

little or no changes in the serum level of high density lipoprotein. Crude alkaloid extract of *Garcinia kola* seeds produced a significant (P<0.05) increase in serum triglyceride (0.87±0.06 mmol/L) relative to control(0.57±0.06 mmol/L) rats. The diet of *Garcinia kola* seeds on the other hand produced non- significant (P>0.05) decrease in serum total cholesterol, low density lipoprotein and triglyceride (2.40±0.17; 0.56±0.04; and 0.55±0.07mmol/L) relative to control (2.90±0.30; 0.69+0.29 and 0.57±0.06 mmol/L) respectively.

Table 2 displayed the effect of diet and crude alkaloid extract of *Garcinia kola* seeds on selected serum enzyme of liver function. Both the diet and the crude alkaloid revealed significant (P<0.05) reduction in serum Alanine aminotransferase (9.66±3.50; 8.00±2.00 IU/L) relative to control (16.00±1.41 IU/L) rats. Whereas the diet produced a non- significant (P>0.05) reduction in serum Aspartate aminotransferase (31.33±4.50 IU/L), crude alkaloid extract caused a significant (P<0.05) increase in serum Aspartate aminotransferase (39.67±6.35 IU/L) relative to control (27.00±0.01 IU/L) rats but both produced a non – significant (P>0.05) changes in serum Alkaline phosphates levels (40.00±1.41 IU/L; 32.67±7.09 IU/L) relative to control (36.50±3.54 IU/ rats.

DISCUSSION

Cholesterol, a structural component in cell membrane and plasma lipoproteins, is both absorbed from the diet and synthesized in the liver and other peripheral tissues (Stanley *et al.,* 2005). The quantitative analysis of serum cholesterol (measures the circulating levels of free cholesterol and cholesterol esters which is a reflection of the two forms in which this biochemical compound appears in the body (Debayo, 1997). The result of the 28 days feeding trials of crude alkaloid extract of *Garcinia kola* seeds revealed a significant (p<0.05) reduction in serum total cholesterol and low density lipoprotein (LDL) of rats relative to control. This observation is in agreement with the report of (Steinberg *et al.,* 1989, Frei, *et al.,* 1990) who observed a limited development of atherosclerosis in animals exposed to various antioxidant agents. *Garcinia kola* seeds have been reported to possess antioxidant activity (Hungaria, *et al.,* 1991 ; Iranloye *et al.,* 2006). This may have contributed to the lowering effect it exhibited on serum total cholesterol and low density lipoprotein (LDL).

The non-significant (P>0.05) changes caused by crude alkaloid extract with respect to high density lipoprotein lend credence to the report of (Wilson *et al.,* 1988; Gordon and Rifkind, 1989) on the protective effect of HDL on coronary heart disease. The significant (P<0.05) increase in triglyceride may be an undesirable effect likened to anion exchange resin that binds to bile which lowers both serum total cholesterol and (LDL) but cause an undesirable rise in triglyceride without significantly affecting the HDL (Sunil *et al.,* 1998).

The diet of *Garcinia kola* seeds produced non-significant (P>0.05) changes in serum lipid profiles confirming the fact that the lowering effect on serum total cholesterol and low density lipoprotein is more confined to the crude extract rather than the diet. This agrees with report of (Dalziel, 1956) who reported that the active principle was contained in the resin present in the seed or other alkaloid being found on analysis.

The enzymes of liver function assayed were those of Alanine aminotransferase (ALT), Aspartate aminotransferase AST and Alkaline phosphatases ALP. Of these enzymes, Alanine aminotransferase is the most sensitive and specific. The significant ($p<0.05$) reduction in serum (ALT) is in conformity with the report of (Iwu, 1984 Braide, 1991) on antihepatotoxic effect of *Garcinia kola* seed. It is worthy to note that both the diet and crude alkaloid extract caused significant reduction in (ALT). The significant ($p<0.05$) increased serum AST produced by crude alkaloid extract may be attributed to relatively low organ specificity of AST (Stanley *et al.,* 2005) which does not enable one to conclude that such increase may be due to crude alkaloid extract. The diet however, does not produce any significant (P>0.05) changes in serum AST. The non-significant ($p>0.05$) changes in serum alkaline phosphatase (ALP) produced by both the diet and crude alkaloid lends credence to the fact that antihepatotoxicity of *Garcinia kola* seeds resides in its biflavonoid fraction or Kolaviron (Braide, 1991; Iranloye *et al.,* 2006).

The crude alkaloid extract is both sensitive in altering the biochemical indices of coronary heart disease as well as those of liver function whereas the diet is non-sensitive to biochemical indices of coronary heart disease but only sensitive to selected enzymes of liver function.

CONCLUSION

Extrapolating the result in human one may conclude that crude alkaloid extract of *Garcinia kola* seeds may attenuate coronary artery disease and hepatotoxicity of liver parenchyma whereas the diet may be effective as antihepatotoxic agent.

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