Research Article

Atherosclerosis regression of intima-media wall thickness of internal carotid artery with long term use of flavonoids & antioxidants

Swaran Jit.[1], Puri P. L[2], Sidhu R. S.[3]

[1]Dr Swaran Jit., Associate Professor Department of Physiology, Government Medical College, Amritsar (Punjab), India, [2]Dr Prem Lata Puri, Associate Professor MAMC, Agroha Hisar (Haryana), [3]Dr R. S. Sidhu, Additional Professor, Department of Physiology, Government Medical College, Amritsar (Punjab), India.

Address for Correspondence: Dr Swaran Jit. E-mail: jswaran09@gmail.com

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Abstract

Introduction: Free radicals of oxygen causes an abnormal intravascular lipid peroxidase activity and start process of atherosclerosis within blood vessels. Atherosclerosis reversal has been studied in humans and animals. Flavonoids are of plant origin and these are special antioxidant molecules. Arthrosclerosis regression was studied with long term use of Flavonoids and other antioxidants. **Material and Methods:** This prospective controlled trial study was done on 80 ischemic heart disease patients with effort angina having ST segment depression or elevation of more than 1mm in their normal electrocardiogram. 46 subjects of Group A taken as STUDY group were given Flavonoids Antioxidant combination for a period of 9 months as an adjuvant therapy along with routine medical management. Results of baseline study were compared with follow up results of the same group and also with another 27 subjects of Group B taken as control. **Results:** The results showed improved myocardial functions, improved lipid profile picture and highly significant reduction of Intima-media thickness of Common carotid artery examined with High resolution B-mode ultrasonography. Mean values of Intima-media thickness (mm) in case of experimental group were 0.92±0.11 at baseline and 0.84±0.10 at follow up. These results in control group at baseline were 0.93±0.12 and at follow up were 0.96±0.15. Data is statistically highly significant ($p<0.001$) in case of experimental group and not significant ($p>0.05$) in case of control group. **Conclusion:** The results of study have clearly reflected that these compounds were able to reduce atherosclerotic plaque present in major and minor arteries.

Keywords: Intima-media thickness, Altherosclerosis, B-mode Ultrasonography, Myocardial functions, Flavonoids.

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Introduction

Oxygen free radicals are Reactive Oxygen Species (ROS) which are the molecules with an extra electron that makes them unstable. Our natural anti-oxidant defense mechanism like Superoxide Dismutase (SOD) compete with them and so do the anti-oxidant vitamin stores like Vitamin C & E, A and Selenium. With age low levels of SOD and depletion of anti-oxidant vitamins stores have long been an object of study both in experimental animals as well as in humans [1] [2]. Free radicals of oxygen cause an abnormal intravascular lipid peroxidase activity and start process of atherosclerosis within blood vessels [3]. The development of atherosclerosis depends on the balance between pro-inflammatory and anti-inflammatory chemicals along with anti-oxidative defense mechanisms [4]. This is shown by many studies by decreased risk of Coronary Artery Disease (CAD in men and women by taking larger amount of antioxidants [5].

Hyperlipidemia and hypertension are the risk factors which are modifiable and can be early listed as factors involved in prevention of IHD. The traditional definition of Hyperlipoprotenemia has been based on plasma cholesterol levels exceeding the 95th percentile value for age and sex in comparison with measurements in a comparable non restricted population [6].

Oxidation- anti-oxidation imbalance has been implicated in causation of various pathological conditions like cancer and heart disease [7] [8]. With

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more free radical production and compromise of anti-oxidant defense there is an extensive all tissue damage [9] [10] [11]. Poly unsaturated fatty acids exist as a major part of the low density lipoproteins (LDL) in blood and oxidation of these lipid components in LDL play a vital role in causation of atherosclerosis [12]. Free radicals has been incriminated in causation of "Reperfusion Injury" and oxidative modification of LDL- C [13]. The oxidized LDL by tissue macrophages migrates to sub-intimal space leads to formation of "fatty streaks" which is the primary lesion of atherosclerosis [14]. Lipid peroxidation is a chain reaction which is initiated by attack on membrane lipids by oxygen free radicals. Antioxidant vitamins as well as flavonoids break this chain [15].

Atherosclerosis reversal has been studied in humans and animals. Here atherosclerosis is induced in them and allowed to regress [16]. There are autopsy studies of starved humans as well as angiographic studies testing anti-atherosclerosis treatment [17]. Flavonoids are of plant origin and these are special antioxidant molecules [18]. These act as a radical trap for free radicals. This is the key reversal mechanism studied in many atherosclerosis reversal studies like CLAS and MARS [19].

Ischemic Heart disease continue to be the biggest killer responsible for about half of all the deaths. Heart disease and cancer remained the 1st and 2nd leading causes of death, respectively, over the 75-year period [20].

There is always mental, physical or financial trauma implicated in popular revascularization surgery. This leaves a patient in distress for many years. Recent evidences suggest that both depression and anxiety increase the risk for mortality and morbidity after CABG surgery independent of medical factors [21]. To find a viable alternative remedy without increasing mortality and morbidity of heart patients is the need of this hour.

The selection of alternative therapies should be based on not only the prevent progression of heart disease but also somehow a reasonable regression of atherosclerosis process which is basic cause of heart disease. For that anti-oxidant flavonoids offer some hope in oxidative damage prevention. Along with that moderate amounts of physical exercise, breathing exercises and free radical scavengers in the form of addition of dietary flavones and flavonoids [22] along with antioxidant vitamins.

Material and Methods

This prospective controlled trial study was done on 80 ischemic heart disease patient with effort angina both pre and more than 3 months old post myocardial infarction cases. Another choice criteria was all patients were having ST segment depression or elevation of more than 1mm in their normal electro cardiogram.

1. Experimental group (A) comprising 50 subjects
2. Control Group comprising 30 subjects (B)

But out of this number 46 subjects up in A group and 27 subjects in C group reported for follow up after 9 months. Total dropout rate in this study was 8.75%. They were divided into two groups. Group A (46) subjects taken as STUDY group were given Capsule Flavogard Plus BID containing Flavonoids (Luteolin+ Arjunalone) - 100 mg with combination of Vitamin A – 2000 IU, Vitamin E – 80 mg and Vitamin C – 20 mg per day for a period of 9 months as an adjuvant therapy in addition to routine hospital management. They were not asked to stop following disease modification advices by diet or exercise. 27 patients were taken as CONTROL group (Group B) and these subjects got only hospital medical management without giving any "Flavonoid or Antioxidant combination". Even the routine addition by prescribing doctor was not given. But lipid lowering agents were continued in both groups as per prescribed management.

The patients having Severe Congestive heart failure, Chronic obstructive pulmonary disease, myxoedema, gout and any malignancy were excluded from study. Study and control group patients were randomly selected who were both, male and female, alcoholic and non-alcoholic, smokers and non smokers, vegetarian or non-vegetarian.

They were examined for:
(i) Systemic examination,
(ii) Lipid profile,
(iii) Fasting blood sugar level

(iv) Ultra-sonographic Examinnation of Distal Common Carotid Artery for Intima media thickness on High resolution B-mode Ultra-sonography with an Ultrasound scanner (bi-sound 2000II s.a. with an 7.5 MHZ transducer). The attention was focused to demonstrate as clearly as possible the six interfaces (Fig.1) required for the measurement of the arterial wall thickness within right distal common carotid artery. A careful search was then made to search for thickest interface between 2-3 and 4-5 sites anteriorly and then posteriorly. Without moving the transducer, probe was moved cranially until bifurcation was rested within the central region of the monitor and the superior arc of the flow divider was adjacent to the cursor. The site 1cm below the lowest level of bifurcation was marked and interface associated with near and far anterior walls was optimized. Far and near wall intima plus media thickness of common carotid from the thickest interface 2-3 and 4-5 as well as total arterial wall thickness from the thickest interface 1-3 and 4-6 were measured. This examination was repeated after 9 months addition of Flavonoid-Antioxidant combination in their prescribed management. Baseline results were compared with within both groups as well as between experimental and control group.

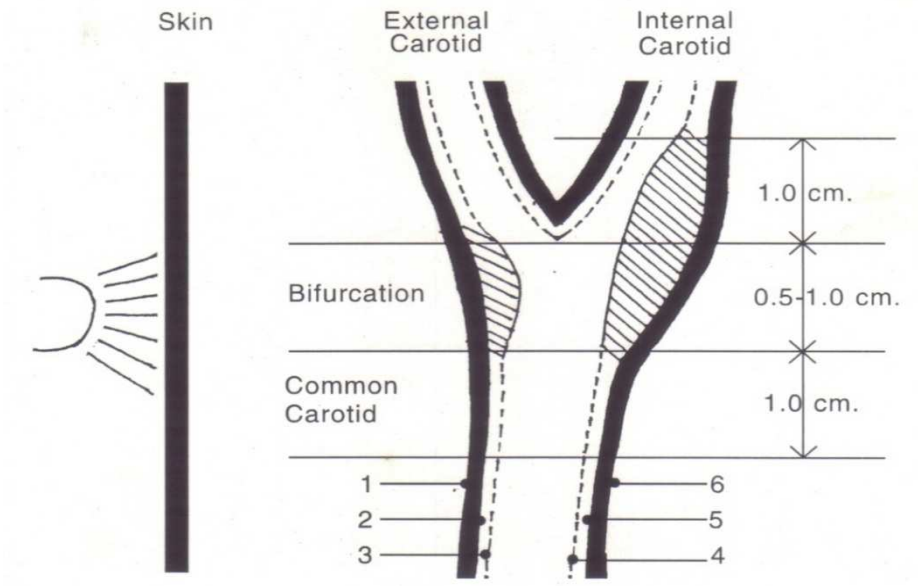

Figure-1: Schematic diagram of Carotid Artery Ultrasonographic examination with lesions in the bifurcation. Near wall interfaces are numbered as: 1-Periadventetia-adventitia 2- Adventitia-media 3. Intima-lumina 4- Lumen-intima 5- Media-adventitia 6- Adventitia-periadventitia (adapted for study from Arch. Intern. Med. 1993;153:501-506)

Observations and results

Table-1: Comparisons of lipid profile in experimental group at baseline and after 9 months follow up.

n=46 (A) n=27(B)

S. No.	Parameter	Baseline		Follow up		p-value	Significance
		Mean	S.D.	Mean	S.D.		
1.	Total Serum Cholesterol	237.32	±37.77	224.67	±19.27	<0.05*	S.
2.	Serum HDL Cholesterol	44.48	±7.64	49.47	±6.82	<0.05*	S.
3.	Serum Triglycerides:	178.75	±44.90	163.02	±31.62	>0.05	N.S.

H.S.= Highly significant S.= Significant N.S.= Non-significant BL= Baseline FU= Follow up

Data shows significant (p<0.05*) lower mean values for Total Serum Cholesterol and higher values of Serum HDL-Cholesterol in case of study group after 9 months therapy of Flavonoids and Antioxidant combination. Serum Triglycerides showed non significant results.

Table-2: Comparison of lipid profile in control group at baseline and after 9 months follow UP.

n=46 (A) n=27(B)

S. No.	Parameter	Base line		Follow up		p-value	Significance
		Mean	S.D.	Mean	S.D.		
1.	Total Serum Cholesterol	230.81	±29.06	241.56	±29.59	>0.05	N.S.
2.	Serum HDL Cholesterol	43.28	±7.03	44.12	±6.62	>0.05	N.S.
3.	Serum Triglycerides:	165.85	±45.49	176.66	±48.99	>0.05	N.S.

H.S.= Highly significant S.= Significant N.S.= Non-significant BL= Baseline FU= Follow up

Data shows non-significant (p>0.05) follow up results of Total Serum Cholesterol and Serum HDL- Cholesterol and Serum Triglyceride in control group. Here only routine medical management was carried out for 9 months follow up.

Table-3: Comparison of baseline results of ultrasonographic study of distal common carotid artery in experimental group with follow up results after 9 months.

n=46 (A) n=27(B)

Parameter	Base line		Follow up		p-value	Significance
	Mean	S.D.	Mean	S.D.		
Combined thickness of arterial wall (mm)	1.30	±0.12	1.23	±0.11	<0.05*	S.
Intima- media Thickness	0.92	±0.11	0.84	±0.10	<0.001**	H. S.

H.S.= Highly significant S.= Significant N.S.= Non-significant BL= Baseline FU= Follow up

Baseline results of Combined thickness of Carotid arterial wall (mm) in case of study Group when compared with follow up results are significant statistically (p<0.05*). Data is statistically highly significant (p<0.001**) in case of intima-media wall thickness. The follow up results were taken after 9 months intake of Flavonoids antioxidants combination.

Table-4: Comparison of baseline results of ultrasonographic study of distal common carotid artery in control group with follow up results after 9 months.

n=46 (A) n=27(B)

Parameter	Baseline		Follow up		p-value	Significance
	Mean	S.D.	Mean	S.D.		
Combined thickness of arterial wall (mm)	1.30	±0.13	1.31	±0.13	>0.05	N.S.
Intima- media Thickness	0.93	±0.12	0.96	±0.15	>0.05	N. S.

H.S.= Highly significant S.= Significant N.S.= Non-significant BL= Baseline FU= Follow up.

In Control Group baseline & follow up results show non-significant change (p>0.05) in cases of combined thickness of arterial wall and intima- media Thickness. Follow up results were obtained after 9 months of routine medical management.

Discussion

It is an undisputable fact that intravascular atherosclerosis is implicated in the causation and progression of coronary heart disease. This study points out to establish the inverse relationship between intake of antioxidants and flavonoids and progression of atherosclerosis in confirmed coronary artery disease (CAD) patients. Atherosclerosis is an inflammatory disease and not merely the passive accumulation of lipids within artery walls. This may be caused as a response to the oxidative components of modified low-density lipoprotein (LDL), or to chronic infection, free radicals, or other factors [23].

This prospective controlled trial study done on 80 known Ischemic Heart Disease patients by doing Ultra-sonographic Examinnation of Distal Common Carotid Artery for Intima media thickness on High resolution B-mode Ultra-sonography both at baseline and after 9 months of addition of alternative therapy as adjuvant establishes once again already documented effect of anti-oxidants and flavonoids on reversal of coronary heart disease [24]. (Table IV)

Anti-atherogenic effect of flavonoids had been also seen in rabbits [25] while our study establish this effect in case of humans too. This may be the outcome of hypo-cholestrolaemic effect of flavonoids and antioxidants as reported earlier [26]. This study has further validated this fact clearly.

Flavonoids are scavengers of oxygen free radicals. These are natural components of our diet since millennia. Consumption of fresh fruits, vegetables and tea since early ages had been adding large amounts of flavonoids and preventing diseases like heart disease and cancer. These provide protection both against coronary heart disease and stroke [27]. Out results have clearly reflected that flavonoids were able reduce intima media thickness of common carotid artery which clearly indicate the reversal of atherosclerosis process. The results have validated the efficacy of flavonoids as anti-athrogenic agents. (Table I, II).

A modified diet plan with increased amounts of dietary flavonoids found in fruits and vegetables and certain plants like T. arjuna and with less amount of low density lipids and saturated fats, produce the hypo-cholestremic effect and this has been established in our study. (Table II).

Low coronary artery disease mortality in France is generally attributed to consumption of red wine [28]. Flavonoids in our study were used to stop further progression of atherosclerosis. Significant reduction in the size of wall of common carotid artery proves efficacy of flavonoids lowering lipid levels and decreasing cholesterol levels in the blood. A number of studies [29] [30] [26] have reported similar beneficial anti-lipidemic and hypocholesterolemic effects of flavonoids in T. arjuna plant.

Side effects: Oral flavonoids antioxidant combination was well tolerated with very fewer side effects like mild headache, nausea and dryness of skin.

Conclusion

Fresh fruits, vegetables, medicinal plants products like T. arjuna and green tea add large amounts of flavonoids in our diet. These provide good protection against coronary heart disease and stroke by partial reversal and modification of atherosclerosis process. The results of study have clearly reflected that these compounds were able to reduce atherosclerotic plaque present in major and minor arteries.

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Abbreviations: BL- Baseline results, **FU**- Follow up results, **CAD**- Coronary Artery Disease, **IHD**- Ischemic Heart Disease, **LDL**- Low density lipoproteins, **HDL**- High Density Lipoproteins.

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Bibliography

1. Tsay HJ, Wang P et al. Age-associated changes of superoxide dismutase and catalase activities in the rat brain, J Biomed Sci. 2000 Nov-Dec; 7(6):466-74.

2. Sevil Gonenc et al. The effect of moderate swimming exercise on anti-oxidant enzyme and lipid peroxidation levels in children. Ind J.Phy Pharm.2000;44(3):340-344.

3. Scott J. Pathophysiology and biochemistry of cardiovascular disease. Cur. Opion. Genet. Develop. 2004;14:271–9.

4. Mehta JL. Mehta J. Antioxidant vitamins in your cardiac patient- are they helpful? Cardiology Review, 1999 Jan Feb; 7 (1) : 56-61.

5. Diane L. Tribble Antioxidant Consumption and Risk of Coronary Heart Disease: Emphasis on Vitamin C, Vitamin E, and β-Carotene. Circulation.1999; 99: 591-595doi: 10.1161/01.CIR.99.4.591.

6. Farmer John A et al. Risk factors for Coronary Artery Disease, Heart Disease: A textbook of Cardiovascular Medicine (Ed. Braunwald) 4th Ed, 1992; 1125-1160.

7. James E. Klaunig, Lisa M. et al. Oxidative Stress and Oxidative Damage in Carcinogenesis Toxicologic Pathology, 2010;38: 96-109.

8. Noriko Noda, Hiro Wakasugi, Cancer and Oxidative Stress JMAJ 2001;44(12):535–539.

9. B González-Flecha, J. C. Cutrin. Time course and mechanism of oxidative stress and tissue damage in rat liver subjected to in vivo ischemia-reperfusion. J Clin Invest.; 1993;91(2): 456–464.

10. Ramos D, Martins E.G. Biomarkers of oxidative stress and tissue damage released by muscle and liver after a single bout of swimming exercise. Appl. Physiol. Nutr. Metab, 2013;38(5): 507-11.

11. Gonenc Sevil. The effect of moderate swimming exercise on anti-oxidant enzymes and lipid peroxidation levels in children Ind. J. Physiol. Pharmacol; 2000; 44(3): 340-344.

12. Esterbauer H, Puhl H. Effect of antioxidants on oxidative modification of LDL. Ann Med. 1991; 23 (5):573-81.

13. Mehta JL, Iris Orbach. Role of new risk factors in coronary artery disease I. H. J. 1997; 51:261-267.

14. Stary HC, Chandler AB. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association, Circulation. 1994; 89 (5) : 2462 - 78.

15. Lobo V. Patil A. Free radicals, antioxidants and functional foods: Impact on human health Pharmacogn. Rev. 2010 Jul-Dec; 4(8): 118–126.

16.Prithvi Raj T. et al. Reversal of Atherosclerosis: Fact or fiction? Cardiol.Today 2000 IV/2; :97-100.

17.Blankenhorn DH, Hodis HN. Atherosclerosis-reversal with therapy.West JMed;1993;159(2):172–179.

18.Rahman Khalid, Studies on free radicals, antioxidants, and co-factors, Clin. Interv. Aging. 2007 Jun; 2(2): 219–236.

19. Hodis HN. Reversibility of atherosclerosis evolving perspectives from two arterial imaging trials: The CLAS and MARS studies, J. Cardiovasc. Pharmacol 1995; 25(suppl. 4): 525-531

20. Hoyert Donna L. 75 Years of Mortality in the United States, 1935–2010 NCHS Data Brief No. 88 March 2012.

21. Tully Phillip J and Baker Robert A. Depression, anxiety, and cardiac morbidity outcomes after coronary artery bypass surgery: a contemporary review J Geriatr Cardiol.2012; 9(2): 197–208.

22. Miller Allan L. Botanical influences and cardiovascular disease, Alter. Med. Review 1996 (3); 6: 422-431.

23. Rodolfo P. Antonio M. Gotto Jr. Atherosclerosis: Evolving Vascular Biology and Clinical Implications Inflammation in Atherosclerosis and Implications for Therapy, Circulation.2004 109 [suppl III]; III-20–III-26

24. Jain V. et al. Effect of *T. arjuna* in patients of angina pectoris, Ind. Med. Gazette 1992 (new); 36:56-59

25. Shaila HP et al. Hypolipidaemic activity of three indigenous drugs in experimentally induced atherosclerosis, International J. Cardiol 1998.; 67: 119-124

26. Ram Alpana et al. Hypochloestremic effects of T. arjuna tree bark, J. Ethnopharmacol.1997; 55: 165-169.

27. Moline J. et al. Dietary flavonoids and hypertension: Is there a link? Med. Hypothesis2000; 55(4): 306-309.

28. E. N. Frankel et al. Inhibition of oxidation of human low density lipoproteins by phenolic substances in red wine The Lancet Vol. 341: 454-457, Ann. Int. Med. 1996; 124(4): 548-56

29. Padmaja UK et al. Safety and efficacy of Hartone in stress angina pectoris- an open comparative trial JAPPI 1999; 47(7): 685-689

30. Harborne JB William CA. Advances in Flavonoids research since 1992, Phytochem. 2000;55(6), 481-504.

31. Ram Alpana et al. Hypocholesterolaemic effects of T. arjuna tree bark, Journal Ethnopharm, 1997; 55:165-169.

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