COMPARISM OF THE HYPOCHOLESTEROLEMIC EFFECT OF KOLAVIRON (A GARCINIA KOLA SEED EXTRACT) WITH QUESTRAN

[1]Nwaneri, V.O.; [2]Anyanwu, K. C; [2]Adaramoye, O.A. and [2]Emerole, G.O
Salem University, Lokoja, Kogi State
Department of Biochemistry University of Ibadan, Ibadan
Email: victoriaoby@yahoo.com

ABSTRACT

The cholesterol lowering potentials of kolaviron; a biflavonoid complex from Garcinia kola seeds and Questran; a hypolipidemic therapeutic drug, administered orally to cholesterol-fed rats for a period of 8 consecutive weeks was investigated.Thiobarbituric acid-reacting substances (TBARS), cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglyceride levels were determined in the plasma. The result showed that kolaviron 100 and 200mg/kg produced over 80% reductions in plasma cholesterol while questran produced 20% decrease in plasma cholesterol. Similarly, kolaviron at the same doses decreased plasma LDL-C levels by over 90% whereas questran exhibited 24.1% reduction in plasma LDL-C. Furthermore, kolaviron inhibited lipid per oxidation by over 40% while quest ran exhibited 21.5% inhibition. The result of the present study demonstrates that kolaviron exhibits a greater efficacy in lowering plasma cholesterol levels than questran and thus suggest a possible use as a dietary supplement for the management and control of hypercholesterolemia.

KEY WORDS: Kolaviron, Questran Hypolipidemic, hypercholesterolemic, garcinia kola.

INTRODUCTION

Flavonoids are ubiquitous group of polyphenolic substances, diverse in chemical structure and characteristics which are present in most plants, concentrating in seeds, fruit skin, vegetables and flowers. A great number of plant medicines contain flavonoids, which have been reported to have a wide range of biological effects including; antibacterial,antiviral (Hanasaki,etal.,1994), anti inflammatory(Middleton andKandaswani,1993), anti thrombotic and vasodialatory actions. They exert these effects by acting as antioxidants capable of scavenging hydroxyl radicals, superoxide anions and lipid peroxy radicals(cavallini and siliprandi,1978) The above free radicals have been implicated in a number of disease processes including cancer (Ginter, 1995), liver diseases (Lebuission et al; 1986) and coronary heart diseases (Gerster, 1989).

Coronary heart diseases and atherosclerosis had continued to be a major cause of mortality in the united state, 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. Thus in recent years, there is a gradual shift from orthodox pharmaceutical products to medicinal plants as alternative medicines especially in the developing countries (Fansworth, 1991), (Murray, 1994).

Gacinia kola Heckel otherwise known as bitter kola is a highly valued ingredient in African traditional medicine. The plant is cultivated mainly for its edible fruits and seeds which have also been employed in folk medicine as a rejuvenating agent and a general antidote.
Kolaviron - a biflavonoid complex from Garcinia kola seeds have been demonstrated to exhibit many pharmacological effects. These include: anti inflammatory, anti oxidant (Farombi et al; 2000), anti diabetic (Iwu, et al; 1990), anti hepatotoxic (Iwu, et al; 1987), (Farombi, et al; 2000). However, to our knowledge, no work has been reported, comparing the hypolipidemic efficacy of kolaviron with any known hypolipidemic drug.
Therefore the present study is designed to examine the ability of kolaviron and questran to attenuate hypercholesterolemia in rats rendered hypocholesterolemic by the administration of dietary cholesterol for a period of 8 consecutive weeks.

MATERIALS AND METHODS:

Plant Materials

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. Briefly, powdered seeds were extracted with light petroleum ether in a soxhlet extractor. The defatted dried marc was repacked and then extracted with methanol .The extract was concentrated and diluted to twice its volume 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.

Repartition of Animal Groups

Twenty eight male albino rats (wister strain), weighing between 150-120g were used. 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 seven groups of four animals each. Group A, serve as the control group and received olive oil. Rats in group B and C (positive control) received questran and kolaviron (100mg/kg) respectively. Rats in group D received cholesterol only (hypercholestreolemic animals). Rats in group E, F, and G were treated orally with questran and kolaviron at either 100 or 200mg/kg respectively and were simultaneously administered cholesterol. Olive oil served as the vehicle for kolaviron, quest ran and cholesterol. Dietary cholesterol was administered orally at a dose of 30mg/0.3ml per animal (Bhandari and sharma 1999). Questran (a hypolipidemic drug) was administered orally at a therapeutic dose of 0.26mg/kg and kolaviron was administered at doses of 100 and 200mg/kg (IWU, 1985); (Farombi et al; 2000). Questran, kolaviron and cholesterol were administered five times a week for a period of 8 consecutive weeks.

Chemicals

Questran (Bristol-myers squibb Hounslow, uk) was purchase from a local chemist in Ibadan, Nigeria. Dietary cholesterol was procured from Aldrich chemical (Millwauke, wi, USA). Thiobarbituric acid (TBA) was purchase from sigma chemical (st louis, MO, USA)). Diagnostic kits for cholesterol, triglycerides and high density, lipoprotein-cholesterol (HDL-C) precipitants

were procured from Boehringer Mannhein Diagnostica (Manbnhein, Germany). All other reagents were of analytical grade and the purest quality available.

Methods

Plasma triglyceride and cholesterol levels were assayed using commercial kits. The lipoproteins (measured using the enzymatic colorimetric method) very low density lipoprotein (VLDL) and low-density lipoprotein (LDL were precipitated by the addition of phospho tungstic acid and magnesium chloride.

After centrifugation at 3000g for 10min at 25^0c, the clear supernatant contained high density lipoprotein fraction (HDL) which was assayed for cholesterol with the diagnostic kit. The LDL-C was calculated using the formula of friedewald et al., (1972). Lipid per oxidation (LPO) was assessed by measuring thiobarbituric acid reactive substances (TBARS) formation, as described by farombi et al., (2000).

Sample Collection

In the 8^{th} week, the rats were fasted for about 12hrs prior to sacrifice. They were sacrificed by cervical dislocation and blood was collected by cardiac puncture into EDTA containing tubes. Plasma was prepared by centrifugation of tubes at 3000g for 10mins in an MSC centrifuge.

Statistical Analysis: Results were expressed as mean ±SD (n=4).One way analysis of variance (ANOVA) was used for data analysis. Duncan's multiple range test at $P<0.05$.

RESULTS

Grouping	HDL-C(mmol/l)	LDLC(mmol/l)	Cholesterol(mmol/l)	TG(mmol/l)	LPO(µmolMDA)
Group A	0.08±0.01	1.35±0.26	1.62±0.36	0.43±0.20	0.64±0.22
Group B	0.06±0.05	1.48±0.41	1.88±0.48	0.59±0.17	0.76±0.09
Group C	0.07±0.07	1.06±0.36	1.48±0.42	0.56±0.13	0.63±0.09
Group D	0.07±0.07	2.05±0.19	2.73±0.47	0.94±0.49	0.85±0.33
Group E	0.08±0.04	1.56±0.38	2.20±0.14	0.87±0.07	0.67±0.12
Group F	0.09±0.02	0.15±0.89	0.34±0.04	0.90±0.12	0.48±0.02
Group G	0.09±0.05	0.06±0.01	0.31±0.07	0.89±0.29	0.45±0.02

Table 1: Effect of kolaviron and questran on plasma lipid profile of cholesterol-fed rats.
Results are the mean ±SD (n=4).$P<0.05$ compared with control.

HDL-C; high density lipoprotein-cholesterol, LDL-C; low density lipoprotein-cholesterol, TG; triglyceride, LPO; lipid peroxidation.

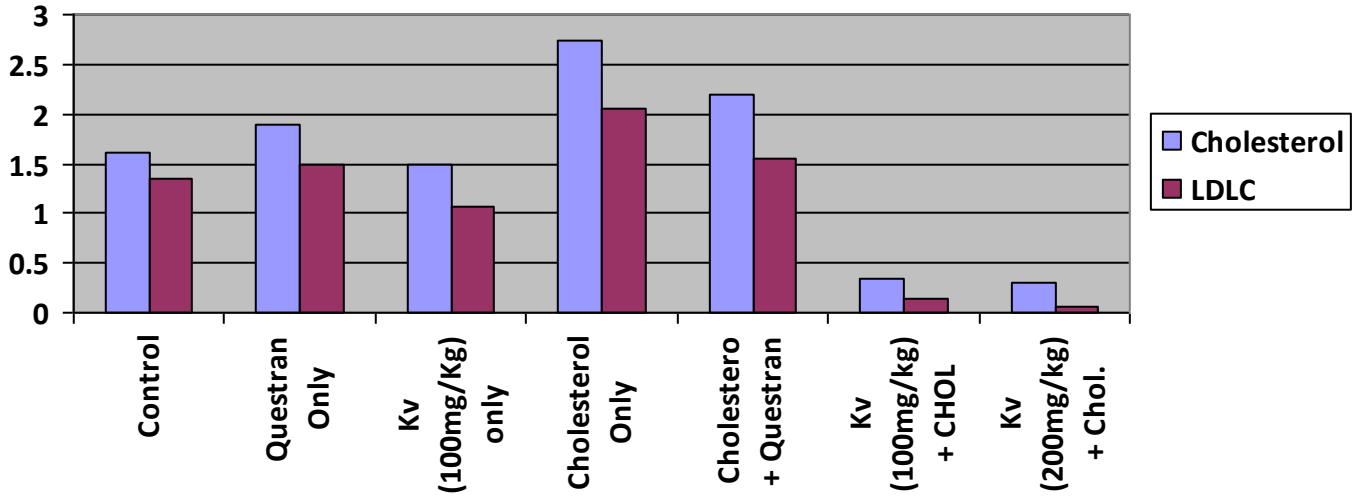

Fig.1 Effect of kolaviron and questran on plasma cholesterol and low density lipoprotein cholesterol in cholesterol fed rats. Data are mean ±SD P<0.05 compared with the cholesterol only fed rats .KV1 and KV2 are 100 and 200mg/kg kolaviron respectively. Chol, cholesterol.

Table 1 and fig.1 shows the effect of kolaviron and questran on plasma lipid profile of cholesterol-fed rats. Dietary cholesterol administered at a dose of 30mg/kg,five times a week for eight consecutive weeks resulted in a significant increase($p<0.05$) in plasma cholesterol levels of the hypercholesterolemic animals compared with the control. However, co-treatment with kolaviron 100 and 200mg/kgproduced 87.6% and 88.8% reductions respectively in the plasma cholesterol levels of the treated animals (groups F and G), compared with the untreated animals (group D).similarly, questran administration (0.26kg/kg) produced a 20% decrease in plasma cholesterol levels of the co-treated rats (group E) compared with the untreated rats (group D). Furthermore, kolaviron at the same doses significantly ($p<0.05$) decreased plasma low density lipoprotein –cholesterol (LDL-C) levels by over 90% in the treated animals compared with the untreated hypercholesterolemic animals. similarly, questran co-treated animals exhibited 24.1% reduction in plasma LDL-C .

Kolaviron 100 and 200mg/kg produced 43.85 and 46.9% decrease respectively in the levels of lipid peroxidation in the plasma of the treated rats (group F and G) compared with the untreated group D. Questran likewise produced a 21.5% decrease in the level of plasma lipid per oxidation compared with the untreated animals. The significantly ($P<0.05$) higher values of plasma triglycerides obtained in cholesterol –fed animals compared with the control animals were unaltered following co-treatment with kolaviron and questran.

DISCUSSION

Results of the present investigation show that kolaviron at the doses of 100 and 200mg/kg produced over 80% reduction in the level of plasma cholesterol while qusetran produced only about 20% decrease in plasma cholesterol levels. It has been reported(Hashim and Vanitallie,1965) that the mechanism of action of most hypolipidemic drugs is to increase cholesterol catabolism to bile acids which is subsequently removed in faeces.

However, increased catabolism of cholesterol leads to increased activity of hepatic β-hydroxy, β-metyl glutaryl-coA reductase, the rate limiting enzyme in denovo cholesterol synthesis. It is therefore speculated that the increased synthesis of cholesterol was presumably sufficient to compensate to some extent for the increased catabolism and thus plasma cholesterol level was not reduced to a greater extent by questran.

The effect of kolaviron as seen in this study conforms with the findings of Muramatsu et al, 1986 who reported that tannic acid and catechins (flavonoids) produced a hypocholesterolemic effect. The result is also in accord with the findings of Nityanaud and Kapoor, 1971, Suheesh et al, 1997, who reported the hypocholesterolemic effect of alcoholic extracts of commiphora mukul and solanium melanogena respectively, in cholesterol fed rats. The result is also in conformity with the study of Koshy et al, 2001 who reported that flavonoids from Garcinia Cambogia,another Garcinia specie elicited hypolipidemic effect as a result of low rate of lipogenesis.

Furthermore, kolaviron at doses 100 and 200mg/kg decreased plasma low density lipoprotein cholesterol (LDL-C) by over 90%. Questran on the other hand, produced 24.1% reduction in the level of plasma LDL-C. Elevated plasma LDL-C is associated with accelerated atherosclerosis(Sparrow et al,1989). Lowering the levels of LDL-C has been established to lower the risk of coronary heart diseases (CHD) (Kashyap,1994). The result of the present study indicates that kolaviron is highly potent in reducing the incidence of CHD. This agrees with the result of Fuhrman et al, 1995; whitehead et al, 1995 who reported that polyphenols from red wine decrease plasma LDL-C and prevent their oxidation invivo. It can as well be speculated that questran's inability to produce a higher percentage reduction in the plasma LDL-C levels could be as a result of its incapability to effectively inhibit the oxidizability of the plasma LDL.

Excess free radicals leads to lipid peroxidation and hence pathological conditions such as atheroscelerosis and cancer(Mora et al 1990). In the present study, kolaviron inhibited lipid per oxidation by over 40%.This result agrees with the report of torrel et al,1986 that flavonoids inhibit lipid peroxidation invitro by acting as a scavenger of superoxide anions and hydroxyl radicals. It is also consistent with the study of Farombi et al, 2000 who reported the inhibition of LPO invivo by kolaviron. Questran however elicited 21.5% inhibition of lipid per oxidation (LPO).

CONCLUSION

Evidence from the present study confirms the greater efficiency of kolaviron over questran in reducing plasma cholesterol and LDL-C levels .Considering the numerous side effects of

hypolipidemic therapeutic drugs which include; formation of cholesterol gall stones, (medical letter,1977),constipation, impaired absorption of fat soluble vitamins, interference with the absorption of many anionic drugs etc and the high hypocholesterolemic potentials of kolaviron, coupled with the non toxicity of Garcinia kola (Braide,1990); kolaviron shows a more promising therapeutic use in the prevention of atheroscelerosis and coronary heart diseases.

However, further work is required to elucidate the possible cardiotonic effect of this extract.

REFERENCES

Hanasaki,Y. Ogawa,S. and Fukui, S.(1994). The correlation between active oxygen scavenging and antioxidant effects of flavonoids. Free radical Biol.Med. **16:** 845-850.

Middleton, E. Jr. and Kandaswamii, C. (1993).The impact of plant flavonoids on mammalian biology and cancer; in the flavonoids: Advances in research (J.B.harborne ed.) Chapman and Hall London. 619-652.

Farombi,E. O.,Tahnteng,J. G., Agboola, A.O., Nwankwo,J.O., and Emerole,G.O.(2000). Chemoprevention of 2- acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron-a Garcinia seed extract.Food and Chem.toxicol.**38:** (6),535-541.

Gryglewski,R.J., Korbut,R.,Robak,J.and Swies,J.(1987).On the mechanism of antithrombotic action of flavonoids.biochem.Pharmacol.**36:** 317-322.

Cavallini.,Bindoli,A.,and siliprandi,N.(1978).Comparative evaluation of antiperoxidative action of silymarin and other flavonoids.Pharmacol.Res.commun.**10:** 132-136.

Salvayre,R.,Negre,A.,Affany,A.,Lenoble,M. and Donsteblazy,L.(1988).Protective effect of lpant flavonoids-analogs and vitaminE against lipid peroxidation of membranes. In plant flavonoids in Biology and Medicine.Biochemical cellular.Newyork,USA.

Ginter,E.(1995).The role of antioxidants in the prevention of tumors.Bratisi leklists. **96:** 195-206.

Lebuisson,D.A., Leroy, L.,Rigal,G.(1986). Treatment of senile macular degeneration with Ginkgo biloba extract.A preliminary double-blind drug vsplacebo study.Presse Med.**15:** 1556-1558.

Gerster, H. (1989).Antioxidant vitamins in cataract prevention. Z. Ernahrung Swiss **28:** 56-75.

Sparrow,C.P.,Parthasarathy,S.,and steinberg,D(1989). A macrophage receptor that recognizes oxidized low density lipoproteins but not acetylated low density lipoprotein. J.Biol.chem**. 264**: 2599-2604.

Turner,P.R., Laville,A., Revil,I.(1984).Metabolic study of variation in plasma cholesterol in normal man. Lancet **22:** 663-665.

Brannwald, E. Shattuk(1997). Lecture- Cardio vascular medicine at the turn of the millinium. Concerns and Opportunities. N. Engl. J. Med. **337:** 1360-1369.

Iwu,M.M., Igboko,O.A.,Okunji,C.O.,Tempesta,M.S.(1990). Antidiabetic and aldose reductase activities of biflavonones of Garcinia kola. J.Pharm.pharmacol**. 42:** (4) 290-292.

Friedewald,W.T., Levy,R.L., and Fredrickson,D.S.(1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge. Clin.Chem. **18:** 499.

Bhandari, U., Sharma,J.N.(1999). Comparism of hypolipidemic activities of cyamopsis tetragonoloba(guar gum) and zingiber officinate(ginger) in experimental animals. Indian Vet.J. **66:** 149-153.

Iwu,M.M.,(1985). Antihepatotoxicity of Garcinia kola seeds.Experientia **41:** 679-700.
Hashim,S.A., and vanitallie,T.B.(1965).Cholestyramine resin therapy in hypercholesterolemia .JAMA. **192:** 289.

Muramatsu,K., Fukuy,M. and Hara,Y. (1986). Effects of green tea cathechins on plasma cholesterol level in cholesterol-fed rats. J.Nutr.Sci.Vitaminol. **32:** 613-622.

Sudheesh, S., Presannakumar,G., Vijayajumar, S., Vijayalashmi, N.R.(1997). Hypolipidemic effect of flavonoids from Solanum Molongena. Plant foods.Hum.Nutr. **51:** 321-330.

Torel,J., Cillard,J., and Cillard,P.(1986). Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochemistry **25:** 383-385.

Braide,V.B.(1990). Pharmacological effects of chronic ingestion of Garcinia kola seeds in the rats. Phytotherapy Research. **4:** 39-41.