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Cardioprotective effect of kolaviron (a Garcinia kola seed extract) in cholesterol-fed rats.

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Abstract

Flavonoids – a group of polyphenolic substances are naturally present in vegetables, fruits, seeds and beverages such as tea and wine. Studies have shown that flavonoid intake is inversely correlated with mortality from coronary heart diseases and myocardial infaction. The effect of kolaviron (a flavonoid complex) extracted from garcinia kola seeds on the plasma and PMF phospholipids and proteins and on the organ weights (lungs, kidneys, heart, spleen and liver) of rats administered with cholesterol, five times a week, for eight consecutive weeks was investigated. The phospholipid levels was relatively stable in both the plasma and the PMF.There was no significant difference (p

Introduction

Coronary heart diseases had continued to be a major cause of mortality in the United states, Europe and much of Asia despite changes in life style and the introduction of lipid lowering drugs (Brannwald, 1997). The developing countries are not totally spared as a result of the influence of the western culture and dietary habit. However, a long standing tenet of nutrition holds that people with diets rich in fruits and vegetables enjoy better health than people eating few fruits and vegetables. Consequently, research has sought for the components or compounds present in these fruits and vegetables which are responsible for this apparent health benefit. Besides, since the discovery of the French paradox; that is, the low cardiovascular mortality rate observed in Mediterranean populations in association with red wine consumption (Formica and Regelson, 1995) Research in the field of flavonoids which are present in red wine has been on the increase. Furthermore, epidemiological studies suggest a protective role of dietary flavonoids against coronary heart diseases (De Groot and Rauen, 1998; Knekt et al., 1996). More than four thousand varieties of these flavonoids have been identified, many of which are responsible for the attractive colours of flowers, fruits and leaves (De Groot and Rauen, 1998). The widespread distribution of these flavonoids, their variety and their relative low toxicity compared to other active plant compounds mean that many animals, including humans ingest significant quantities in their diet.

Several other beneficial properties of flavonoids have since been ascertained. They are most commonly known for their antioxidant activity, reduction of oxidative stress and inhibition of low density lipoproteins (LDL) oxidation (Hanasaki et al, 1994). They also act as vasodilators of blood vessels, anti inflammatory and anti tumour agents (Knekt et al., 1997).

Kolaviron is a biflavonoid complex extracted from the seeds of *Garcinia kola* heckel which is commonly known as bitter kola. Bitter kola is a highly valued ingredient in African traditional medicine (Ayensu, 1978). Kolaviron has been shown to exhibit many pharmacological effects such as anti oxidant (Farombi et al, 2000), anti atherogenic (Adaramoye et al., 2005), anti hepatotoxic (Iwu et al., 1987; Farombi et al., 2000), anti diabetic (Iwu et al., 1990). In the present study, the cardioprotective potential of this extract was investigated in rats rendered hypercholesterolemic by the administration of dietary cholesterol for eight consecutive weeks.

Materials and Methods

Preparation of plant extract:

Garcinia kola seeds were obtained locally in Ibadan, Nigeria. A total of 3kg of peeled seeds were sliced, pulverized with electric blender and then air dried in the laboratory. Extraction of kolaviron was achieved using the method of Iwu, et al., (1990). Powdered seeds were extracted with light petroleum ether in a soxhlet extractor. The defatted dried extract was repacked and then extracted with 70% methanol .The extract was concentrated and diluted to twice its volume at ratio 1:2 with distilled water and extracted with ethylacetate (6x250ml). The concentrated ethylacetate fraction gave a yellow solid known as kolaviron. The extract was prepared into two concentrations (100 and 200mg/kg) using olive oil as a vehicle.

Animals and treatments:

Twenty male albino rats (Wister strain), weighing between 130 -200g were used .The animals were housed in standard polypropylene cages and kept under controlled room temperature of 25ºc. The animals were fed on normal laboratory chow, purchased from Ladokun feeds Ibadan. Animals were given access to food and water ad libitum. They were distributed randomly into five groups of four animals each and an acclamatization period of seven days was allowed for the animals before the commencement of treatments.

Group A serve as the control group and received olive oil. Rats in group B (positive control) received kolaviron 100mg/kg. Those in group C received cholesterol only (hypercholesterolemic animals). Rats in group D and E were treated orally with kolaviron at 100 and 200mg/kg respectively and were simultaneously administered cholesterol. Olive oil serves as the vehicle for kolaviron and cholesterol.

Dietary cholesterol was administered orally at a dose of 30mg/0.3ml per animal (Bhandari and Sharma, 1997). Kolaviron was administered at doses 100 and 200mg/kg (Iwu, 1985; Farombi et a.l, 2000). Kolaviron and cholesterol were administered five times a week for a period of eight consecutive weeks.

Chemicals:

All reagents used were of analytical grade and the purest quality available.

Collection of samples:

In the 8^{th} week, the rats were starved for about 12 hours prior to sacrifice. They were sacrificed by cervical dislocation; the thoracic region was cut open and the tissues to be used (lung, kidneys, heart, spleen and the liver) were rapidly excised and immediately placed in ice-cold 0.25m sucrose to wash off the excess blood and cool off the tissue. The tissues were then blotted dry and weighed using a weighing balance (Sartorius 2354).

Preparation of plasma

The blood collected with the anticoagulant was centrifuged with a table top centrifuge KA 1000 at 3000g for 10 minutes. The supernatant (the plasma) was then collected carefully using a syringe and needle into sample bottles and stored in the freezer for subsequent analysis.

Preparation of sub cellular fractions of tissue homogenate

For the preparation of the post mitochondrial supernatant, the liver tissue homogenate was centrifuged at 12,500g for 15 minutes to pellet intact cells, cell debris, nuclei and mitochondria using an MSE refrigerated centrifuge. The resultant supernatant (the post mitochondrial fraction; PMF) was carefully decanted and stored in the freezer for subsequent analysis.

Determination of phospholipid (pl)

Plasma and PMF phospholipids were estimated by the method of Chen. Jr. Torabara and Huber Warner; (1956). 0.5ml of microsomal/plasma suspensions was added to 9.5ml of ethanol-ether, in a test tube, and heated at 80ºc till boiling. 1ml of digestion mixture was added to 5ml of the above solution and gently heated for 45minutes. The test tubes were cooled and 1ml of distilled water was added and then boiled for 15minutes to conver pyrophosphate to orthophosphate. 4ml of reagent C was then added to 4ml of the above treated samples and the mixture incubated at 37ºc for 1.5 – 2hours. The blue colour developed was read against blank containing distilled water at 600nm.

Determination of protein content by biuret method

PMF and plasma protein were estimated by the method of T.E. Weichselbaum 1946 and Josephson et al., 1957. 20µl of sample and standard was mixed with 1000µl of the colour reagent, incubated for 10 minutes at 20 – 25ºc and the absorbance of the sample and standard were measured against the reagent blank within 30 minutes at a wavelength of 546nm.

Statistical analysis

Results were expressed as mean ±SD (n =4). One way analysis of variance (ANOVA) was used for the data analysis. Duncan's multiple range tests at $p< 0.001$ and $p<0.05$.

Results

Table 1 depicts the effect of kolaviron (kv) on body weight, visceral organ weights and relative organ weight of cholesterol – fed rats.

Table 1:

Effect of kolaviron (kv) on body weight, organ weight and the relative weight of visceral organs in cholesterol – fed rats.

Data are the mean ±SD (n=4). P<0.001 compared with control. Chol. Cholesterol, kv kolaviron.

Cholesterol administration at a dose of 30mg/day for eight consecutive weeks caused a significant increase (p<0.001) in the relative heart weight of the hypercholesterolemic animals (group C) when compared with the control group (A). Co-treatment with kolaviron (groups D and E) significantly (p<0.001) reduced the cholesterol induced enlargement of the heart. This reversal also seemed to be dose dependent. However, the increase in the relative weight of the liver caused by cholesterol administration was not significantly (p<0.001) reversed following co-treatment with kolaviron at the doses of 100 and 200mg/kg. There was no significant effect produced by kolaviron administration at the same doses on the other visceral organs.

Table 2 shows the effect of kolaviron on the plasma and PMF phospholipid levels. No significant difference (p<0.05) was observed in the plasma and PMF phospholipid levels of the cholesterol – fed rats (Group C) when compared with the pretreated groups (D and E).

Grouping	Plasma phospholipid (mg/dl)	PMF phospholipid (mg/dl)
Control (A)	8.50 ± 0.50	8.64 ± 2.16
Kv100mg/kg (B)	7.00 ± 1.00	8.63 ± 0.75
Cholesterol (C)	7.67 ± 0.47	8.86 ± 0.45
Chol. +kv100mg/kg (D)	8.50 ± 0.50	8.64 ± 0.53
Chol. +kv200mg/kg (E)	7.00 ± 1.00	8.32 ± 0.81

Table 2:

Effect of kolaviron (kv) on plasma and PMF phospholipids.

Data are mean ± SD (n=4). P< 0.05 compared with control. Chol. Cholesterol, Kv. Kolaviron.

As shown in Table 3, there were no significant differences (p<0.05) on the plasma and PMF protein concentrations between the control (Group A) and the pretreated groups (D and E).

Grouping	Plasma protein concentration (mg/dl)	PMF protein concentration (mg/dl)
Control (A)	2.00 ± 0.25	0.119 ± 0.01
Kv100mg/kg (B)	18.52 ± 2.63	0.04 ± 0.02
Cholesterol (C)	2.00 ± 0.06	0.04 ± 0.01
Chol. +kv100mg/kg (D)	2.00 ± 0.52	0.04 ± 0.01
Chol. +kv200mg/kg (E)	22.52 ± 2.55	0.05 ± 0.01

Table 3:

Effect of kolaviron on plasma and PMF protein concentrations.

Data are mean ± SD (n=4). P< 0.05 compared with control. Chol. Cholesterol, Kv. Kolaviron.

Discussion

Flavonoids – a group of natural substances with variable phenolic structures, are abundantly present in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine (Kennedy et al., 2002; Slimestad et al., 2007). Invitro experimental studies have shown that flavonoids posses anti inflammatory, anti allergic, anti viral and anti carcinogenic properties (Middleton, 1998).

Kolaviron – a complex mixture of biflavonoids, benzophenones and xanthones (Waterman and Hussein, 1983) has been demonstrated to be anti hepatotoxic (Akintonwa and Essien, 1990), anti inflammatory (Igboko, 1987), anti oxidant (Farombi et al., 2000).

Dietary anti oxidants have been observed to increase low density lipoprotein oxidative resistance in vitro (Esterbaur et al., 1991), (Reaven et al., 1993). It is postulated that ingesting antioxidants and minimizing free radical exposure may reduce low density lipoprotein's contribution to atherosclerosis and hence coronary heart diseases (Esterbaur et al., 1991).

In the present study, cholesterol administration produced a significant increase in the relative heart weight of the hypercholesterolemic animals. This could be as a result of cholesterol deposition on the arterial walls of these animals, leading

to increased resistance to blood flow. Thus the heart in an effort to pump blood through the narrow arteries becomes enlarged and hence the increased weight.

However, the co-treatment with kolaviron at 100 and 200mg/kg reversed this increase in the relative heart weight of the pre treated animals. It has been reported (Hammon, 1996) that flavonoids present in hawthorn berries have a favourable effect on blood pressure by its dilation of the blood vessels resulting in reduced peripheral resistance and increased coronary circulation. Hence hawthorn berries are utilized as cardiotonics, coronary remedies and antihypertonics (Grainger, 1994). Kolaviron probably working in the same way prevented the cholesterol induced enlargement of the heart, indicating that regular intake of kolaviron can protect against heart defects and failures. This result conforms to epidemiological studies by (Degroot and Rauen, 1998) which suggests a protective role of dietary flavonoids against coronary heart diseases. The result also agrees with the report of Hertog et al., 1995 which established an inverse relationship between flavonoid intake and mortality due to coronary heart diseases.

Besides, garcinia biflavonoids have been found to inhibit lipid peroxidation invivo (Farombi et al., 2000), (Adaramoye et al., 2005). Lipid peroxidation has been implicated in many pathological conditions that include coronary heart diseases and cancer (Mora et al., 1990).

The phospholipid levels as observed in this experiment were relatively stable in both the plasma and the PMF. There was no significant difference between the controls and the pretreated groups, suggesting that the pretreatments somehow exerted a protective effect on membrane phospholipids, thus indirectly protecting the membrane bound enzymes of the mixed function oxidase system; since it has been reported that any interference with the phospholipid component of the membrane affects the activities of the membrane bound enzymes of the mixed function oxidase (Emerole and Thabrew, 1983; Brenner, 1984). This result seemed to agree with the findings of Hirosuke et al., (1988) that concentration of hepatic triglyceride and phospholipid was not affected by dietary manipulations. This result goes further to illustrate the speculation that kolaviron acts as a membrane stabilizer and also prevents the distortion of cellular ionic environment (Iwu et al., 1990).

Providing white tea or green tea polyphenols in the drinking water of animals has been found to significantly increase the activity of phase 11 enzymes in the liver (Santana-Rios et al., 2001). Conversely Bravo et al., 1994 who studied the degradability of polyphenolic compounds (catechins and tannic acid) in the intestinal tract of rats reported that there were no interactions between phenolic compounds and protein digestion. However in the present study, there was no significant difference in the protein concentration in both the plasma and PMF of Kv- fed group and the controls; indicating that Kv might not be exerting any significant effect on protein metabolism.

Conclusion

The present study revealed the cardioprotective influence of kolaviron on cholesterol-fed rats. Studies have shown that the biflavonoids of *Garcinia kola* are pharmacologically active with several pharmacokinetic advantages over simple monomeric flavonoids. *Garcinia kola* seeds therefore could be a potent preventive agent for coronary heart diseases.

However, further work need to be done to ascertain the long term effect of kolaviron on these visceral organs.

References

1. Akintonwa A, Essien AR. 1990. Protective effects of garcinia kola seed against paracetamol – induced hepatotoxicity in rats. J. Ethnopharmacol., 29:207-11.

2. Adaramoye OA, Nwaneri VO, Anyanwu KC, Farombi EO and Emerole GO. 2005. Possible anti-atherogenic effect of kolaviron (a garcinia kola seed extract) in hypercholesterolemic rats. Clinical and experimental pharmacology and physiology 32:40-46.

3. Ayensu ES. 1978. Medicinal plants of West Africa. Reference publication Algonac.

4. Brannwald E. Shattuk. 1997. Lecture – Cardiovascular medicine at the turn of the millennium; concerns and opportunities. N.Engl.J.Med. 337:1360-1369.

5. Bhandari U and Sharma JN. 1999. Comparism of hypolipidemic activities of cyamopsis tetragonoloba (guar gum) and Zingiber officinals (ginger) in experimental animals. Indian Vet. J. 66:149-153.

6. Bravo L, Abia R, Eastwood MA and Sauracalixto F. 1990. Degradation of polyphenols (catechins and tannic acid) in the rat intestinal tract. Effect on colonic fermentation and feacal out. Bri. J.Nutr., 71:933-946.

7. Brenner RR. 1984. Effect of unsaturated acids on membrane structure and enzymatic kinetics. Progress in lipid research 23:69-96.

8. De-groot H, Rauen U. 1998. Tissue injury by reactive oxygen species and the protective effects of flavonoids. Fundam Clin Pharmacology 12: 249-255.

9. Esterbauer H, Puhl H, Dieber-Rotheneder M, Waeg G, Rabl H. 1991. Effect of antioxidants on oxidative modification of LDL. Annals of Medicine 23(5):573-81.

10. Esterbaur H, Dieber-Rotheneder M, Striegl G, Waeg G, 1991. Role of vitamin E in preventing the oxidation of low density lipoprotein. Am J Clin.Nutr., 53(1 suppl):314-321.

11. Emerole GO and Thabrew MI. 1983. Changes in some hepatic microsomal componenets induced by prolonged administration of chloroquine. Biochem. Pharm., 32(2):3005-3009.

12. Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO and Emerole GO. 2000. Chemoprevention of 2 – acetylaminofluorene- induced hepatotoxicity and lipid peroxidation in rats by kolaviron- a garcinia kola seed extract. Food and Chem.Toxicol., 38(6):535-541.

13. Formica JV, Regelson W. 1995. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicology 33:1061-1080.

14. Grainger NB. 1994. Herbal drugs and phytopharmaceuticals. CRC press, Boca Raton.

15. Gilberto Santana-Rios, Gayle A. Orner, Adams Amantana, Cynthia Provost, Shiau-Yin Wu and Roderick H. Dashwood. 2001. Potent antimutagenic activity of white tea in comparison with green tea in the Salmonella assay. Mutation Research 495:61-74.

16. Hamon NW. 1996. Hawthorns: The genus crataegus. Can. Pharm. J. 121:708-709.

17. Hertog MG, Kromhout D, Aravanis C. 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Medicine 155:381-386.

18. Hanasaki Y, Ogawa S. Fukui S. 1994. The correlation between active oxygen scavenging and antioxidative effects of flavonoids. Free Radic Biol Med., 16: 845-850.

19. Hirosuke Oku, Nobuhiro Fukuda, Mariko Mori, Maki Kinjyo, Hiroshi Motono and Isao Chinen. 1988. Hypocholesterolemic effect of Bagasse alkaline extract in rats fed artherogenic diet. Nutrition Reports International 38:843-852.

20. Igboko OA. 1987. Anti inflammatory flavonoids and constituents of garcinia kola heckel. PhD Thesis, University of Nigeria Nsukka.

21. Iwu MM, Igboko OA, Okunji CO, Tempesta MS. 1990. Anti diabetic and aldose reductase activities of biflavonoids of garcinia kola. Pharm. Pharmacol., 42(4):290-292.

22. Iwu MM. 1985. Antihepatotoxicity of garcinia kola seeds. Experimentia 41:679-700.

23. Iwu MM. 1986. Biflavonoids of garcinia: Pharmacological and biological activities In plant flavonoids in biology and medicine. Alan R. Liss. NewYork. 485-488.

24. Knekt P, Jarvinen R, Reunanen A, Maatela J. 1996. Flavonoids intake and coronary mortality in Finland: a cohort study. BMJ 312:478-481.

25. Kennedy JA, Matthews MA, Waterhouse AL. 2002. "Effect of Maturity and Vine Water Status on Grape Skin and Wine Flavonoids". Am. J. Enol. Vitic. 53(4):268-74.

26. Middleton EJ. 1998. Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biology 439:175-182.

27. Mora A, Paya M, Rios JL and Aleanaz MJ. 1990. Structure activity relationships of polymethoxy flavones and other flavonoids as inhibitors of non enzymic lipid peroxidation. Biochem. Pharmacol., 40:793-795.

28. Reaven PD, Khouw A, Beltz WF, Parthasarathy S, Witztum JL. 1993. Effect of dietary antioxidant combinations in humans: Protection of LDL by vitamin E but not by beta-carotene. Arterioscler. Thromb., 13:590-600.

29. Slimestad R, Fossen T, Vågen IM. 2007. "Onions: a source of unique dietary flavonoids". J. Agric. Food Chem., 55(25):10067-80.

30. Waterman PG and Hussain RA. 1983. Systemic significance of xanthones, benzophenones and biflavonoids in garcinia. Biochem. Syst. Ecol. 11:21-30.